AUTONOMIC FUNCTION IN ATRIAL FIBRILLATION

VARUN MALIK

MBBS (HONS), BMEDSCI (HONS), FRACP

CENTRE FOR HEART RHYTHM DISORDERS

DEPARTMENT OF CARDIOLOGY

ROYAL ADELAIDE HOSPITAL

&

SCHOOL OF MEDICINE

THE UNIVERSITY OF ADELAIDE

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Dr Varun Malik, PhD thesis, 2018 - 2021

To my parents Drs' Pramod K & Manjushree Malik, my wife Dr Phillippa Malik & my beloved daughter Miss Hannah Anoushka Malik

"Research is to see what everybody else has seen, and to think what nobody else has thought." Albert Szent-Györgyi

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Abstract

At the population level, the prevalence of Atrial fibrillation (AF) is escalating. AF burden also increases in individuals, due its self-perpetuating, progressive nature. While AF itself has been implicated in its self-maintenance, mechanisms responsible are not fully determined.

The efferent arm of the Autonomic nervous system (ANS) alters atrial electrophysiology, triggering AF. At the population-level, imbalances of autonomic tone are prospectively associated with AF. Whilst it is well known that cardiovascular risk factors promote AF, how they interact is uncertain, though there is a potential role of the ANS. AF itself, causes autonomic dysfunction, altering atrial innervation and heightening efferent sympathetic activity. Thus, a bidirectional relationship is likely between AF and the ANS. Whilst considerable effort was placed on the efferent arm of the ANS, the role of the afferent, regulatory arm in AF pathophysiology is unknown. Especially cardiovascular reflexes regulating blood pressure and volume. Interestingly, blood-volume regulating (low-pressure) baroreceptors are co-located in pulmonary vein-atrial tissue, which contain drivers for AF.

This thesis undertakes a series of studies that delineate the role of the afferent arm of the ANS in AF. Using a systematic review and meta-analysis, we demonstrate that AF is independently associated with falls and syncope in older adults. AF can cause orthostatic intolerance (a clinical manifestation of autonomic dysfunction).

Next, we examined low-pressure baroreceptor function in paroxysmal or persistent AF patients, studied in sinus rhythm (SR), identifying novel blood volume regulating reflex abnormalities using Low-level Lower Body Negative Pressure (LBNP).

Then, we performed a series of autonomic reflex tests to delineate the function of low-pressure volume regulating (LBNP), high-pressure blood pressure regulating baroreceptors (Valsalva) and both (Isometric handgrip reflex, IHR). Testing cardiovascular reflexes in the presence of AF is challenging, however, we utilised continuous beat-beat haemodynamic monitoring and venous occlusion plethysmography to evaluate these in patients with AF studied in-AF, and in SR. We demonstrated dysfunction of the volume-regulating LBNP reflex in-AF, for the first time and confirmed diminished LBNP reflexes in-SR. IHR was diminished in-AF, however blood pressure baroreflexes were preserved.

In subsequent studies, we proceeded to show that cardioversion improved LBNP reflex abnormality, as did LLTS (low-level vagal nerve stimulation) a novel neuromodulating technique (LLTS), aiding localisation to the afferent level. Further, we tested whether catheter ablation of AF might further destroy such afferent receptors in pulmonary veins, not having found evidence for this.

Given loss of homeostasis (blood-volume dysregulation) in-AF, we propose an intersect between autonomic and anatomic remodelling (atrial dilatation), possibly representing a mechanism behind AF progression.

In the last study, we tested the interplay between anatomic and autonomic remodelling in Postural tachycardia syndrome (POTS), a known dysautonomia, where the heart is thought

normal. We present new evidence of cardiac remodelling, utilising electrocardiogram markers (also associated with AF).

AF has several sequelae; falls, syncope, dementia, and heart failure, notwithstanding, its own progression, all of which, increase morbidity and all-cause mortality. We open a new scientific avenue to explore mechanisms for these and highlight the role of rhythm management and neuromodulation to treat AF.

Statement of Original authorship

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 give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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Publications and Communication to the scientific community

Chapter 1: Introduction and literature review

Manuscripts:

1. Malik V, Mishima R, A DE, D HL, Sanders P. The "Road" to Atrial Fibrillation: The Role of the Cardiac Autonomic Nervous System. J Atr Fibrillation 2020;13:2400.
2. D. Linz, A.D. Elliott, M. Hohl, V. Malik, U. Schotten, D. Dobrev, S. Nattel, M. Bohm, J. Floras, D.H. Lau, P. Sanders. Role of autonomic nervous system in atrial fibrillation. Int J Cardiol 2019;287:181-188.

Chapter 2: Atrial fibrillation is associated with syncope and falls in older adults: A systematic review and meta-analysis: clinical context to the concept of autonomic dysfunction due to Atrial Fibrillation

Manuscripts:

1. V. Malik, C. Gallagher, D. Linz, A.D. Elliott, M. Emami, K. Kadhim, R. Mishima, J.M.L. Hendriks, R. Mahajan, L. Arnolda, P. Sanders, D.H. Lau. Atrial Fibrillation Is Associated With Syncope and Falls in Older Adults: A Systematic Review and Meta-analysis. Mayo Clin Proc 2020;95:676-687.

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Published Abstracts:

1. V. Malik, C. Gallagher, D. Linz, A. Elliott, T.Agbaedeng, M. Emami, R. Mishima, J. Hendriks, R. Mahajan, L.F. Arnolda, D.H. Lau, P. Sanders. Atrial fibrillation is an independent predictor of falls risk, syncope & orthostatic intolerance in older adults: a systematic review and meta-analysis. EP Europace 2019; pp. ii858-ii866.
2. V. Malik, C. Gallagher, D. Linz, A. Elliott, M. Emami, K. Kadhim, R. Mishima, J. Hendriks, R. Mahajan, L. Arnolda, P. Sanders, D. Lau. Atrial Fibrillation is Independently Associated with Both Syncope and the Risk of Falling in Older Adults: A Systematic Review and Meta-Analysis. Heart, Lung and Circulation 2019;pp. S202-S203.

Presentations at Scientific Sessions:

1. European Heart Rhythm Association (EHRA) annual scientific session, 2019.
2. Cardiac Society of Australia and New Zealand annual scientific session, 2019.

Prizes:

1. European Heart Rhythm Association (EHRA), Focus On Award: Best abstract from Australia, 2019.

Chapter 3: Novel clinical evidence of autonomic dysfunction in patients with Atrial Fibrillation studied in sinus rhythm: A new clinical entity?

Manuscripts:

1. V. Malik, D.J. McKitrick, D.H. Lau, P. Sanders, L.F. Arnolda. Clinical evidence of autonomic dysfunction due to atrial fibrillation: implications for rhythm control strategy. J Interv Card Electrophysiol 2019;54:299-307.

Published Abstracts:

1. V. Malik, D.J. McKitrick, D.H. Lau, P. Sanders, L.F. Arnolda. P2883 Novel clinical evidence of autonomic dysfunction due to atrial fibrillation; with partial reversal after successful pulmonary vein isolation: implications for an early rhythm control strategy. European Heart Journal 2018;pp. ehy565.P2883-ehy565.P2883.

Presentations at Scientific Sessions:

1. European Society Cardiology (ESC) annual scientific session, 2018.

Prizes:

1. Central Adelaide Local Health Network, Nimmo Prize Finalist, 2018.

Chapter 4: Abnormal cardiac volume-sensitive reflex in the presence of Atrial Fibrillation: implications on atrial remodelling

Chapter 5: Cardioversion reverses abnormalities of the cardiac volume-sensitive reflex due to Atrial Fibrillation: impact of rhythm

Chapter 7: Low-level Tragus Nerve Stimulation (LLTS) can reverse abnormalities of the cardiac volume-sensitive reflex due to Atrial Fibrillation: Impact of neuromodulation strategies

Manuscripts:

1. Malik V, Elliott A, Thomas G, Mishima R, Pitman B, Middeldorp E, Fitzgerald JL, Young G, Roberts-Thomson K, Arnolda LF, Lau DH & Sanders P. Autonomic afferent dysregulation in Atrial fibrillation. J Am Coll Cardiol EP 2021; Accepted manuscript.

Published Abstracts:

1. V. Malik, R. Mishima, A. Elliott, M. Emami, K. Roberts-Thompson, R. Mahajan, L. Arnolda, D. Lau, P. Sanders. 70 Autonomic dysfunction in atrial fibrillation (AF) patients: absent vasomotor reflex to decreased cardiac venous return during AF in comparison to sinus rhythm; implications for earlier rhythm control. EP Europace 2020;22:euaa162. 274.

2. V. Malik, Adrian D. Elliott, Mehrdad Emami, Kurt Roberts-Thomson, Rajiv Mahajan, Leonard F. Arnolda, Dennis H .Lau, Prashanthan Sanders. Autonomic dysregulation of cardiac volume during AF in comparison to sinus rhythm; implications for rhythm control. Heart Rhythm 2020;17(5),S291-S382.
3. V. Malik, A. Elliott, G. Thomas, L. Arnolda, D. Lau, P. Sanders. AF Begets Autonomic Nervous System Dysfunction: A Possible Contributor to "AF begets AF". Heart, Lung and Circulation 2021;30,S101-S102.
4. V. Malik, A.D. Elliott, G. Thomas, R.S. Mishima, B.M. Pitman, M.E. Middeldorp, J.L. Fitzgerald, K.C. Roberts-Thomson, G.D. Young, L.F. Arnolda. B-YIA1-01 Abnormal cardiac volume-sensitive reflex in patients with AF: impact of rhythm and implications on atrial remodelling. Heart Rhythm 2021;18(8),S48. Heart Rhythm Society.

Presentations at Scientific Sessions:

1. European Heart Rhythm Association (EHRA) annual scientific session, 2020. **Selected as congress highlight.**
2. Heart Rhythm Society annual scientific session, 2020.
3. Cardiac Society of Australia and New Zealand annual scientific session, 2021.
4. Heart Rhythm Society annual scientific session, 2021.
5. Central Adelaide Local Health Network Research Week, What's New in Heart Disease? RAHSearch, 2021.

Prizes:

1. Central Adelaide Local Health Network, Nimmo Prize, Clinical research (Full time), 2021.
2. Cardiac Society of Australia & New Zealand, Heart Rhythm Prize, 2021.
3. Heart Rhythm Society, Young Investigator Award (Clinical Research), 2021.

Chapter 6: Catheter ablation of Atrial Fibrillation is not associated with destruction of cardiac afferents driving volume-regulating autonomic reflexes: Impact of ablative rhythm intervention

Manuscripts:

1. Malik V, Elliott A, Thomas G, Mishima R, Pitman B, Middeldorp E, Fitzgerald JL, Young G, Roberts-Thomson K, Arnolda LF, Lau DH & Sanders P. Catheter Ablation of Atrial Fibrillation is Not Associated With Cardiac Volume-regulating Afferent Nerve Disruption. Under review.

Published Abstracts:

1. V. Malik, A. Elliott, G. Thomas, L. Arnolda, D. Lau, P. Sanders. Catheter Ablation of Atrial Fibrillation is Not Associated With Cardiac Volume-regulating Afferent Nerve Disruption. Heart, Lung and Circulation 2021;30,S153-S154.

2. V. Malik, A.D. Elliott, G. Thomas, R.S. Mishima, B.M. Pitman, M.E. Middeldorp, J. Ariyaratnam, K.C. Roberts-Thomson, G.D. Young, L.F. Arnolda. B-PO05-138 Catheter ablation of atrial fibrillation is not associated with destruction of cardiac afferents driving volume-regulating autonomic reflexes. Heart Rhythm 2021;18(8),S428.

Presentations at Scientific Sessions:

1. Cardiac Society of Australia and New Zealand annual scientific session, 2021.
2. Heart Rhythm Society annual scientific session, 2021.

Chapter 8: Abnormal cardiac electrical remodelling in Postural Tachycardia Syndrome (POTS): more evidence of a link between cardiac autonomic & anatomic remodelling

Manuscripts:

1. Malik V, Nguyen MT, Seeley, MC, Thiyagarajah A, Elliott AD, Arnolda LF, Sanders P & Lau DH. Abnormal Cardiac Remodelling in Postural Orthostatic Tachycardia Syndrome: Further Insights into its Cardiac Origin. Under review.

Published Abstracts:

1. V. Malik, A. Thiyagarajah, D. Munawar, D. Linz, A. Elliott, M. Emami, R. Mishima, R. Mahajan, P. Sanders, D. Lau. Abnormal Cardiac Electrical Remodelling in POTS:

Mechanistic Insights on Potential Autonomic Dysregulation. Heart, Lung and Circulation. 2019;S199-S200.

2. V. Malik, A. Thiyagarajah, D. Munawar, D. Linz, A. Elliott, M. Emami, R. Mishima, R. Mahajan, P. Sanders, D. Lau. Abnormal Cardiac Electrical Remodelling in POTS: Mechanistic Insights on Potential Autonomic Dysregulation. Asia Pacific Heart Rhythm Society Annual Scientific Meeting 2019.

Presentations at Scientific Sessions:

1. Cardiac Society of Australia and New Zealand annual scientific session, 2019.
2. Asia Pacific Heart Rhythm Society annual scientific session, 2019.

Other Publications & Presentations during the PhD period

Manuscripts:

1. Malik V, Ganesan AN, Selvanayagam JB, Chew DP, McGavigan AD. Is Atrial Fibrillation a Stroke Risk Factor or Risk Marker? An Appraisal Using the Bradford Hill Framework for Causality. Heart Lung Circ 2020;29:86-93.
2. Malik V, Lau DH, Linz D, Middeldorp ME, Sanders P. Is the stomach a way to one's heart? Gastric autonomic activity and AF susceptibility in gastroesophageal reflux disease. J Cardiovasc Electrophysiol 2019;30:2271-2273.
3. R. Wells, V. Malik, A.G. Brooks, D. Linz, A.D. Elliott, P. Sanders, A. Page, M. Baumert, D.H. Lau. Cerebral Blood Flow and Cognitive Performance in Postural Tachycardia Syndrome: Insights from Sustained Cognitive Stress Test. J Am Heart Assoc 2020;9(24),e017861.
4. Sanders P, Malik V, Lau DH. Glycemic Control in Atrial Fibrillation: A Sweet Spot of Risk Factor Modification. JACC Clin Electrophysiol 2019;5:904-906.

Published abstracts:

1. Malik V, Mohammadi FA, Chew DP, et al. A case of recurrent ventricular fibrillation and intractable coronary spasm abolished by sympathetic denervation: autonomic dysfunction a shared mechanism? Paper presented at: Heart Rhythm2019.
2. Malik V, Mohammadi FA, Chew DP, et al. Sympathetic nervous system modulation to abolish recurrent ventricular fibrillation & intractable coronary spasm. Paper presented at: EP Europace2019.

Presentations at Scientific Sessions:

1. Heart Rhythm Society annual scientific session, 2019.
2. European Heart Rhythm Association annual scientific session, 2019.

Chapter 1: Atrial fibrillation & the autonomic nervous system. An introduction & literature review

1.1 Atrial fibrillation (AF)

1.11 The burden of AF

The prevalence of atrial fibrillation (AF) has surged over the last two decades, such that it has become a significant burden to healthcare.[1] From a global perspective, in 2010, it is estimated that there were 33.5 million people living with AF of whom 20.9 million were males and 12.6 million, women.[2] Thus, it is the most common clinical arrhythmia, and is associated with both morbidity (including from stroke and heart failure) and mortality.[2-4] In comparison to estimates of age adjusted prevalence rates from 1990, in 2010, there was an increase of +26.7 per 100 000 in males and +13.2 per 100 000 in females. Indeed, it is estimated that in 2019, the global burden of AF was 59.7 million people[5]; just under double that in 2010 and increasing at a rate even greater than the already staggering historical predictions made regarding the burden of AF by 2050.[2]

Aside from the astonishing increases in the prevalence of AF, there have been increases in both morbidity and mortality associated with AF (approximately 19%) from 1990-2010, as estimated by disability-adjusted life-years.[2] In Australia, over a 20-year period from 1993-2013, the annualised increase of hospitalisation with AF was 5.2%.[1] More than double that of myocardial infarction (2.2% increase per year) or heart failure and, by 2013, AF-related hospitalisations had surpassed presentations for either myocardial infarction or heart failure.[1]

Associated with this, the cost of AF-related hospitalisations increased by 479%.[1] AF is thought to contribute to approximately 1% total health expenditure. Concerningly, GARFIELD-AF,[3] a large worldwide registry of 17 162 patients with non-valvular AF, showed that over a 2-year period, cardiovascular death was more common than ischaemic stroke and systemic embolism and this was not abated by anticoagulation. Thus, identifying a strong need for comprehensive strategies to manage this epidemic.

Finally, not only is the global prevalence of AF increasing, at an individual level, AF is also associated with an escalating burden from time of first diagnosis, due its self-perpetuating, progressive nature.[6] Therefore, a better understanding of the factors that promote and maintain AF, both at a population-level and at an individual-level is critical to addressing this global health concern.

1.12 Risk factors that promote and maintain AF

In several other chronic cardiovascular illnesses, especially coronary artery disease, it is well established (and widely understood) that cardiovascular risk factors promote these chronic diseases and are modifiable targets that are imperative to treat. In regard to AF, this concept is still fairly recent, however there is now compelling evidence to support addressing risk factors in the management of AF.[7-9] Indeed, management of obesity and associated risk factors in affected individuals has received a Class I recommendation in the management of AF.[10] At the individual level, at the onset of AF, triggering factors (producing ectopy from the pulmonary veins[11]) feature in the genesis of AF, however, over time, modifiable risk factors are thought to predominate.[12] Risk factors associated with AF are obesity, hypertension, physical inactivity (and in some cases extreme physical activity), obstructive sleep apnoea, diabetes mellitus, hyperlipidaemia, alcohol and smoking.[7] Indeed, the prevalence of these risk factors themselves are on the rise.[13, 14]

The mechanisms by which modifiable risk factors promote AF are incompletely understood,[12] and therefore this is an important avenue of further research. However, here, the main mechanisms responsible for each risk factor's role in the promotion of AF, aside from the role that the Autonomic nervous system (ANS) plays, are summarised. The role of the ANS in the link between risk factors and AF requires specific attention and will be presented in the next sections of this chapter.

The mechanisms associating obesity to AF are multifactorial.[7] Electrophysiologic changes such as conduction slowing and heterogeneity and electrical fractionation[15, 16], structural remodelling (increased left atrial pressure leading to left atrial enlargement and left

ventricular hypertrophy),[17] and finally, possible paracrine effects of epicardial fat on atrial fibrosis due to inflammatory changes, consequently affecting atrial electrophysiology, can all contribute to the development of AF.[7, 18, 19]

Hypertension results in atrial dilatation and remodelling through reduced atrial compliance. It also results in higher levels of Angiotensin II, resulting in atrial fibrosis and the development of atrial electrophysiologic substrate for AF.[7, 13] Physical inactivity can promote AF through the development of obesity. Exercise can offset this risk.[7, 20] Obstructive sleep apnoea leads to large fluctuations in intrathoracic pressure which results in increased atrial filling, increased atrial pressure and structural remodelling (enlargement, fibrosis and associated electrophysiologic remodelling).[7, 12] Diabetes mellitus and alcohol, both, also induce electrophysiological remodelling as well as fibrosis.[7, 12] Lastly, smoking can induce inflammation, thrombosis, oxidative stress, endothelium dysfunction and fibrosis in the atria; which could produce electrophysiologic vulnerability to AF.[7, 21]

1.13 The mechanisms of AF

The pathophysiology of AF is complex.[12, 22] Aside from risk factors that can promote changes in atrial electrophysiology, the ANS plays a role[23] (and will be discussed in much more detail in subsequent section) and there are genetic variants that can promote AF.[22] Additionally, AF itself plays a role in its own pathophysiology.[24, 25] Each of these interact to produce electrophysiologic characteristics in the atria that can trigger and then perpetuate AF. At disease onset, there may be triggering factors for AF that arise from the initiation of atrial ectopic beats, firing, from the pulmonary veins.[11] Then, as AF progresses, the triggers become less relevant and there is a shift to substrate development that can maintain AF.[6, 12, 24, 25] Such substrate have been classified according to those that are a. modifiable (from risk factors, as discussed above); b. due to AF-related electrophysiologic and structural remodelling (which may be amenable to rhythm control interventions); and c. non-modifiable risk owing to age, sex and genetic factors.[12] Indeed, the presence of multiple co-morbidities seem to co-exist with increasing individual burden of AF.[6]

The triggering mechanisms, from an electrophysiological perspective are generally produced by enhanced automaticity, or triggered activity (after depolarisations), which produce focal ectopy. Localised re-entry (or micro re-entry) is also a mechanism by which AF can be triggered. These can be initiated by a number of factors (including the ANS) that promote abnormalities in cellular calcium handling.[6, 12] The pulmonary veins are anatomically quite susceptible to AF, owing to the disorganised myofiber arrangement of the muscle sleeves surrounding the ostia of each pulmonary vein as they enter into the posterior left atrium, thus promoting anisotropy. They also possess electrophysiologic characteristics such as shorter refractory periods, with differences (heterogeneity) in comparison to the refractoriness of the

rest of the atrial tissue, thus promoting AF vulnerability.[26] These characteristics can be made worse by the ANS, age and other factors that can initiate AF.

Aside from the electrophysiologic changes due to AF itself,[24, 25] which will be addressed in much more detail subsequently in this chapter, the historical proposed mechanisms for the maintenance of AF included re-entrant mechanisms and multiple daughter wavelets.[27] The atria have rich autonomic innervation; which can trigger automaticity, afterdepolarisations (triggered activity) and re-entry facilitating AF.[23, 28] The role of the ANS as a trigger of AF is highlighted in this thesis and will be presented in more detail in subsequent sections of this chapter.

From an electrophysiologic perspective, at the cellular level there are changes in the ion channel currents whereby alterations in the balance of potassium channels produce automaticity. Strong inward rectifying potassium channel (I_{K1}) activity, in excess of the activity of automatic (pacemaker) currents (I_f) in the pulmonary veins, normally prohibit automaticity. These are reversed in AF.[29] Prolongation of the atrial action potential duration, resulting in inward movement of calcium ions through L-type calcium channels provoke early depolarisations within the repolarisation phase.[29] Delayed after depolarisation occurs through intracellular calcium overload from the sarcoplasmic reticulum; through defective function of ryanodine receptors.[29] Once again, the ANS can trigger AF via this cellular electrophysiologic “interface” and is discussed further below.

The switch from ectopic drivers for AF to more maintained AF arises from electrophysiologic substrate in the AF, some of which, as discussed above occur due to AF and some through the concomitant presence of modifiable risk factors. Re-entrant

mechanisms can maintain AF from the ectopic firing "drivers" in the pulmonary veins and can take the form of certain patterns; such as a leading circle or spiral wave fronts.[29] These rely on atrial substrate to produce certain conditions for re-entry whereby there are functional zones of block, recovering or excitable tissue and refractory tissue. These depend on tissue properties such as conduction velocity, autonomic tone, ion channels and alterations that occur through substrate-based remodelling. Conduction velocity and refractory period interact to maintain AF. Thus, shorter refractory periods and diminished conduction velocity can produce an increased number of re-entrant circuits, maintaining AF.[29] These mechanisms can be altered by rhythm controlling (anti-arrhythmic medications) to halt and produce conditions that are not favourable for AF. AF can induce electrical ion channel remodelling that perpetuate AF.[25, 29] Structural remodelling, particularly fibrosis that develops due to the presence of modifiable and non-modifiable risk factors for AF, appears to play an important role in the maintenance of AF through interference of myocyte electrical coupling (slowing conduction velocity through the atrium) and promoting re-entry and ectopy and indeed, this may be responsible for a positive feedback mechanism that results in the progression of AF.[22, 29]

Multidimensional electrophysiologic mapping techniques (panoramic mapping) have allowed us further insights into the electrophysiologic basis of AF maintenance and substrate-based persistence of AF.[30] Rotors (rotational activations or micro re-entrant circuits) have been thought to be present in patients with persistent AF, however, these are dependent on the type of mapping used and have not been universally defined. Additionally, targeting these via ablation strategies has yielded mixed results.[30] Endo-epicardial dissociation of electrical activation with wavefronts that breakthrough from areas other than what was mapped, is another mechanism by which AF could persist and indeed, seems to increase in complexity

with underlying substrate remodelling.[30] The specific factors that influence this and how to clinically map and treat this functional mechanism remains to be elucidated.

1.14 Therapeutic goals in the management of AF

The therapeutic goals of AF management can be divided into 4 "pillars".[13] Namely, risk factor management, anticoagulation to reduce stroke risk, rate control or rhythm control. We have discussed the importance of risk factor management above, however the mechanisms by which these converge to produce AF and therefore, by corollary, how treatment of each risk factor, or in combination can reduce AF do require further delineation. We shall present, later in this chapter, our review on possible ANS mechanisms that lead to AF from each of these risk factors.[31] In this thesis, we shall present data that pertains to risk factor management, as above as well as ANS factors that occur due to AF and may influence rhythm management decisions. Therefore, this section will summarise the current therapeutics available to manage AF and highlight the need for new targets, such as the ANS.

Historically, whether one should pursue a rhythm management approach was questioned, with two randomised studies showing no reduction in stroke risk or mortality benefit.[32, 33] However, these early studies highlight the limited value of anti-arrhythmic medications used to manage rhythm in AF[12] and the need for better strategies to maintain rhythm. Rate control can manage symptoms, however, in certain conditions such as in concomitant AF and heart failure, ß-blockade in the control of rate has not shown to have any mortality effect.[12, 34] Further, there are, as we shall present subsequently, in this chapter as well as in Chapter 2, other clinical effects of AF that were not captured in these early studies. In the last few decades, a catheter based procedure to manage rhythm has emerged.[11] Ablation of the tissue in the left atrium, surrounding the pulmonary veins, in order to electrically isolate the drivers in these regions that produce and maintain AF (PVI; Pulmonary vein isolation) appears to be the most effective of these strategies, although additional lesions sets in the atria have been

proposed and investigated.[35] Although current guidelines suggest trial of antiarrhythmic agents prior to PVI[10, 12], novel data from two studies recently, have shown that PVI may be more efficacious as a first line strategy, earlier in the course of the disease process,[36, 37] whereas in chronic AF where there is a more established substrate and AF is more persistent, catheter ablation is not as effective.[30]

Finally, aside from an understanding of the role that the ANS plays in AF mechanisms, it is potentially a target in risk factor management[31] (see below) and may be a therapeutic target in reducing the burden of AF in some individuals.[38]

1.2 The role of Autonomic nervous system (ANS) in AF

It is well known that AF can manifest from autonomic perturbations;[39-41] which several risk factors of AF could also trigger.[42-50] In this section, we discuss the cardiac neuroanatomy, how perturbation in the ANS can trigger AF, the population-level data linking the ANS with AF, how potential risk factors can promote the development of AF and finally whether AF could potentially promote its own progression through the ANS.

1.21 Cardiac neuroanatomy

A thorough understanding of the cardiac neuraxis and an appreciation of its complex anatomy is paramount. Both adrenergic and cholinergic (efferent, or motor) neurons innervate the atrium together with a complex circuitry that involves afferent or regulatory neurons as well as intrinsic cardiac (local circuit) neurons.[51] These neurons form part of a neural hierarchy that serves to provide beat-beat integrative control of cardiac function.[52] Although, often oversimplified to promote the concept of targeting specific areas of the ANS for specific indications, the neuroanatomy of the intrinsic cardiac nervous system (ICNS) promotes a rather stochastic operation.[51]

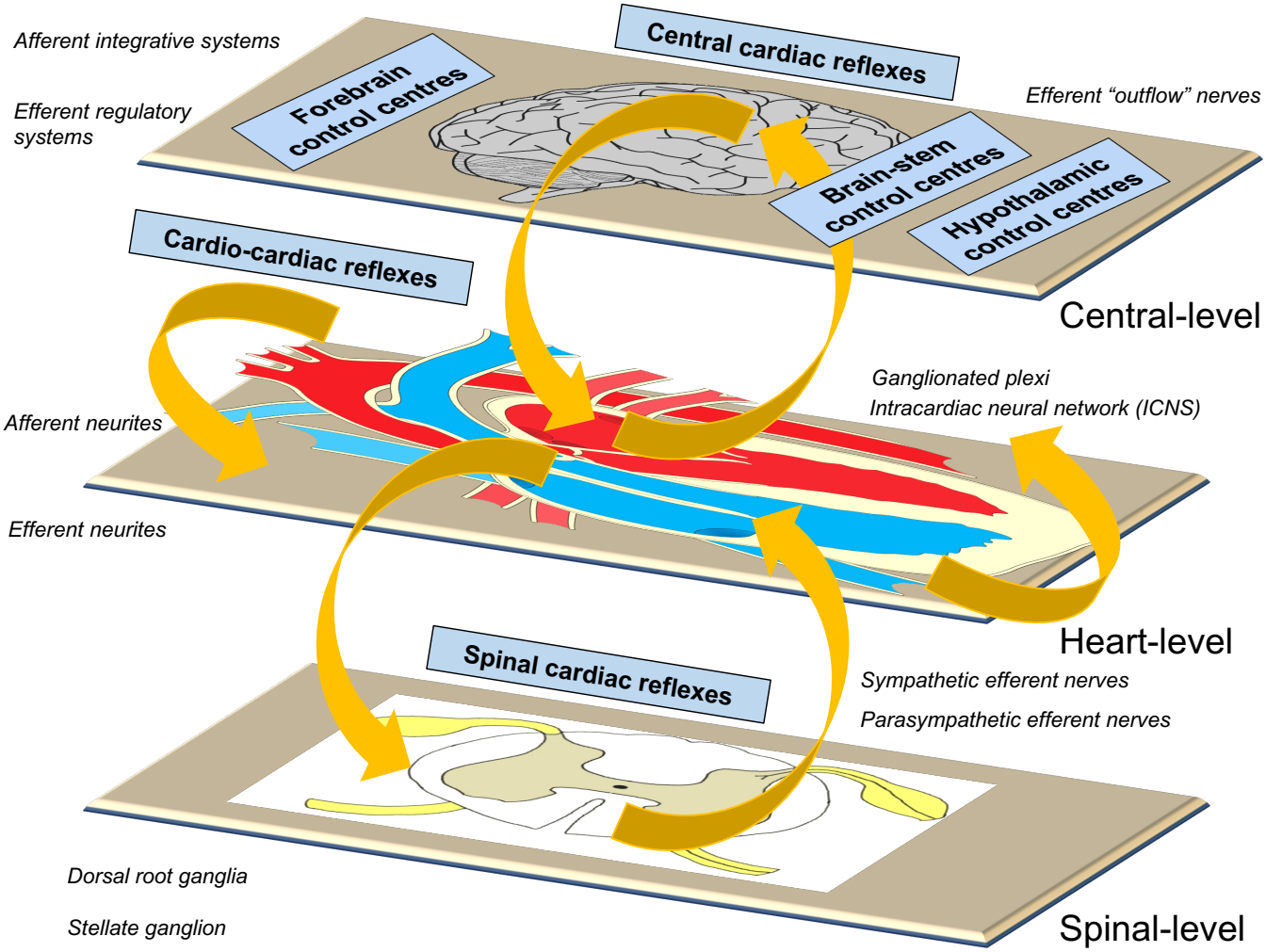
The best descriptor for the hierarchical control of the heart, from multiple layers of feedback loops comes, is the term “nested series of interacting feedback loops”[52] These not only influence atrial electrophysiology to provide a vulnerability to cardiac arrhythmia (including AF), they are also vulnerable to the effect of the dysrhythmia on the ANS (neural remodelling).

At the level of the heart, afferent, motor (efferent sympathetic and parasympathetic), peptidergic and local circuit neurons (that form part of the ICNS) converge on ganglionated plexi that are found in cardiac muscle as well as in epicardial fat pads surrounding the heart [51-53]

Afferent information is collected from afferent mechano-sensitive or chemo-sensitive neurites that are found in abundance in the heart.[52] These signals are conveyed to multiple neuraxial layers of feedback control loops that are able to interact with each other and then

determine the overall extent of efferent outflow (be that sympathetic outflow from intrathoracic extracardiac ganglia, such as the stellate ganglia or parasympathetic outflow from the vagus nerve) to the heart itself (chronotropy and inotropy), blood vessels (vasomotor tone) and neuroendocrine glands (adrenal glands). Thus, there is a beat-beat resolution in terms of integrative neural control. Further, these multiple feedback loops involve local neurons within the heart, between the heart and surrounding intrathoracic ganglionated plexi (*cardio-cardiac reflexes*), dorsal root ganglia, spinal cord, sympathetic ganglia (stellate ganglia); *spinal and intrathoracic reflexes* and then, finally, feedback loops involve higher centres of cardiovascular integrative control in the mid brain (hypothalamus, medulla) as well as the telencephalon (forebrain, cortical brain structures). Thus, there is a series of feedback "rings" from the heart (and initially only involving cardiac neurons) and progressively involving extracardiac loops within the thorax and ultimately encompassing the central nervous system. Figure 1A.

Figure 1A: "layers" of neural control of the heart



There are multiple layers of feedback control circuits; a. within the heart; b. intrathoracic (extracardiac and spinal-level); and c. central layers. Each of these integrate afferent with efferent and sympathetic with parasympathetic efferent neurons to the heart to regulate its function with every cardiac beat.

Sensory transduction from the heart (the afferent neurites)

Sensory transduction of mechanical information (i.e., stretch) is the predominant focus of the experiments performed as a part of this thesis and thus, it is imperative that the detailed neuroanatomy of the afferent neurites is presented. In terms of the literature surrounding the influence of the ANS and AF; this important aspect of the ANS has been thus far neglected. We will present, in further (experimental) chapters why it is imperative that further work is carried out to elucidate a. the effect of AF on afferent neurite function and b. the effect of abnormal afferent function on the development (and or maintenance of AF). There is precedent for abnormalities of cardiac afferent function in cardiovascular disease from studies in hypertension and left ventricular dysfunction[54], heart failure[55] and after cardiac transplantation.[56, 57] It is recognised for more than a century that areas in the heart and lungs can produce dramatic reflexes that alter heart rate and blood pressure.[58] On one hand, veratridine, injected into the coronary arteries produces powerful depressor effects (Bezold Jarisch reflex)[59] and, on the other, Bainbridge showed a reflex tachycardia from the infusion of saline.[60]

Afferent neurons are classified according to the nature of the stimulus: a. mechanosensitive; b. chemosensitive and c. both mechano- and chemosensitive.[53] Associated somata (cell bodies) can be found either; centrally (in the medulla); at the dorsal root ganglion in the spinal cord, or nodose ganglia (intrathoracic, extracardiac); or at the level of the heart (intrinsic cardiac ganglia).[51, 53] A large number of nerve endings respond to both chemical substances; such as bradykinin, potassium and hydrogen ions, adenosine, adenosine triphosphate, free oxygen radicals and arachidonic acid metabolites as well as mechanical stimuli from intense ventricular activity.[53, 61] They can generally course within the

subendocardial layers and can have either myelinated or unmyelinated fibres.[62-64] The reflex effects associated with these nerve endings can be abolished by applying (painting) phenol to the endocardial surface.[65, 66] In general terms, the myelinated endings (A-fibres) have a more rapid conduction rate, lower physiological thresholds and higher discharge rates in comparison to the unmyelinated C-fibre endings, which are often irregular in their discharge patterns.[53]

Cardiac sympathetic afferent nerves

There are cardiac sympathetic neurons, which appear to participate in pain and sympathetic-type responses that have been more difficult to define; however they may participate in the development of arrhythmia; particularly lethal ventricular arrhythmias in that they can provoke efferent sympathetic cardiac activity.[53] "Sympathetic afferents from the heart (predominantly from the epicardial surface of the ventricles[67]) ascend via the dorsal columns, spinothalamic and spinoreticular tracts to terminate in the cerebral cortex. [53, 68] They also have projections in the mid and hindbrain autonomic regulatory areas (paraventricular nucleus[69-71] and nucleus tractus solitarius, NTS[72, 73]). The afferent endings in the heart express transient receptor potential vanilloid 1 (TRPV-1).[74] The elicited reflexes are often termed Cardiac Sympathetic Afferent reflexes, CSAR. Of interest, these cardiac sympathetic afferent nerves can depress baroreflex function and heighten chemoreflexes since they share input into the central regulatory areas described above (particularly the NTS) with baroreceptor and chemoreceptor inputs.[72, 73] Under normal conditions, such reflexes are not thought to have any significant influence on sympathetic activity.[67]

However, cardiac sympathetic afferent neurites can be sensitised in cardiac diseases; in which there is depressed baroreceptor function together with heightened sympathetic efferent

activity (with resultant increases in heart rate, arterial pressure, and cardiac contractility). As such, CSAR are provoked by ischaemia, metabolic mediators as well as cardiac enlargement (structural remodelling), which can further trigger autonomic remodelling.[61, 67, 75-77]

In a rodent heart failure model, produced by coronary artery ligation, the potent toxin, resiniferatoxin (RTX, 50μg/ml), which is an analogue of capsaicin, was applied on the epicardial surface of both ventricles in order to selectively destruct cardiac sympathetic afferents, known to express transient receptor potential vanilloid 1 (TRPV-1). TRPV-1 is activated by capsaicin; and destroyed by its ultrapotent analogue RTX. The main findings of this study[75] were near complete abolishment of CSAR, reduced efferent sympathetic activity (renal and cardiac nerves), improved baroreflex sensitivity, prevention of elevated left ventricular filling pressures, reduction of pulmonary oedema and reversed adverse cardiac remodelling (reduction of fibrosis, apoptosis, fibrotic markers such as transforming growth factor as well as hypertrophy and finally there was also evidence of a reduction in cardiac enlargement. Thus, these autonomic feedback loops can not only alter overall autonomic tone via dysregulation of other autonomic pathways, but they can also influence structural changes. This may be an important feature in cardiac arrhythmias; where the development of atrial dilatation can influence Atrial fibrillation progression and, perhaps, also the development of heart failure.

CSAR participate in a positive feedback loop[61] and under normal physiological conditions there is a fine regulatory balance that ultimately govern cardiovascular physiology on a beat-beat basis, and which can "prescribe" a certain set of conditions for each haemodynamic stressor, dialling down one set of regulatory systems and enhancing another set. In cardiovascular disease (and this possibly extends to Atrial fibrillation as well), there is likely

a loss of balance in such afferent regulatory processes – whereby neurohormonal activation persists, likely due to the predominance of positive feedback loops, such as in heart failure[67, 72, 73, 75] together with a loss of baroreflex control. It is hypothesised that ventricular arrhythmias arising from myocardial ischaemia can result from a positive control mechanism activating cardiac afferents, resulting in augmentation of sympathetic efferent activity.[78] Importantly, these can be abated by thoracic dorsal rhizotomy[79] or stellectomy.[80, 81] I am not aware of any work that specifically addresses the concept of CSAR dysregulation in Atrial fibrillation, and this would be an important avenue to pursue given the overall level of heightened sympathetic activity associated with AF[82] and our findings (presented in subsequent chapters in this thesis).

Arterial (high-pressure) baroreceptors

The arterial baroreflex is fundamental to our survival as an erect species and it is obligatory in blood pressure regulation for cardiac homeostasis.[53, 83] Afferent (mechanosensitive) neurites that transduce changes in stretch, which are found in the outer walls of the aorta and carotid arteries form the information gathering limb of the baroreflex. They input directly to the brainstem. They function in two ways; the first is by firing in patterns (and in groups), transducing the propagating local arterial pressure wave impulses after each cardiac cycle.[53, 84] This information is preferentially carried by large, myelinated "A" fibres and are thought to modulate large or sudden (acute) blood pressure changes.[85] The second is by tracking the baseline blood pressure via small, myelinated or unmyelinated "C" fibres, possibly providing information as to the overall arterial pressures at a certain steady state.[85] Owing to their central regulation, as well as their operation over the whole arterial waveform, specific functions can only be deduced and there is no certainty as to the specific role of each of these receptor sub types.[85] Nevertheless, it appears that the majority of the fibres in the arterial

baroreceptor complex are composed of C fibres.[86] These C fibres are able to produce strong cardiovascular reflexes with lower discharge frequencies than myelinated receptors.[87] The central input of arterial high-pressure baroreceptors is the Nucleus tractus solitarius (NTS) and it is interesting that whilst each of the fibres converge in similar areas, they converge in anatomically distinct areas and therefore, there are likely differential effects of each of these arterial baroreceptor forms.[88, 89] Within the NTS, there may be second order neurons that receive baroreceptor information, and from the NTS, there is signal transduction to other central areas such as the nucleus ambiguus and ultimately to the RVLM, the effector for sympathetic efferent outflow. The Valsalva manoeuvre is a test of the gross integrity of these receptors[90] and can be tested during AF, however techniques exist to determine sensitivity or gain (more subtle characteristics of arterial baroreceptor function in the context of fluctuating blood pressures), though these rely on the presence of sinus rhythm.[83, 90-92] Arterial high-pressure baroreceptors are thought to be structurally very similar to low-pressure baroreceptors (see below).[93] In general, in the literature, there is much more data regarding the structure and function of arterial baroreceptors in comparison to their low-pressure cardiopulmonary counterparts.

Arterial chemoreceptors

Peripheral (arterial) chemoreceptors are located in the carotid and aortic bodies and there are also central chemoreceptors processing similar information in the medulla.[94, 95] These respond to changes in oxygen and carbon dioxide saturation. These receptors are of great importance generally, however in they are not of considerable relevance to this thesis and thus these have only been mentioned here for the sake of completeness. There is a potential role that these chemoreceptors play in the development of AF from obstructive sleep apnoea.

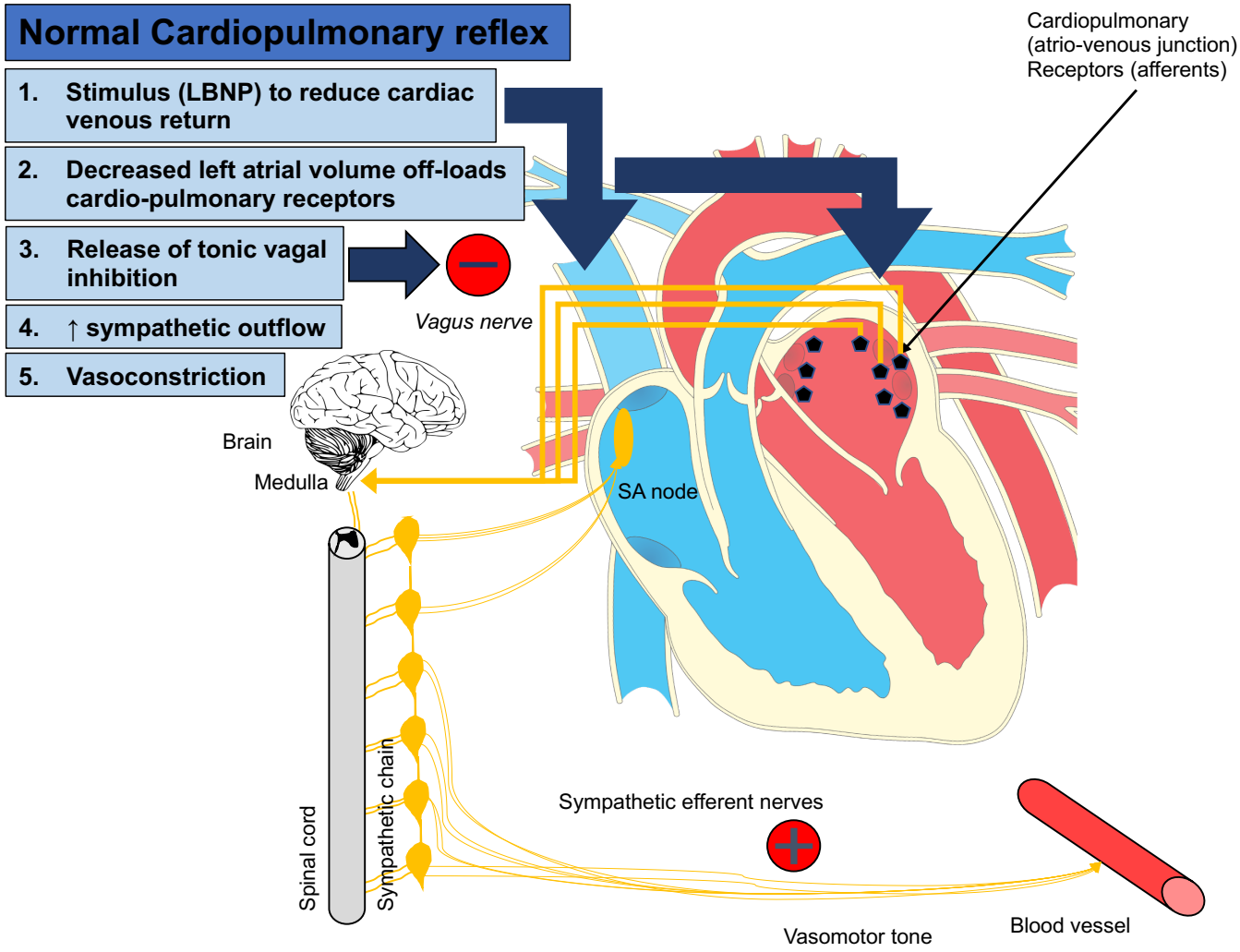
This is presented below. There is a layer of integration with baroreceptors of both the high-pressure arterial type and the low-pressure cardiopulmonary type.[53]

Cardiopulmonary (low-pressure) baroreceptors

These low-pressure baroreceptors predominantly sense changes in cardiac volume. Their physiologic function has been well-studied through decades of physiologic experiments and the techniques used to decrease blood volume and their normal responses have been well-validated.[96] The specific techniques that have been used are presented in this chapter and as such form a large part of the scientific background for the experiments contained within this thesis. The most pragmatic method by which to determine the physiologic role of these receptors is to produce changes (decreases) in central blood volume without altering mean arterial pressure.[93] The early methods, in animal models were models of haemorrhage, whereby blood was withdrawn from the animals, and the function of these receptors measured before changes in arterial pressure occurred.[93] In humans, a technique to simulate the effect of haemorrhage via redistribution of blood from the heart to the peripheries, via negative pressure applied to the lower limbs (Lower body negative pressure; LBNP) has been able to achieve a similar objective.[57, 58, 96, 97] There are multiple effects of LBNP (at varying levels) and duration.[96] At low-levels, where blood pressure does not fall, this technique can be quite useful (especially in addition to the testing of arterial baroreflexes in another way as we have done in the experimental chapters of this thesis) to differentiate high-pressure from low-pressure baroreceptors. However, it should be duly noted that if interpreted in isolation, the differential effects of each of these receptors on LBNP is not absolute.[58] At low-levels of LBNP, mainly acute neural function is tested. At higher levels, arterial pressure falls, and the reflexes involved in severe haemorrhage can be tested. With short durations (as we have utilised in our studies), LBNP mainly tests autonomic (acute) nervous reflexes. Longer

durations of LBNP can induce neuroendocrine changes.[96] LBNP is further discussed in Chapter 4, however we have summarised the acute autonomic nervous reflex elicited by low-level LBNP in humans in Figure 1B.

Figure 1B: The acute autonomic (nervous) reflex elicited by low-level, short duration LBNP



In healthy adults, LBNP at low-levels where arterial pressure remains unchanged and with short duration produces an acute autonomic neural reflex; whereby offloading cardiopulmonary receptors in veno-atrial junctions of the heart results in tonic vagal withdrawal and consequently, an increase in central sympathetic outflow (to the heart and the blood vessels). The measurable effect of this reflex is peripheral vasoconstriction and is discussed in detail in subsequent chapters.

From a physiologic perspective, the earliest description of the receptors participating in the regulation of cardiac volume is probably the curious finding of a reflex tachycardia induced by venous filling by Bainbridge.[60] Much later, it was subsequent experimental work by Linden and Kappagoda who showed that this reflex was initiated by discrete distension (and activation) of presumed receptors in the location of pulmonary vein-atrial or vena cava-atrial junctions of the heart by the inflation of small balloons in these areas.[98-100] Seminal work from Paintal[101-103] has identified these receptors in the atria by producing single filaments of vagal nerve slips and measuring the electrophysiologic properties of these receptors. He identified atrial volume as the primary stimulus of these receptors.[103] Further convincing data comes from Gupta *et al*.[93] who compared the relative contributions of arterial high-pressure and atrial low-pressure baroreceptors in response to progressive haemorrhage as well as infusion in 15 anaesthetised dogs. They have demonstrated, reasonably conclusively, that during moderate changes in volume, arterial receptor discharges remained constant, whereas atrial low-pressure receptors increased activity four-fold with a 20% increase in blood volume and, decreased activity by 80% following 20% decrease in blood volume (moderate). Interestingly, it has been shown that dogs who had a higher blood volume (through ingestion of high salt diets) in comparison to dogs with lower atrial volume (from low-salt diets) were found to have a higher activity of these atrial receptors, where atrial pressures between the groups were similar.[104] Indeed, in the dogs with higher atrial receptor activity, sensitisation to changes in atrial pressure were also seen.

There has also been some historical work to define the structural appearance of such veno-atrial receptors. The first histologic description of the receptors that respond to changes in atrial stretch arise from work using Cajals staining methods to elucidate cardiac afferent arborisations by Nonidez.[105] These receptors were initially thought to represent the sensing

mechanism for the Bainbridge reflex- whereby an increase in heart rate occurs in response to increased caval distension and distension of the heart,[60, 105] however, as discussed above, they respond to both increases and decreases in cardiac volume. These have been localised to veno-atrial junctions in the heart and in particular, the pulmonary vein-atrial junctions, as they are found more in the left atrium than in the right.[97, 105, 106] Histologically, they appear to be myelinated, with a complex receptor ending that is unmyelinated and arborises in the endothelium.[58, 105] Coleridge *et al*.[107] have provided quite strong evidence to support the function of these receptors, by identifying the electrophysiologic characteristics by isolating nerve filaments from the vagus nerve, testing atrial mechanotransduction (pressure) and associated discharge firing rates of these receptors and then subsequently analysing this area histologically and showing histologically that atrial receptors similar to those identified by Nonidez[105] are responsible for the vagal discharges identified using electrophysiologic techniques. Thus this, together with other work, as above, demonstrates the role of these identified receptors as low-pressure-sensitive mechanoreceptors (respond to changes in volume).[58, 106]

The structure and function of these receptors in both health and disease states is not completely demonstrated. Indeed, in the scientific literature, these seem to mostly have become forgotten. The most useful study in terms of correlation of the histology and its function in heart failure was performed by Zucker *et al*.[108] In this study, a canine model of heart failure was produced by surgical creation of an infra-renal aortocaval fistula. They were able to demonstrate that the heart failure dogs, for any change in atrial pressure, had depressed low-pressure atrial baroreceptor function and that this was associated with reduced atrial compliance as well as histological abnormalities in the unencapsulated receptors (Paintal type B, or low-pressure volume-regulating atrial baroreceptors). Specifically, they

showed a loss of end arborisation, histologically. This mechanistic data is consistent with the finding that humans with heart failure have deficiencies in the reflex response to LBNP.[55] Although there is scant evidence of the function of such atrial receptors in cardiac arrhythmia, particularly AF, in one open-chest experiment using dogs, Atrial flutter of fibrillation induced by mechanical stimulation, was associated with increased firing discharges of these atrial receptors. It was thought to represent the mechanism by which AF can induce a reflex diuresis.[109] Indeed, there is evidence to indicate that atrial receptor stimulation results in decreases in antidiuretic hormone.[58] Indeed, conversely, longer, low-levels of LBNP (offloading atrial receptors) without decreases in blood pressure (or arterial high-pressure baroreceptor stimulation), results in an increase in antidiuretic hormone as well as renin levels.[110]

Muscle sympathetic afferent nerves

These are complex neural systems that transduce both chemical and mechanical information in skeletal muscle, integrating these with the autonomic nervous system at multiple "nexus" points.[53] Their integrative role in cardiovascular regulatory physiology (homeostasis) is important in the regulation of cardiac function during activity or exercise. They can act preferentially; in one organ system. For example, cardiac sympathetic outflow is increased preferentially over peripheral (vasomotor) sympathetic activation.[53] These somato-sympathetic reflexes (as measured by increases blood pressure and heart rate) are proportional to the maximal strength rather than the muscle mass or the absolute tension produced by muscle contraction. Therefore, small muscle groups (in the digits) can evoke similar responses to the larger muscles of the thigh.[111] Changes at the level of the muscle due to mechanical contraction or chemical changes (decreases in pH and increases in lactic acid, potassium ions and hydrogen ions) can activate receptors in the perivascular space that then

travel to the spinal cord (group IV afferents) that enter into the spinal cord through the lateral division of the dorsal roots, synapsing in the ipsilateral substantia gelatinosa (dorsal horns) of the spinal cord. [112, 113] Then the way in which these afferent neurons interconnect at varying levels or "nexus points" in the central (supraspinal) structures is less clear and may involve areas in the motor cortex, hypothalamus and other areas.[53, 111] Nonetheless, it seems critical for such afferent information to be conveyed to the Rostral ventrolateral medulla (RVLM) where the bulbo-spinal efferent sympathetic outflow neurons leave (preganglionic vasomotor neurons) and which are, thus, responsible for the expression of the reflexes.[111] Indeed, contralateral transection, chemical inactivation of radiofrequency ablative energy delivery obliterates these reflexes.[114, 115] As final effector pathway, other cardiovascular homeostatic reflexes, including the baroreceptor reflexes, which regulate blood pressure and volume, are also, thus, produced by the RVLM. This allows for integrative control and thus these somato-sympathetic reflexes are influenced also by baroreceptor function.[111, 116] Isometric handgrip exercises are a commonly used technique to measure cardiovascular responses to these somato-sympathetic reflexes[111, 117] and the combined responses to a variety of tests of the integrity of cardiovascular afferent (regulatory) function can assist us in the differentiation of where any putative deficits may be in interpreting baroreceptor responses. This test is further detailed in our testing protocol in the experiments presented as part of this thesis.

Renal afferent nerves

These afferent neurons are both chemo-sensitive and mechanosensitive. Renal mechano-sensitive neurons are found in the renal parenchyma and the walls of the renal pelvis, participating in reno-renal (mediated centrally) reflexes that depress sympathetic efferent outflow.[118] Chemosensitive neurons are predominantly located in the renal parenchyma and pelvis where they participate in spinal-level reflexes that elevate sympathetic efferent

outflow.[53, 118] These receptors are not relevant to this thesis and are therefore only mentioned briefly here.

Efferent sympathetic innervation to the heart

The ultimate neural circuitry "end-point" resides with efferent neurons (both sympathetic and parasympathetic) terminals innervating cardiac myocytes, coronary and peripheral vasculature.[53] Also, there is a direct effect of circulating catecholamines (produced in the adrenal gland) in the "terminal" portion of cardiovascular output.[53, 67] It is imperative to note that the historical context was to consider these opposing "sympathies" – activation of either one or other (reciprocally). This has been debunked and it is now quite clear that there are a multitude of "nexus points"[52, 53] that can interact either within or between themselves; that are either intracardiac, extracardiac but intrathoracic; central; or a combination of each. These hierarchical control circuits can activate either sympathetic neurons; parasympathetic neurons or, as is most common; both, at varying levels in order to integrate autonomic physiology with cardiovascular homeostasis.[53] Put in another way, it is possible for cardiac regulation to occur independently (within cardiac/thoracic levels) without involving central brain inputs.[52, 53, 80] and it is also possible to preserve cardiac control with multiple layers of redundancy present in the system given the hierarchical neuronal efferent control.[119-121]

Cardiac sympathetic efferent neurons

Pre-sympathetic efferent neurons originate in areas of the brainstem and project to cell bodies of sympathetic pre-ganglionic neurons in the intermediolateral cell column of the spinal cord.[122, 123] These cell bodies have axonal inputs to the thoracic ganglia (sympathetic chain including the stellate ganglia) that are located paravertebrally.[52] The peripheral efferent innervation of the heart arises from the cervical levels (C7/C8) up to T4[23] of the spinal cord

and the sympathetic preganglionic neurons that influence efferent vasomotor function predominantly arise from the thoracic levels.[124]

Tonic activity of sympathetic outflow occurs even in basal states.[122] There are three main types of sympathetic efferents that innervate the heart and blood vessels; determined by their sensitivities; baroreceptor sensitive, glucosensitive and thermosensitive.[122] Under basal conditions, baroreceptor sensitive afferents, and their tonic inhibitory activity, mostly, restrain cardiac sympathetic efferent outflow from the RVLM.[53, 122] There are midbrain circuits that can regulate central sympathetic outflow too.[125-127] There is a degree of laterality, however, mostly, innervation to the heart comes from somata in each major ganglion in order to maintain control even if one of those areas is compromised.[119-121, 128-132] These can innervate the extracardiac or intrinsic cardiac ganglia and thereby converge on to the local cardiac network of nerves.[53]

Cardiac parasympathetic efferent neurons

Cardiac parasympathetic preganglionic neurons have somata within the medulla (mostly nucleus ambiguus)[133-135] and innervate the multitude of ganglionated plexi in the atria and the ventricles.[136-138] These intrinsic cardiac ganglia can regulate cardiac function in both the atria and ventricles[132, 139, 140] in a bilateral manner.[121, 141, 142] Indeed, these act as a "collective" to control cardiac function.[143] This local neuronal circuitry could act independently of central control.[144-146] A lot of the integrative function is provided by local circuit neurons within these cardiac ganglia in the heart.[53]

Renal efferent nerves

There are some studies that have evaluated efferent renal denervation in animal models of AF and demonstrated reduced AF complexity and atrial autonomic changes after renal

denervation.[147, 148] There has also been some clinical data to suggest that in hypertensives with AF renal sympathetic efferent denervation may be additive in the treatment of AF; however, care needs to be exercised in this study owing to the lack of a placebo or sham renal denervation arm.[149] These neurons are only mentioned here briefly, for the sake of completeness and given some limited data in AF.

1.22 The ANS as a mechanism (trigger) of atrial fibrillation

The question as to whether AF may be related to acute perturbations in the ANS arose from early observational data that assessed the time of onset of a paroxysm of AF during a 24 hour period and found that there was bimodal distribution strongly resembling the circadian rhythm.[41] The cardiac ANS comprises of sympathetic and parasympathetic divisions that can be afferent/signal processing or efferent nerves together with a large network of interneurons that form a plexus often denoted "the little brain on the mammalian heart."[51, 53] Extrinsic sympathetic innervation to the heart arise mostly from the extra vertebral sympathetic chain, among which, the stellate ganglion is chief.[28] However, the vagus nerve (which contains mostly parasympathetic nerves) has also been found to augment sympathetic tone, with immunohistochemical assessment of the vagus showing adrenergic neuron staining.[28] The vagus nerve contains a mix of both motor and sensory neurons that innervate structures in the heart (sino-atrial and atrio-ventricular nodes, both atrial and ventricular myocardium as well as intrinsic cardiac ganglia).[28] Afferent (sensory) inputs into the vagus nerve can arise from the pulmonary vein- left atrial junctions, sites known to be critical for the pathogenesis of AF.[106]

The downstream effects of efferent atrial nerves on cellular electrophysiology to cause AF are well known and have been detailed previously.[23, 28] Adrenergic nerves release noradrenaline, which stimulates ß-adrenoreceptors through G-coupled proteins. The principal arrhythmogenic effect of adrenergic stimulation comes through its fundamental purpose enhancing myocardial calcium handling in order to produce cardiac contractility in the face of a "fight or flight" situation. [28] Adrenergic nerve- mediated activity of L-type calcium channels, increases calcium influx, resulting in action potential duration changes to enhance early after- depolarization. Owing to the increase in intra-cellular calcium, abnormalities in calcium-handling develop.

First, there is calcium overload, resulting in the extrusion of calcium from the sarcoplasmic reticulum. Second, ryanodine type-2-receptor dysfunction occurs, exacerbating this process. The sodium/calcium exchanger ion channel (NCX), owing to its stoichiometry, *disproportionately* offloads 1 calcium ions from the cell in exchange for 3 sodium ions. This produces a net inward current responsible for triggering delayed after depolarization. Finally, adrenergic stimulation can also enhance automaticity. All these effects can occur at the level of the atrium and the pulmonary veins, triggering AF. [23, 28] Cholinergic (parasympathetic) stimulation can result in shortening of the atrial effective refractory period by increasing the activity of I_{KACh} (acetyl-choline receptor mediated inward rectifying potassium channel). This effect is heterogenous owing to the spatial differences in parasympathetic atrial innervation. [23] The combined contribution of both sympathetic and parasympathetic arms appears to be important in the development of AF with some caveats. In younger patients without structural heart disease, a spectrum of vagal triggers such as vaso-vagal syncope, after ingestion of a meal, at night, or during the recovery phase of exercise, can bring about a paroxysm of AF. [150]

1.23 Cardiac autonomic dysfunction and the risk of incident Atrial fibrillation

Although there have not been a great number of large-scale studies that have directly addressed this question, there is mounting indirect evidence through clinical sequelae of autonomic dysregulation to suggest that there is an association between the incidence of AF and cardiac autonomic dysfunction. The first indirect evidence from large population level prospective cohort studies came from those that assessed whether the presence of orthostatic hypotension (OH) was associated with an increased future risk of AF. The first of these studies, a prospective analysis of 33 346 community dwellers in the city of Malmö, Sweden[151] demonstrated that the presence of OH; defined as a decrease in systolic blood pressure of ≥ 20 *mmHg* and/or a decrease in diastolic blood pressure of ≥ 10 *mmHg* within 3 *minutes* of standing was independently associated with the risk of AF during approximately 24 years follow up. A subgroup analysis indicated that hypertensive individuals with co-existing OH drove this effect.

In a prospective analysis of the 12 071 participants of the Atherosclerosis Risks in Communities (ARIC) study, there was a substantially increased risk of incident AF in patients with OH (18.4%) compared to those without (11.6%), over an 18-year follow up.[152] When the authors adjusted for age, sex and the presence of concomitant risk factors (which perhaps also contribute to autonomic dysfunction), this relationship persisted and was similar in risk to either diabetes or hypertension in their multivariate model.

Psychological stress, in particular anger or hostility, is known to produce perturbations in autonomic activity.[153] Here a surge in sympathetic activity and rise in catecholamines together with decreases in vagal activity have been observed in the laboratory setting in humans. In

animals, correlated with an increase in catecholamines and reduced heart rate variability, protocols that reproduce social stress can trigger arrhythmia.[154] In humans recent data by Lampert *et al*. [153] demonstrate that AF was more likely to occur during anger or stress and that this association was significantly attenuated on patients on ß-blockers (excluding sotalol). At a population level, the Framingham offspring cohort, which included 3873 participants in whom psychosocial questionnaires at a baseline visit were completed, showed that the incidence of AF at up to 10 years follow up was higher in those with higher anger and hostility measures.[155]

Perhaps the only direct measure of autonomic function at a population level, albeit limited to spectral heart rate characteristics (from short 2-minute electrocardiograph recordings) also comes from an analysis participants of the Atherosclerosis Risks in Communities (ARIC) study by Agarwal *et al.*[156] This large prospective cohort study (11 715 participants) also had a long follow up of approximately 20 years. Here, the major finding was that, despite adjustment for a variety of variables, lower resting heart rates and a lower overall variability of resting heart rate were highly suggestive of cardiac ANS dysfunction being associated with an increased risk of incident AF. Power spectral density – frequency domain characteristics of both heightened parasympathetic (High Frequency; HF) as well as sympathetic tone (ratio of Low frequency: High Frequency = LF:HF) were associated with incident AF. Given that the measurements are at rest and predict future incident AF; they are unlikely to implicate simple surges in the activity of both arms of the ANS as causative in in the development of AF. Rather, it is more likely that there is an underlying dysfunction of the ANS present even during sinus rhythm and even at rest. This independent association with future incident AF boosts the hypothesis that chronic disturbances of the cardiac ANS, rather than acute shifts, contribute to the development of AF. These studies are summarised in Table 1A.

Table 1A: Autonomic dysfunction and the incident risk of AF at a *population* level

Study	Measure	Population	Study size	Mean Age	Average follow up duration (years)	Multivariate adjusted risk of the incidence of AF
Direct evidence of autonomic dysfunction						
Agarwal *et al*.[156]	Heart Rate Variability	Atherosclerosis Risks in Communities (ARIC)	11, 715	54±6	20	1.14 (CI; 1.08 – 1.21): per each SD lower HRV.
Indirect evidence of autonomic dysfunction						
Fedorowski *et al*.[151]	Orthostatic hypotension	Malmö, Sweden	33, 346	46±7	24	1.30 (CI; 1.05 – 1.61)
Agarwal *et al*.[152]	Orthostatic hypotension	Atherosclerosis Risks in Communities (ARIC)	12, 071	55±6	18	1.4 (CI; 1.15-1.71)
Eaker *et al.*[155]	Psychosocial: Anger and Hostility measures	Framingham offspring	3873	49±10	10	1.2 (CI; 1.0-1.4): Anger & 1.3 (1.1-1.5): Hostility

SD = Standard Deviation. CI = confidence Interval. HRV = Heart Rate Variability (SD normal-normal RR interval)

1.24 The role of the ANS as the "road to AF" from risk factors

Atrial remodelling due to risk factors for AF has been shown to be reversible; however, the mechanisms by which these risk factors interact to produce atrial remodelling and subsequent AF are unclear.[7] This intersection is critical to the development of strategies to combat this disease. The parallel increases in the prevalence of both, the development of AF as well as its risk factors may be underpinned by a shared mechanism. Here, we review the potential role of the autonomic nervous system (ANS) as an intermediary between risk factors and the development of AF.

Obesity

The role of obesity in the development of AF is well established. Firstly, through a strong epidemiological link, second through electrophysiological studies demonstrating atrial substrate development in obesity[13, 15, 16] and finally, through the finding that weight loss together with a comprehensive risk factor management strategy can result in improved freedom from AF after catheter ablation[9] and may even reverse the progression of AF.[157] It is also well established that obesity[42, 158-162] including visceral obesity[50] is associated with an increase in sympathetic tone, as assessed by direct muscle sympathetic nerve activity (MSNA). Importantly, 10% weight loss after gastric banding procedure in severely obese patients led to a reduction in MSNA concomitant with improved cardiac and sympathetic baroreflex function.[163]

Obstructive Sleep Apnoea

Obstructive sleep apnoea (OSA) has been identified as an independent risk factor for AF.[7, 43] The acute effect of obstruction results in thoracic impedance changes which increase cardiac

venous return and left atrial stretch, with associated electrophysiological changes such as reduced atrial effective refractory period and consequent AF vulnerability.[13, 164] In parallel with these acute effects, there is also increased ganglionated plexus activity with reduced susceptibility to AF after ganglionated plexi ablation, atropine and vagotomy.[13] These observations strongly implicate the ANS in the pathophysiology of AF due to OSA. Additionally, an acute obstructive event also initiates the diving reflex, hypoxia and arousals that induce surges in both parasympathetic and sympathetic activity, which could promote the onset of AF.[23] Finally, repetitive airway obstruction can result in chronic effects that include the development of atrial fibrosis and electrical remodelling, both of which contribute to AF maintenance,[13] OSA can also result in autonomic remodelling with both sympathetic and parasympathetic atrial hyperinnervation, baroreceptor dysfunction, and chronic sympathetic activation, all of which can produce AF.[165] Interestingly, in addition to ganglionated plexus ablation, a variety of strategies to modulate the ANS, such as ß-blockers, renal denervation, low-level tragus stimulation and baroreceptor stimulation, have been shown to reduce AF vulnerability to OSA in pre-clinical studies.[165] Further work is required to determine whether neuromodulation can be a useful adjunct in the treatment of OSA, particularly in those intolerant or non-adherent to continuous positive airway pressure treatment.

Hypertension

Epidemiological studies have consistently identified hypertension as an important risk factor for AF.[166, 167] Although, several mechanistic studies have identified a hypertension related atrial substrate, there is cumulative evidence that in this group, sympathetic overdrive may be a significant contributor to this risk.[13, 168-171] The ANS has achieved significant attention in the pathogenesis of hypertension.[44] Techniques such as sympathetic nerve activity using MSNA, the spill-over of noradrenaline and heart rate variability have clearly shown a sympathetic "overdrive" that occurs at all severities of elevated blood pressure.[44] Additionally, the increase in sympathetic activity parallels blood pressure increases; implicating a cause/effect link.[172] Indeed, these findings have been further extended to show that sympathetic over-activity is associated with and may be contributory to hypertension related end-organ damage (vascular remodelling, left ventricular hypertrophy, and perhaps even diastolic dysfunction).[44]

In a small group of pre-hypertensive individuals, MSNA at baseline and at 8 year follow-up demonstrated a correlation between MSNA and BP increase.[173] In a comparison of young black men, who have a raised risk of hypertension, to white men, there was an exaggerated vasomotor response to sympathetic activity: where MSNA, itself, was not different between the groups.[174] This suggests factors other than gross sympathetic motor tone are important in mediating the role of increased sympathetic nerve activity and high blood pressure. Although arterial baroreceptors and chemoreceptor deficits in heart rate control have been well established; there is no impairment in baroreceptor control of vasomotor tone.[44, 172] Cardiopulmonary receptors; found typically in veno-atrial junctions of the heart,[108] that respond to changes in blood volume and inhibit efferent sympathetic outflow[175] have been shown to be reversibly impaired in patients with hypertension and left ventricular hypertrophy.[54]

Diabetes

The epidemiological association of AF with diabetes is well described and it is considered an independent risk factor for AF.[166, 176, 177] Recent work has demonstrated that in patients undergoing catheter ablation for AF, glycaemic management results in significantly improved outcomes,[178] highlighting the need for suitable targets to optimise (>10% reduction in HbA1c and a target of <6.5%).[179] Although there are likely a number of responsible mechanisms;[179] of which none have been clearly elucidated, it has been shown that diabetes (which is well known to cause autonomic neuropathy) can result in cardiac autonomic dysfunction; assessed using tests such as Valsalva manoeuvre, heart rate variability during deep breathing, heart rate and blood pressure responses to standing and the isometric handgrip test.[180] It is proposed that cardiac autonomic dysfunction can cause tachycardia, reduced exercise capacity, orthostatic intolerance (together with peripheral blood vessel sympathetic denervation) and perhaps even silent myocardial ischemia. [180]

In an animal model of diabetes, atrial refractoriness, atrial conduction velocity and AF inducibility were not different to control rats at baseline; however, diabetic rats showed a heightened susceptibility to AF from sympathetic stimulation, together with histologic evidence of sympathetic nerve remodelling.[48] This link has also been demonstrated in humans, where the burden of AF in diabetics was strongly correlated with LF/HF ratio, a frequency domain spectral characteristic of heart rate variability thought to represent a marker of sympathetic activity.[181] Overall (time-domain) heart rate variability was not reported in this study, which is a significant limitation. Direct measures of sympathetic nerve activity (MSNA and noradrenaline spill over) show that diabetes is associated with both an increased central sympathetic drive as well as an impairment in the sympathetic responses to a carbohydrate

load.[49] Therefore, there is modest evidence that implicates autonomic dysfunction as a mediator of the elevated risk of AF associated with diabetes.

Alcohol excess

The role of alcohol as a dose-dependent risk factor for AF is well known.[47] A recent meta-analysis has demonstrated that moderate levels of alcohol consumption are associated with increased risk of AF.[182] A randomised intervention study has shown that in patients with AF, abstinence results in reduced recurrence at 6 months follow up.[183] A variety of mechanisms have been proposed. However, the direct effect of alcohol as neurotoxin[184] resulting in autonomic dysfunction is likely to be important. Acute ingestion of alcohol is associated with a decrease in heart rate variability[185, 186] and diminished vagal heart rate modulation in healthy adults.[185] Sympathetic hyperactivity was observed in patients with coronary artery disease[186] and with a history of AF, in whom alcohol ingestion was associated with increased sympathetic tone (assessed using heart rate variability) together with increases in ß-adrenergic density.[187] A number of studies also report an increase in MSNA due to alcohol.[188]

Finally, acute alcohol ingestion, which is a cause of syncope and orthostatic intolerance, is associated with an impaired homeostatic reflex response to decreased venous return, elicited by a non-invasive technique (Lower Body Negative Pressure; LBNP).[188, 189] Whilst under normal circumstances, blood pressure is maintained due to vasoconstriction,[96] alcohol ingestion results in significant decreases in blood pressure and absent vasoconstriction. Although this reflex deficit could potentially be confounded by the known vasodilatory effect of alcohol, Carter *et al*.[188] showed a concomitant deficit in the MSNA response to LBNP, which provides strong evidence to the contrary. Moreover, separate studies report baroreceptor dysfunction due to alcohol,[190] implicating cardiac afferent neurotoxicity. Therefore, not only

can alcohol cause perturbations in autonomic tone and autonomic remodelling, but it may also lead to autonomic deficits, thus, providing a pathway to the development of AF.

Smoking

There is an epidemiologic link between smoking and the long term risk of developing AF, with the highest risk in those with the largest intake, and somewhat decreased risk in those who quit.[191] There is no direct evidence linking the ANS to smoking as a risk factor. The pathophysiologic link is presumed to be related to the development of other risk factors in smokers – such as inflammation, hypertension, and vascular disease. Nevertheless, there is strong evidence that cigarette smoking causes an increase in sympathetic activity.[45] Middlekauff *et al*.[45] provide a comprehensive review of the role of nicotine as well as fine particulate matter from tobacco in the development of autonomic dysfunction. Both acute and chronic effects result in an increase in sympathetic nerve activity, which can at least partially account for the epidemiologic association of smoking with AF.

Smoking causes acute increases in heart rate, blood pressure and contractility due to the effect of nicotine to increase noradrenaline from peripheral sympathetic efferent nerve terminals[192, 193] as well as a rise in sympathetic nerve activity. Whilst several mechanisms may be responsible for chronic increases in SNA, several studies demonstrate a consistent attenuation of afferent nerves, particularly the baroreceptors found in the heart and blood vessels. While under normal circumstances, the baroreflex would counter the effect of nicotine on sympathoexcitation, smoking results in dysfunction, permitting unchecked sympathetic activity.[45, 194] This effect appears to be reversible; encouraging the role of smoking cessation as a component of risk factor management.

Physical inactivity

Physical activity is associated with a reduced risk of AF in a number of studies and it can offset AF risk in obese individuals.[195, 196] A sedentary lifestyle is not only strongly linked to both cardiovascular disease as well as mortality, it is associated with autonomic dysfunction.[46] Similar to the other risk factors, as described above, physical inactivity is associated with sympathetic hyperactivity, and baroreceptor dysfunction owing to an enhanced baroreflex initiated sympathetic outflow. More recently, destructive changes in the central nervous system centres that control sympathetic outflow (RVLM; Rostral Ventrolateral medulla) have been described that result in an increase in sympathetic nerve activity.[46] Indeed, it is proposed that physical inactivity may contribute to the development of hypertension due to the increased sympathetic activity.[46]

Although the benefits of physical activity, especially in terms of AF risk are widely accepted; excessive physical activity, particularly endurance exercise, is also associated with an increased risk of AF.[197] The mechanisms underlying this association are not well understood; although heightened parasympathetic activity that result in electrophysiological changes in the atrium (shortening refractory periods) due to acetylcholine dependent potassium channels may play a role.[197, 198] A rat model of endurance training has demonstrated a temporal association with vagal activation (both efferent atrial effects and baroreceptor augmentation) coupled with AF inducibility; that wanes with cessation of excessive physical activity.[199]

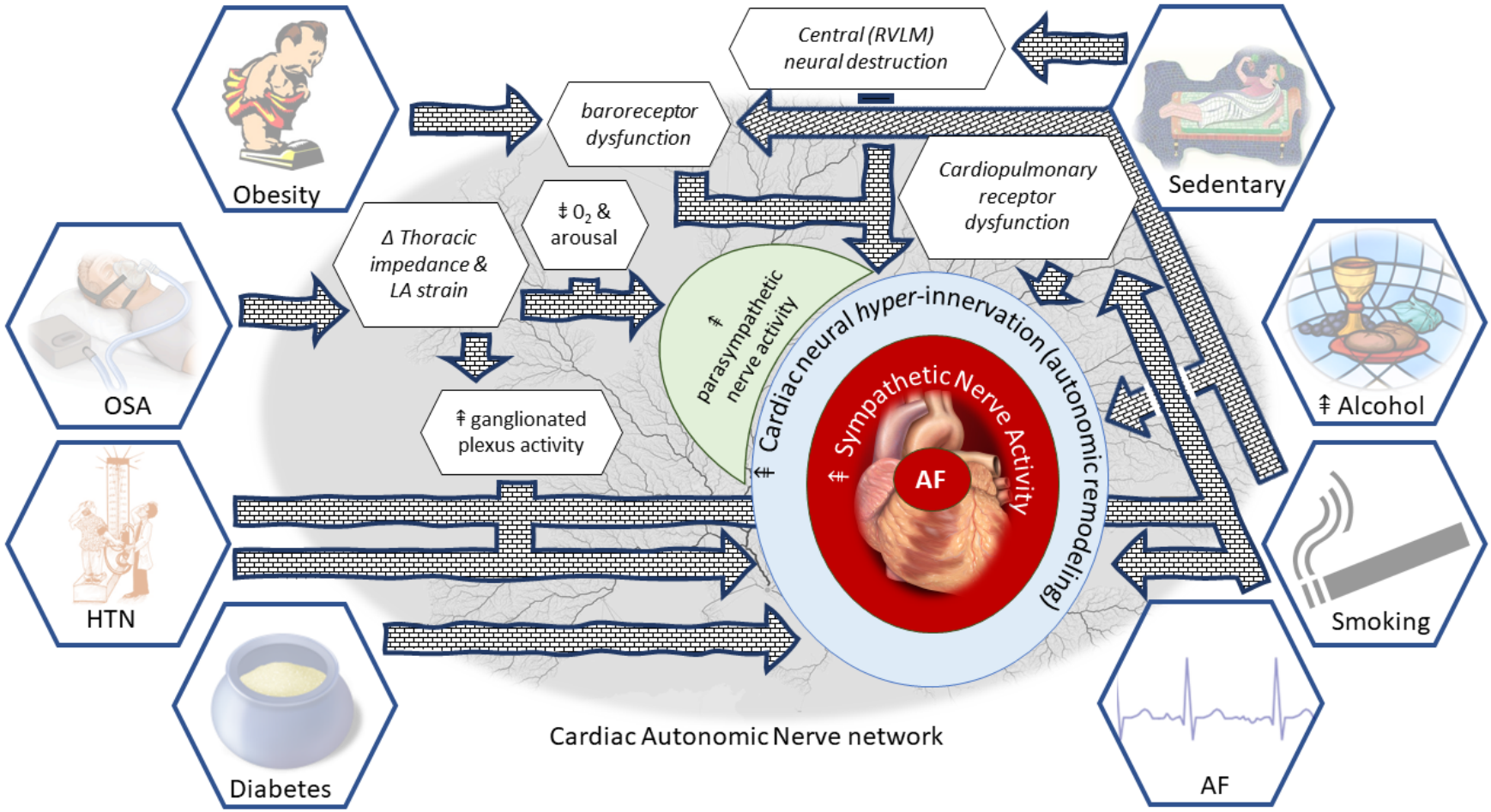
Through a combination of epidemiological association as well as mechanistic studies, there is considerable evidence to suggest the concept that the ANS may form an integral link between lifestyle-based risk factors and the development of AF. Potentially this relationship may extend

to be one of the drivers of disease progression. There is a surprising amount of evidence linking autonomic dysfunction due to risk factors to AF and are summarised in Figure 1C.

Therefore, we propose that the role of the ANS is quite literally an important section of the "road" that leads to AF.

Dr Varun Malik, PhD thesis, 2018 - 2021

Figure 1C: "All roads lead to AF"



A "map" of the road to risk factors leading to autonomic dysfunction and then to AF. From Malik et al.2020.[31]

1.25 Potential role of the ANS in the progression of AF?

The effect of autonomic tone on atrial electrophysiology, and, as a trigger for AF is fairly well established. I have already discussed this concept; which has been reviewed extensively by our group; in collaboration with others.[23] In this section I aim to discuss the essential limitation of prior work in this area; which generally confines the role of the autonomic nervous system and its perturbations to the direct, *efferent* effects of autonomic tone. Identification of this knowledge gap; in my opinion is of vital importance in accurately determining the role of the ANS in the interplay between risk factors (atrial substrate) as well as propensity to progression (atrial remodelling) in the disease process that characterises AF.

The classical experiments performed by Wijffels *et al*.[24] were the first to demonstrate the now well-known dictum; "AF begets AF". 12 goats were prepared by suturing epicardial electrodes overlying their atria under general anaesthesia via an open thoracotomy. These electrodes were externalised and connected to an electrical stimulator (Medtronic SP3084, Minnesota) with long cables attached with springs to the ceiling of the housing for each goat. This allowed free movement of the awake animal. The electrical stimulator was used to produce a 1s biphasic stimulus (20ms interval at 4 times the diastolic threshold). The system was able to easily differentiate AF from sinus rhythm; SR by assessing the local signal isoelectric segments (much longer during SR than during AF). AF could therefore be accurately determined and maintained as required. The control experiment (single atrial burst – 1s, 50Hz) produced short bursts of AF (6 ± 3s); whereas increased duration of artificially maintained AF progressively lengthened sustained AF. Artificial atrial fibrillatory activity of approximately 7.1 ± 4.8 days in 10 of 11 goats produced sustained (at least >24 hours) of AF. Additionally, the atria became more vulnerable to AF development with reduced median

fibrillation intervals, enhanced susceptibility of a single atrial extra stimulus to produce AF and reduced atrial effective refractory periods. A subsequent experiment by the same group[25] examined what the mechanism for this self-perpetuation may be. Importantly, they examined the effect of autonomic tone as well as other possible mechanisms such as calcium handling, acute changes in atrial pressure and atrial ischaemia. Here, they reported the results of various pre-treatments in 25 goats prepared in a similar way to the first study[24]: atropine (to block the effects of *efferent* parasympathetic tone), propanol (β-blockade; *efferent* sympathetic blockade), volume loading (0.5-1L Hemaccel; to raise atrial pressure), atrial natriuretic factor (ANF); α-human 1-28 ANF (a direct effect on atrial ion channels or an effect via a neurohormonal mechanism), rapid long-term atrial pacing and glibenclamide (ATP-regulated K^+ channel blocker that was used to test the hypothesis that atrial ischaemia was the cause of AF perpetuation. Dosage; 10μmol/kg).

Atropine (0.1, 0.3, 0.6 and 1.0 mg/kg intravenously, cumulatively every 10 minutes; n = 6) and propranolol (0.1, 0.3 and 0.6mg/kg; n = 6) were administered before AF induction (during sinus rhythm), after 1-3 days of AF (also during sinus rhythm) and once AF was sustained. In 3 goats, atropine (0.2mg/kg) and propanol (0.2mg/kg) were co-administered in order to produce combined autonomic *efferent* blockade. Neither atropine (at maximal dose 1 mg/kg), nor propranolol (at maximal dose 0.6mg/kg) abolished the reduction of atrial refractory period seen due to the preceding 24 hours of AF. In this study, the effect of sustained AF (during AF) was assessed by measuring Atrial fibrillation Intervals (AFI). Whilst profound effects were seen on atrio-ventricular conduction (shortened RR interval with atropine and lengthened with propranolol), there was no clear effect on AFI to suggest that acute autonomic tone had any effect in modulating the atrial electrophysiologic changes

due to perpetuating AF. In this study, autonomic blockade was performed either before any or after completion of electrical remodelling. Therefore, the possibility that autonomic tone could, over time modulate the decrease the atrial effective refractory periods and therefore increase atrial vulnerability to AF induced by AF itself has not been excluded in this study.

Volume loading in 12 normal goats (with a consequent acute rise in both atrial pressure and diameter) did not shorten atrial effective refractory periods. Although ANF in plasma rose from 42 (baseline) – 99pg/mL at after 1 week and up to 4 weeks of AF (n = 6); administration of α-human 1-28 ANF (which causes plasma ANF to rise in the 4 goats in which it was administered) did not have any effect on atrial effective refractory periods. However, in this study, atrial pressure was studied in normal goats and therefore whether atrial pressure contributes to increases in atrial vulnerability at this stage is not known. Nevertheless, any substantive effects of ANS mediated ANF effects on atrial effective refractory periods in normal goats were ruled out by this experiment. This finding is in contrast to prior observations in dogs; where combined vagal and β-adrenergic blockade attenuated the effect of ANF infusion on atrial effective refractory periods and monophasic action potential durations; which can enhance the susceptibility to developing AF.[200]

In 10 goats, rapid pacing for a prolonged period (up to 2 days) at a cycle length of 180-200ms, showed substantial decreases in atrial effective refractory period, and associated bursts of AF. Importantly, there was both, an acute decrease in atrial effective refractory period with commencement of a rapid pacing protocol (pacing cycle length decrease from 360ms with 1:1 AV conduction to 180ms atrial pacing with 2:1 AV conduction) with further shortening over the subsequent 2 days of rapid atrial pacing (with 2:1 AV block). Thereby,

also showing that ventricular response was not relevant in these responses. Finally, with resumption of slower atrial pacing (360ms); atrial refractory periods improved over 2 days.

Thus, the main findings of this study were that the AF -induced electrical remodelling observed was not due to changes in autonomic tone, acute atrial stretch, atrial ischaemia or ANF. High atrial stimulation rates themselves seemed to be a stimulus for a maladaptive change in atrial electrophysiology.

The effect of efferent autonomic tone (via combined β-adrenergic blockade with propranolol and atropine) was tested in closed-chest, atrial paced dogs.[201] Here, combined symopathovagal cardiac efferent blockade did not abolish the electrical remodelling changes seen with rapid atrial pacing. On the other hand, calcium augmented electrical changes and verapamil blocked these changes. Together with these findings, biopsy confirmed calcium overload highly suggestive that the electrical remodelling that occurs due to rapid atrial pacing is not influenced by acute efferent changes in autonomic tone; instead, these changes may occur due to calcium handling in atrial myocytes.

The conclusion from this seminal work is that mechanisms other than the autonomic nervous system influence atrial remodelling due to AF itself; however, the role of other regulatory aspects of the autonomic nervous system have not been studied, nor considered, in this.

1.26 ANS changes attributable to the irregularity of AF: potential mechanism for atrial remodelling?

I have already introduced the concept that whilst acute changes in autonomic tone do not appear to influence the chronicity or progression of AF (electrical remodelling) – there may be changes in autonomic function not previously considered – which may play an important role in the perpetuation of AF (as well as result in clinical sequelae associated with autonomic dysfunction due to AF). One such potential mechanism may be due to the irregularity of the cardiac function (beat-beat variability of heart rate during AF) or indeed this is simulated by the encroachment of rapid atrial pacing on the "vulnerable" period of atrial myocardial repolarisation – which may induce autonomic changes. This is a simulation of short-coupled beats during AF (where the short-coupling would produce beats that occur in the vulnerable period as described above).

There is indeed some physiologic evidence in humans that this is likely to occur. In 8 patients referred to an electrophysiologist for a cardiac electrophysiologic study; left peroneal nerve microneurography was performed during 3 minutes of resting sinus rhythm and induced AF (via rapid atrial burst or programmed extra stimulus pacing). [82] This study demonstrated a clear increase in peripheral sympathetic nerve activity (SNA) during AF in all patients (171±40% from baseline). Further, in 5 patients, a 3-minute protocol of irregular atrial pacing was compared to regular atrial pacing. Again, a rise in SNA was seen with irregular pacing over regular atrial pacing (124±24% versus 91±20%, respectively; P=0.03); highly suggestive that it is the beat-beat irregularity in AF that results in increases in efferent sympathetic activity. This is important for several reasons. The most evident is that the rhythm itself (AF) can perturb autonomic function inducing an increase in sympathetic

activity. The mechanism for this increase is not clear; however, it is evident that acute atrial events (3 minutes of AF) can manifest increases in SNA. The mechanism for such a change has not been documented. The most likely hypothesis is that perturbations in cardiovascular haemodynamics influence overall SNA activity, which in turn would implicate afferent autonomic nerves, i.e., those that respond to changes in central volume and blood pressure. Another study by Segersen *et al*.[202] in 23 patients who underwent electrophysiology studies confirmed these findings and furthered them by demonstrating that the degree of irregularity in atrio-ventricular sequential pacing independently influenced the increase in SNA as assessed by left peroneal nerve microneurography. For every percentage increase in beat-beat irregularity there was a 6.1% increase in SNA: independent of other haemodynamic factors. Of interest, in the study by Wasmund *et al*.[82] it was shown that this increase in SNA occurred despite increases in central venous pressure (which should load low pressure, volume-sensitive cardiopulmonary baroreceptors, found in the pulmonary vein-left atrial junction, resulting in reflex decreases in SNA). Also, the increase occurred despite a lack of decrease in mean blood pressure. The increase in SNA due to beat-beat irregularity was thought unlikely to occur due to cardiopulmonary (low pressure) baroreceptors and more likely due to input from arterial (high pressure) baroreceptors. The authors further postulated that it was a long coupling of the beat-beat variability associated with AF that results in impairment of the ANS- manifest by a reflex increase in SNA due to offloading the arterial (high pressure) baroreceptors and resultant increase in SNA to achieve homeostasis.[203] In prior sections we have already suggested the potential role of some cardiovascular risk factors for AF that heighten SNA and may therefore contribute to the development of maintenance of AF.

In some patients, a heightened adrenergic state from irregular heartbeats (in AF) could also contribute to the development of heart failure. Therefore, the augmentation of SNA from

beat-beat irregularity could also promote a clinical sequela of AF. Indeed, in patients with co-existing AF and heart failure there is higher sympathetic activity than in heart failure patients without AF.[204] These authors, also support observations made by Wasmund *et al*.[82] that beat-beat variability and associated haemodynamic effects are responsible for this rise in SNA. Catheter ablation for AF in heart failure patients with reduced ejection fraction has been shown to improve mortality outcomes as well as reduced hospitalisation for heart failure.[205] It remains to be seen whether rhythm management could ameliorate autonomic remodelling and therefore represent the mechanism underlying the benefit of rhythm management in heart failure associated with AF. Further, the role of neuromodulation in the prevention of heart failure and associated morbidity and mortality in patients with AF is also undetermined.

Thus, evidence supports autonomic efferent (SNA) activity augmentation due to the irregularity of the heartbeat intrinsically associated with AF. Whether or not factors such as the coupling intervals of the beats are important or not- this would implicate haemodynamic changes (both in terms of cardiac and central volume and pressure) and in turn, therefore, reflect the potential importance of the cardiac and cardiovascular afferent (regulating autonomic nervous system) in the shift of sympathetic tone due to AF irregularity.

1.27 Prior ANS testing efforts in AF: Afferent ANS function

The concept that afferent nervous dysfunction may be associated in patients with AF was first investigated in patients with heart failure. A study performed by Gould *et al*.[206] demonstrated impaired sympathetic responses in response to a circulatory challenge exerted by Head-Up-Tilt (HUT) in patients with co-existing AF and heart failure. HUT was performed during AF (where it was present for at least 3 months) and the responses were compared to heart failure patients with no history of AF (who were therefore studied in sinus rhythm). Specifically, there was an attenuated cardiac sympathetic increase (as assessed by trans-cardiac noradrenaline spill-over) in response to 10 minutes of 30° HUT, suggestive of an impaired baro-receptor reflex response to HUT. Although the authors of this study put their findings down to low-pressure baroreceptors found in pulmonary vein- left atrial junctions – HUT is a non-discrete test that can affect both low pressure baroreceptors (found in veno-atrial junctions and predominantly respond to changes in cardiac (atrial) volume) as well as carotid and aortic arch high pressure baroreceptors (which respond to positional changes of these receptors in relation to the heart as well as changes in arterial (central) pressure at their level). Further, this study tested patients with heart failure and persistent AF, during AF. Whether or not these changes represented rhythm only, or a compounding of other co-morbidities, especially heart failure in patients with AF (perhaps due to rapid ventricular response) remains unknown. Despite the limitations in interpretation of these findings; they indicate that, at least in heart failure, AF is likely associated with afferent dysfunction and therefore provides early evidence of a potential mechanistic link between the irregularity of heart beats during AF and efferent autonomic effects.

In patients with AF and without heart failure (which itself could perturb the autonomic system); studies of the role of the afferent nervous system are scant. In study of 73 outpatient participants with paroxysmal AF, a variety of autonomic tests; baroreflex sensitivity, isometric handgrip manoeuvre, deep breathing, posture change (sit-stand, 30:15 ratio) and Head-up tilt were performed.[207] They found that baroreflex sensitivity was decreased in this patient population and that this, together with deficits in some of the other autonomic reflex tests (deep breathing, 30:15 ratio) were associated with poor quality of life measures using the SF-36 questionnaire. Although Head-up tilt was not associated with poorer quality of life measures; a significant proportion (26%) developed hypotension, an abnormal reflex haemodynamic response to this reflex test and 12% were positive (with associated symptoms).

Another small study[203] evaluated high pressure-sensitive baroreceptor function in patients with persistent AF who were restored to sinus rhythm by electrical cardioversion. Patients older than 18 years of age with AF >30 days who had an indication for cardioversion were enrolled. They excluded those with pacemakers, or patients with implantable defibrillators who had any bradycardia pacing indication; significant ventricular ectopy which would affect baroreceptor sensitivity measurements and if cardiac medication were altered from during enrolment. They compared responses in this group to a control group consisting of age and left ventricular ejection fraction-matched patients without AF. Although the groups had similar rates of hypertension and diabetes mellitus, there were differences in the proportion of heart failure as well as the usage of rate and rhythm anti-arrhythmic medications (β-blockers, calcium channel blockers, and other rhythm controlling anti-arrhythmics). High pressure (arterial) baroreceptor sensitivity was measured in those with AF on the day of their cardioversion (after a minimum of 2 hours after sedation was provided for the procedure

(propofol) and at 30 days post cardioversion (if successful in maintaining sinus rhythm). Of 24 enrolled patients; 14 patients completed the day-30 assessment in sinus rhythm. 24 control participants were enrolled and had their baroreflex sensitivity tested once.

The study was performed in two institutions, and, owing to this, there were two methods used to evaluate baroreflex sensitivity. At one centre, the modified oxford technique[92] was utilised and in the other: the sequence method.[91] Both methods compare changes in R-R interval as an index of changes in systolic blood pressure (SBP) and therefore require stable sinus rhythm for the measurement. The unit of the measure (ΔR-R/ΔSBP) is *ms/mmHg*. Briefly, the modified oxford method utilises both a potent vasodilator (sodium nitroprusside) and a pressor agent (phenylephrine) in order to induce 20-30 *mmHg* decreases and increases in SBP; respectively from which corresponding instantaneous R-R intervals can be measured and used to calculate baroreceptor gain. The sequence method utilises spontaneous changes in SBP (>1 *mmHg*/beat for >3 consecutive R-R intervals) followed by >4ms/beat R-R lengthening as well as the reverse (progressive decreases in SBP followed by R-R shortening). In this study, these sequences were automatically identified using a computer algorithm, regression slopes produced and averaged.

The results of this assessment of baroreflex sensitivity in patients with persistent AF immediately after cardioversion and at 30 days are of interest. In the control group baroreflex gain (BRG) was higher than in the AF group immediately post cardioversion (10.8±5.5 vs 5.2±3.6 *ms/mmHg,* $P<0.05$). At day 30; the BRG increased from 4.1±3.7 to 7±6 *ms/mmHg* ($P<0.01$) in the n=14 participants with pre and post data.

There are several important limitations to the interpretation of this study. There were differences in the use of BRG techniques between the groups. The control group all underwent the sequence method. The AF group was split; and in those (n=14) that had repeated measurements; there was an even split of both methods. Importantly, in the repeated group, there was no change in BRG measurement method at the second visit. The continued use of medications may have contributed to differences between the control and AF groups. There were more AF patients on antiarrhythmic agents (both for rate and rhythm control). There was also a higher proportion of heart failure in the AF group; although it is noted that this was not statistically significant, there was left ventricular ejection fraction matching and other conditions such as hypertension and diabetes mellitus were similar between groups. Although, the clinical effects of propofol are very short (< 15 minutes) and the half-life of propofol is short (up to 90 mins);[208, 209] which is less than the time point chosen in this study, there may be persisting effects other than sedation; as the drug us not completely eliminated. In both humans and in animals, propofol has been shown to inhibit muscle SNA, decrease blood pressure and heart rate and importantly, decrease baroreflex sensitivity[210], which could explain the decrease in BRG in AF patients studied 2 hours post cardioversion in sinus rhythm. Indeed, as Liu *et al*.[210] suggest, there is a variability in both duration and magnitude. As the authors[203] themselves suggest as a limitation, other factors such as dehydration, acute stress from having a procedure may also play a role. Finally, the main limitation to this work is that it has been undertaken in sinus rhythm, where really the effect of AF itself on BRG was the question. Naturally, any study exploring the effect of baroreflex sensitivity is most likely to use the relationship of the R-R interval to SBP owing to its non-invasive nature and that it has been validated in most other studies using these measures. An alternative would be to use a more direct (invasive) measure of SNA. Nevertheless, it is likely that there may be persisting effects even in sinus rhythm on BRG that have been appropriately captured in this

study. Furthermore, there is evidence from this study that AF patients who were studied in sinus rhythm (30 days following cardioversion) have a decreased BRG (taken under similar circumstances to the control subjects) and this adds weight to the findings in this study.

Thus, in summary, this study by Field *et al*.[203] demonstrates the possibility of alterations in afferent autonomic function, not previously considered in the study of the ANS and AF and therefore represents an important contribution to the literature in this area. Aside from the proposed effects of AF on afferent dysfunction of arterial (high pressure) baroreceptor function, this study[203] as well as the study by van den berg *et al*.[207] on baroreflex sensitivity reduction and quality of life in paroxysmal AF patients, presented earlier; show that autonomic remodelling that persists even during sinus rhythm in AF patients is highly likely and that there may be a gradient of effects (worst during AF) that may partly reverse after cardioversion – promoting the concept of a rhythm control strategy for AF.

Finally, dysfunction of arterial (high pressure) baroreceptors reported by Field *et al*.[203] is not likely to explain the effects of the irregularity of AF on SNA presented by the same group (and corroborated by others), as described in detail above. The postulated mechanism from these authors was that it is the arterial (high pressure) baroreceptors rather than low pressure (cardiopulmonary receptors) that respond to decreases in arterial pressure owing to long coupled beats during AF- with an associated reflex increase in SNA. The finding that BRG is abnormal due to AF and persists even during SR to some extent suggests that the reason underlying the increased SNA due to AF is complex. Whilst the effects of low volume (cardiopulmonary) baroreceptors in the acute increase in SNA due to AF appeared unlikely as in these studies, owing to high central venous volumes – the function of these receptors has not been tested. Further, their finding that reflex SNA increases due to AF did not accompany

changes in mean arterial pressure may be taken to mean that there are acute effects related to arterial pressure independent of blood pressure changes or neither baroreceptor alone is involved. Whilst the postulated mechanisms implied normal cardiopulmonary function – this has not been specifically tested. Indeed, if there were dysfunction of low pressure (cardiopulmonary) baroreceptors due to AF- an imbalance of function, irregularities in volume handling together with abnormal handling of blood pressure (afferent or regulatory) ANS dysfunction could underly reflex autonomic tone changes (increase in SNA), particularly if this were a chronic form of autonomic remodelling that occurs due to AF.

Ultimately, there appears to be a direct role that AF plays in the development of an increase in sympathetic nerve activity, due to AF itself and these abnormalities may manifest in varying severity – possibly worse during AF and present even during sinus rhythm; reflecting an underlying (chronic) autonomic remodelling that occurs due to AF. Whether this could influence the progression of AF (and indeed of its clinical sequelae such as heart failure) has not been uncovered previously and warrants considerable effort.

It is important to appreciate the challenges of assessment of autonomic function in the presence of AF. Investigations carried out on baroreflex sensitivity would not be feasible in the presence of AF, itself and the effect of AF was presumed from measurements performed immediately after cardioversion to restore sinus rhythm.

1.28 Atrial ANS remodelling due to AF: Potential autonomic substrate for AF

Atrial fibrillation is associated with several adaptive autonomic changes as seen in animal experiments that have studied the effect of rapid atrial pacing induced AF. Most studies have assessed autonomic remodelling at the atrial level. Such studies have identified changes in both sympathetic and parasympathetic type neurons as well as nerve distribution and growth.

Electrophysiologic remodelling, characterised by a shortening of refractoriness, spatial dispersion of atrial refractoriness and conduction velocity[211-217] may be partly mediated by changes in autonomic innervation. In the ventricle, there is evidence implicating increases in sympathetic innervation in the production of heterogenous refractoriness.[218, 219] The first evidence of the potential link between heterogenous refractoriness (together with AF) and heterogenous sympathetic innervation in Atrial fibrillation arises from a study of 26 dogs who had atrial electrophysiologic measurements after either a sham procedure or atrial (topical) phenol application; resulting in heterogenous atrial denervation.[212]

These animals were anaesthetised with isoflurane and intubated.[212] Baseline AF inducibility was tested using a quadripolar catheter inserted into the right atrium. Programmed stimulation (burst) at 50ms cycle length and an output of 10mA for 10s was used to produce AF. The same induction was repeated 10 times; and the duration of AF measured, with a 5-minute break after reversion before the next induction. Immediately after this, they exposed the right atrium, performing a thoracotomy and dissecting the visceral pericardium, rendering the heart exposed, within a cradle of pericardium. 88% phenol was applied using a cotton applicator to the right atrial pericardium in n=15 with n=11 receiving saline. The three right

atrial locations (superior and inferior free wall as well as the anterior aspect of the base of the right atrial appendage – near Bachmann's bundle) were standardised. After application of either saline (sham) or phenol, the animals were closed and allowed to recover for two weeks.

All animals were then subjected to a repeat electrophysiology study using a quadripolar catheter to induce AF in the same manner as described above. Induction of AF >60minutes terminated the protocol. In a subset of animals (n=5 in each group) additional 3-D atrial epicardial mapping was performed during right atrial pacing (200ms) and during AF using placque electrodes (240) placed on the left and right atrial epicardium, which collected unipolar signals (at 1Khz). Mean AF cycle length (4 seconds of AF) was used to infer local refractoriness during AF and conduction velocities were obtained from isochronal maps created during atrial pacing.

In another n=5 in each group; unipolar plunge electrodes (30-guage stainless steel wires) were placed in the areas in which either phenol or saline had been applied (4 electrodes) and in other right atrial areas (2) and the left atrium (2). Additionally, the cervical vagus and thoracic ansae subclaviae nerves were exposed, tied, and ligated. The distal portions were then attached to bipolar electrodes attached to a Grass stimulator for efferent nerve stimulation. Here, the authors chose stimulation characteristics (voltage and frequency) to achieve 50% sinus rate increase (sympathetic stimulation) or decrease (parasympathetic) stimulation. In each electrode site, using unipolar pacing, the authors assessed atrial (local) effective refractory period at baseline, during sympathetic stimulation, parasympathetic stimulation and during combined sympathetic and parasympathetic stimulation. In 2 phenol-treated animals, action potential duration was measured in tissue obtained from 1 phenol and 1 non-phenol site after cardioplegia and cardectomy, using microelectrodes.

Finally, in 10 phenol-treated animals, Positron-emission tomography (PET) scanning was performed of excised atria using [$_{18}$F]Fluorodeoxyglucose, FDG for myocardial viability) and [$_{11}$C]hydroxyephedrine (HED; sympathetic nerve endings) and 5-[$_{11}$C] methoxybenzovesamicol (MOBV; parasympathetic nerve endings). Microscopic examination also took pace (after tissue were fixed in formalin, cut, embedded in paraffin, sectioned, and stained with haematoxylin-eosin).

This thorough experiment, which has been detailed above has yielded several important findings. The first is that of AF inducibility with rapid atrial (burst) pacing. No animal had >60 minutes of AF at baseline. The sham group (which underwent thoracotomy and saline epicardial application) had no change in the duration of AF (longest <1 minute), number of attempts required to produce any AF (median of 10 attempts) and no animal had >60 minutes (sustained AF). In the phenol group, the majority of animals had sustained AF (14 out 15), markedly different to baseline and to the control group. Further, the ease of AF inducibility was also markedly greater (median 5 attempts at burst pacing to produce AF). In the 15th animal – 50 minutes of AF were produced. 3-D Epicardial mapping showed micro-re-entrant wavelengths during AF – with no focal/macro-re-entrant tachycardia arising from areas of phenol application. Microelectrode analysis demonstrated normal action potential durations in phenol-applied tissue, PET imaging showed normal FDG uptake and histologic assessment confirmed normal tissue architecture.

Using PET-HED, phenol-applied tissue demonstrated loss of sympathetic efferent terminals (denervation) without any effect on parasympathetic efferent terminals (PET-MOBV). Functional electrophysiologic testing at baseline, with either sympathetic, or parasympathetic, or both sympathetic and parasympathetic stimulation showed that phenol-

applied areas had a lower effective refractory period (ERP) than non-phenol areas as well as alterations in the autonomic control of ERP. This was correlated with an increased dispersion of refractoriness; that likely facilitates the inducibility of AF that was observed in this study from the application of phenol (which selectively caused sympathetic denervation). Notably, AF was inducible without any autonomic (vagal) stimulation.

Thus, this study ties heterogeneous sympathetic denervation to heterogeneity in atrial refractoriness (where the tissue itself is structurally normal) and then to AF inducibility under baseline autonomic conductions (without the need for autonomic stimulation).

This study was quite thorough, and the experimental design has addressed most potential limitations. Unfortunately, however, it is not completely known whether phenol could affect atrial afferent (baroreceptors) and the immense network of other types of nerves that are both sympathetic, parasympathetic, or mixed. However, what is clear is that sympathetic denervation results in changes in electrophysiologic properties of the atrium (i.e., autonomic remodelling) that rendered the animal vulnerable to AF.

This concept was furthered by the same group in dogs with atrial fibrillation produced by long term atrial pacing.[211] Here, their objective was to identify whether the self-sustaining nature of AF (produced by rapid atrial pacing in dogs) resulted in alterations in atrial autonomic expression – particularly sympathetic heterogeneity. In this study, 9 animals were treated as controls. 6 animals were prepared by implantation of an atrial pacemaker, programmed AOO, or asynchronously at 600bpm and 4 times the diastolic threshold for 6 weeks. At this time, during sustained AF, animals underwent an open chest electrophysiology study via median sternotomy, where the heart was exposed within the pericardial cavity using

epicardial (custom-made) placque electrodes placed on both left and right atria. Animas were anaesthetised using isofluorane. Mapping was undertaken during AF- assessing AF cycle length for 4 seconds. Similar to the previous study,[212] the cervical (sympathetic) ansae and vagus (parasympathetic) nerves were located and transected. As previously described (see above) a custom electrode was used to stimulate each nerve to assess the effect of autonomic efferent stimulation. PET scan using [$_{11}$C] HED to label sympathetic efferent terminals was performed in all animals. To correct for differences in regional delivery of HED, the authors injected [F-18] labelled microspheres of hydroxyapatite to measure atrial perfusion. PET studies were performed in explanted atria. Biopsy samples of each atrial appendage was collected and analysed for noradrenaline content.

By the fourth week, all 6 animals were in sustained AF. At 6 weeks, pacing was programmed off for the open electrophysiology procedure. All control animals were in sinus rhythm with similar ventricular rates to the AF group. PET-HED imaging showed an extensive redistribution of HED retention (sympathetic innervation) in AF. Mean biatrial retention fraction of HED was greater in AF dogs than in controls as was left atrial and right atrial individual HED retention fraction. There was also higher tissue noradrenaline content. There was no difference in the mean HED retention fraction between left and right atria in either group. The variation of sympathetic innervation (spatially) was quantified using a co-efficient of the variability of HED retention. It was greater in the AF group than in controls. Moreover, there was also higher variability in the right atrium than in the left in AF dogs. The control group, however demonstrated similar variability in either atrium. Electrophysiologic studies showed shortening of the mean AF cycle length with vagal stimulation. Stellate stimulation itself did not change mean AF cycle length; however, propranolol lengthened it. There were differences between left and right atria. First, mean AF cycle length was shorter at baseline in

the left atrium. Vagal stimulation shortened right atrial cycle length more than the left, where sympathetic stimulation did not change AF cycle length in either atrium. Finally, propranolol mean AF cycle length increased in both atria. The dispersion of atrial refractoriness (determined using the coefficient of variation of the AF cycle length) was measured during autonomic modulation. A greater dispersion was seen in the right atrium than in the left (in all autonomic states – sympathetic and parasympathetic stimulation as well as propranolol injection).

Thus, in this study the authors demonstrate that a canine AF model has enhanced sympathetic innervation and sympathetic innervation heterogeneity that correlated with electrophysiologic AF cycle length dispersion variability (in the right atrium). In this study, in contrast to Wijffels et al.[25] lengthening was seen of mean AF cycle length with propranolol. The main differences were the fact that in this study the duration of AF was longer (6 weeks vs first 7 days). In this study, electrophysiologic changes were correlated with heterogeneity in atrial sympathetic innervation rather than changes acute autonomic tone. Further, given the differences in the effects of propranolol; and that the effects seem to apply after a longer period in AF; it appears that autonomic efferent remodelling due to AF itself may represent a structural neural abnormality rather than simply a functional effect of autonomic tone.

These findings have been confirmed by Chang et al.[220] who demonstrated that in 6 dogs in whom rapid atrial pacing to sustain AF >48 hours (average of 111±76 days pacing) was performed, there was histologic evidence of sympathetic hyperinnervation together with nerve sprouting. Further, similar to Jayachandran et al.[211] both nerve sprouting and sympathetic hyper-innervation were higher in the right atrium than the left atrium. They also found inhomogeneity of nerves in each tissue. These animals were compared to tissue from a

control group of n=6. This study provided histologic evidence (where prior data arose from PET imaging). The use of the immunocytochemical stain for Growth Associated Protein-(GAP)-43 allowed analysis of nerve sprouting. Here, the authors identified an increase of autonomic nerve growth. However, it remains unknown whether this is the effect of AF itself or whether it occurs due to rapid atrial pacing. There was also no correlation with functional electrophysiologic measures of atrial refractoriness in this study.

In a similar vein – Yu et al.[221] measured sympathetic and parasympathetic nerve density in the epicardial cardiac ganglia of dogs with AF produced by rapid atrial pacing. In this study, n=7 (active group) had an epicardial lead sutured to the left atrial appendage via left thoracotomy and attached to a subcutaneously implanted pacing generator. After a week of recovery from the implantation of the pacemaker; it was turned on to deliver asynchronous pacing (AOO) at 600bpm and digoxin administered to control ventricular rates. Sustained AF was defined as >48 hours of AF without need for induction pacing. Once this occurred, hearts were explanted for immunocytochemical staining. 7 control animal hearts were explanted for staining without pacing procedures. Ventral and dorsal epicardial atrial fat pads together with underlying myocardium as well as the interatrial septum were excised and fixed in formalin. 10μM thick serial sections were analysed using methylene blue to identify cardiac ganglia. Then these sections and surrounding serial sections were further analysed with rabbit anti-rat Tyrosine Hydroxylase (TH) polyclonal antibody and rabbit anti-rat acetyl choline (Ach) polyclonal antibody in order to stain sympathetic and parasympathetic nerves respectively. Positive controls were obtained by staining cerebral tissue.

The authors of this study analysed density in cardiac ganglion locations as identified above. Each area was submitted to computer analysis to identify the area of stained nerves (brown)

to the total area analysed (nerve distribution density). For each slide, three fields with the highest nerve density were manually chosen; and their density automatically measured. Then these were averaged to provide the nerve density for that slide. There were four consistently identified sites; right atrial; ventral left atrial; dorsal atrial (between both atria) and the inferior vena cava-inferior right atrial ganglionated plexi – most of which converged at sites surrounding the pulmonary veins, vena cavae, cranial atrial and septal locations.

This study showed that there was increased density of sympathetic and parasympathetic neurons in the AF dogs compared to control animals. There were also differences in the distribution of TH positive nerves. In the AF group – the left sided plexi had a higher distribution of TH+ nerves than other sites. The Ach+ distribution in the AF group was similar to TH+ nerves (heterogeneous and more left sided), whereas in the control group there was no difference in distribution heterogeneity (all plexi had a similar density). Thus, this provides additional data and contributes to the concept that there is neural cardiac remodelling in animal models of pacing induced AF. This study is limited by the fact that only small areas in ganglionated plexi were chosen – initially manually, based on visual appearance of density. Therefore, there is the potential for bias, and it may be that other areas would have had a different density. Therefore, it remains difficult to draw conclusions from this study regarding distribution of density (heterogeneity) – but the overall data suggesting higher density of both TH+ and Ach+ nerves in ganglionated plexi is more convincing, and it is consistent with other data. This data and other immunocytochemical staining-derived data presented previously should not be overinterpreted. Autonomic neurons, particularly of cardiac ganglia, may exhibit both acetylcholine and catecholamines.[221-223] Thus, what is sympathetic only or parasympathetic only is not well defined. There is cross-innervation of both arms of the ANS and multiple interconnecting layers that add to the complexity of

neural innervation.[52] Nevertheless given that both arms of the ANS contribute to AF mechanism[28] it is less relevant which "arm" of the ANS is responsible and more important to understand and recognise neural control of the cardiac ANS and the effect of AF on this.

Post-mortem isolated cardiac immunohistochemical staining (human tissue) has demonstrated differences in those with documented persistent AF compared to those known to have been in sinus rhythm.[224] In this study, hearts from 39 deceased subjects were isolated and fixed within 24 hours. Recorded history and electrocardiographic data showing at least 2 ECG spaced 6 months apart were required to group into the AF-persistence group. Sections were taken from the pulmonary veins (longitudinally) and included 4mm PV-left atrial tissue; basal and apical components of the left and right atrial appendage; anterior antrum of superior pulmonary veins; and three sites in the posterior left atrium. Elastica-von-giemsa staining demonstrated nerve location and density. Specific sympathetic (tyroxine hydroxylase antibody) and parasympathetic stains (anti-choline acetyl transferase antibodies) were used for immunocytochemical staining of the regions containing nerves. Investigators were blinded in their analysis. There were 15 hearts in the AF-persistence group and 24 hearts in the reference group. There were no substantial differences in baseline characteristics between the groups (similar ages, gender, co-morbidities and left ventricular ejection fraction). There was no overall difference in nerve density between the groups. The overall distribution was also similar, with the highest nerve density in and around the pulmonary veins with the posterior left atrium exhibiting the second highest density. There was, however, a shift in adrenergic nerve predominance (14% compared to 5% in controls) and a concomitant decrease in parasympathetic nerves (2% compared to 9% in controls).

Whilst the last few paragraphs have discussed studies which have demonstrated changes in sympathetic innervation in the atria – contributory to AF mechanism, there are also data to support changes in parasympathetic atrial innervation. In a canine model of rapid atrial pacing,[225] 16 healthy, and 18 AF dogs (implantation of an atrial chamber pacemaker) underwent a variety of analyses. All underwent tissue nerve analysis. 11 of these underwent electrophysiology mapping studies in AF with autonomic blockade under general anaesthesia. Using high density mapping of the pericardium with placque electrodes with 2.5mm-5mm interelectrode spacing, the authors assessed a variety of invasive electrophysiologic parameters during AF surrounding the organisation and frequency of the electrical signals from the left atrium (free wall and posterior left atrial wall) as well as the left atrial appendage. Specifically, these characteristics included frequency domain measures of a. activation rate (DF), organisational regularity (OI), intervals between deflection on the electrocardiogram (FI) which depends on AF cycle length and signal fractionation and finally Shannon's Entropy, a statistical measure of complexity and finally AF cycle length. In the AF mapping studies, animals also underwent parasympathetic blockade with atropine only (0.04mg/kg) or dual blockade with atropine and propranolol (0.2mg/kg).

Tissue was processed from both RAP and control animals to isolate RNA and obtain quantitative PCR. Immunohistochemistry was performed to analyse density and distribution of nerve fibres, bundles, and ganglion tissue. Here, the authors excised the hearts and performed ice cold cardioplegia with a protease inhibitor to remove any blood traces. The atria were excised, and tissue samples obtained from the posterior left atrium, left atrial free wall, left atrial appendage as we as the right atrial free wall, appendage, and posterior segment of the right atrium. Finally, the left stellate ganglion was excised from the posterior chest wall, Samples were either frozen in solution (-80°C) or fixed in 10% formalin and

embedded in formalin. Immunohistochemical staining with either anti-mouse antibody for acetylcholinesterase (Ache; parasympathetic innervation) or anti-rabbit antibody for dopamine β-hydroxylase (DBH; sympathetic innervation) was performed for all the tissue excised from the atria and left stellate ganglion. Using light microscopy, the sections were analysed to obtain the area of myocardial tissue, the number, and the area of the nerve bundles (parasympathetic, stained brown and sympathetic bundles, stained navy blue). Random segments were chosen from each tissue area and the density of nerve bundles in relation to myocardial cells was obtained. The atrial tissue was scanned for the presence of ganglionated plexi. The area of the neuronal cell body in relation to the total ganglion area was calculated. A similar process was undertaken for the left stellate tissue.

In this experiment, after cardioplegia and atrial tissue excision, some atrial tissue was used to isolate viable cardiac myocytes after removal of the cardioplegia solution with immersion in Tyrode's solution at 37°C). Beating tissue was either examined in this way, or with electrical (in vitro) pacing at 1, 2, 3, or 6 Hz for 8 hours using square wave 5 ms, 18V pulses (or alternatively with variable CL (6 Hz with 0, 25%, or 50% variability). After this, RNA was extracted for PCR analysis as described above.

Lastly, a complex computational model was used to simulate sympathetic and parasympathetic effects on atrial myocardial tissue and thus determine the interplay between these two autonomic arms as influencers of atrial electrophysiology as well as to investigate the proposed link between autonomic influences on atrial tissue and the changes that occur on the pattern of cellular ion activation and simulated wave propagation.

There are several important findings from this study. The first is that there are regional differences in autonomic nerve distribution in a normal canine left atrium. The posterior left atrium (PLA) had the largest nerve bundles of all the areas examined, whereas the left atrial appendage (LAA) had the smallest. In contrast, the density was highest in the LAA, with a higher parasympathetic nerve density under normal conditions in each of the areas of atrial myocardium. In the AF model, there was no difference in the overall autonomic bundle density or parasympathetic predominance; however, there were much larger nerve bundles present. This was seen most in the PLA. Additionally, the entire atrium had increased myocardial nerve fibre density -of both sympathetic and parasympathetic nerve fibres. In the AF hearts, there was neuronal hypertrophy of both left stellate ganglion and ganglionated plexi (sympathetic and parasympathetic innervation, respectively). This was attributed by the authors to increased GAP43 mRNA (neuronal growth cone marker) expression and associated nerve sprouting in all areas of the atrium in the AF group. Next, the authors suggest that in their study, the co-efficient of variation across the LAA, LAFW and PLA was similar in the AF group; however, there were differences in parasympathetic nerve fibre innervation density within each area (heterogeneity). They found that the PLA had the highest co-efficient of variation in parasympathetic innervation; larger than the LAA. These changes (parasympathetic distribution heterogeneity) correlated with electrophysiological characteristics of the AF wavefronts in the rapidly paced canine (AF) group in each of these atrial regions. Finally, AF becomes slower, less fractionated, and more organized with autonomic blockade in each region of the atria, once again, highlighting the influence of both atrial autonomic innervation as well as efferent autonomic tone in the electrophysiologic characteristics of AF. In the LAA, for instance, where there is a more homogeneous parasympathetic distribution, EGM during AF are slower, less fractionated, and more organised. Computational modelling data (simulating the action of sympathetic innervation

on calcium-coupling and parasympathetic innervation on inward rectifying acetyl choline activated potassium channels (I_{KAch}) suggests that there is an interplay between increased sympathetic (efferent tone) and parasympathetic atrial innervation heterogeneity (as demonstrated by the immunohistochemical assessment in this study). The combination of both provides substrate for wave break and re-entry and thus could provide a mechanism that initiates *and* facilitates AF.

Lastly, the authors investigated whether nerve growth factor (NGF) expression influences this interplay between autonomic innervation and AF-sustaining electrophysiology. The AF dogs had a 6-fold increase in myocardial NGF expression than controls – highest in the LAA where AF is more organised. In controls NGF expression was similar in all atrial tissue areas examined. The increase was only modest in either group in ganglionated fat pads. Isolated active myocardial cell experiments utilising incremental pacing techniques identified that incremental pacing enhanced NGF expression – much more with regular rather than irregular pacing – explaining, in part, why LAA expression of NGF was highest in AF. They propose that this may play a part in remodelling of other tissue areas (such as the stellate ganglia) and that this may occur due to retrograde neuronal transport of NGF. Thus, the authors, suggest that the autonomic nervous system and associated autonomic remodelling could influence AF maintenance, particularly in the LAA; which could be important from a clinical standpoint and could justify further work to assess the role of LAA isolation (in conjunction with atrial appendage occlusion to reduce stroke risk).

Thus, these data show that AF is associated with the development of ANS remodelling with both sympathetic and parasympathetic innervation alterations, associated with electrophysiological changes which could contribute to the progression of AF.

1.29 Clinical ANS dysfunction as a consequence of AF

Prior sections have detailed the effect of autonomic tone in producing AF as well as the effect of AF on influencing autonomic remodelling. We have introduced the importance of evaluating the "whole" ANS in AF – not only efferent autonomic tone, but also afferent (regulatory) autonomic function. We hypothesise that this "missing link" could provide an explanation for AF maintenance and progression, and we have undertaken the first experimental steps towards this endeavour in the studies (subsequent chapters) that we have performed and documented in this thesis.

In this section, we introduce potential clinical scenarios of autonomic dysfunction that could occur as a consequence of AF. Herein, lie the possible clinical consequences of AF, which could, in part, be attributed to disturbances of regulatory autonomic function. From a scientific perspective, these data will serve to illustrate the importance of carrying out detailed clinical autonomic (reflex or regulatory) assessments in patients with AF and, thus provide some of the rationale as to the experiments that we have performed and presented in subsequent chapters of this thesis. Some of the work in this chapter will form part of a meta-analysis[226] (Chapter 3) and therefore will be discussed in much more detail subsequently. It is imperative to note that there are several potential explanations for the associations introduced here and detailed in subsequent chapters, and, that the link between the ANS (regulatory autonomic arm) and AF is not exclusive and that there are likely to be complex interactions that produce these sequelae, of which, we propose autonomic dysfunction is a part.

Several, predominantly retrospective data, demonstrate an association between AF and an independently increased risk of falls, orthostatic intolerance, and syncope in older adults.[226]

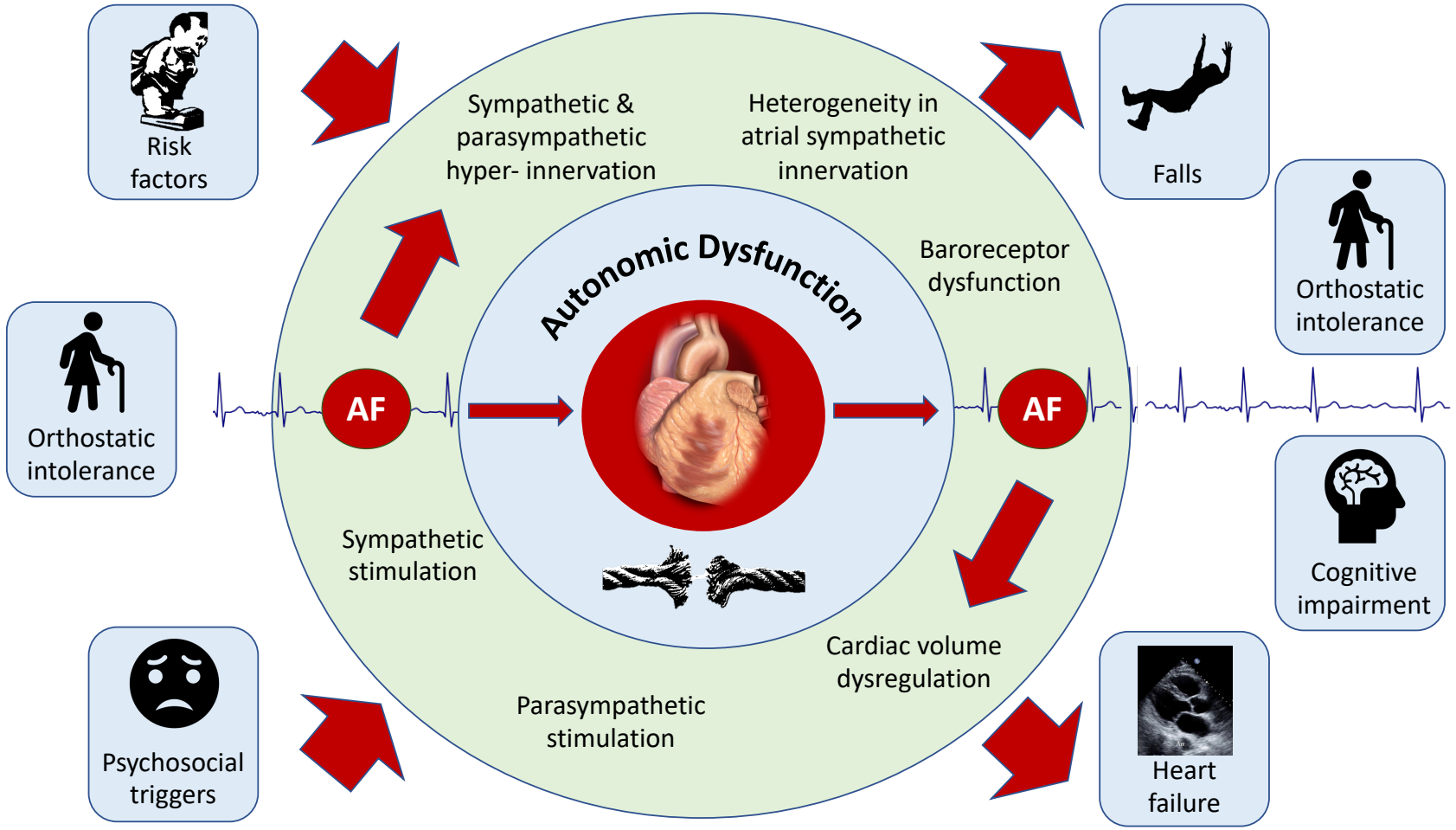
There are also data indicating diminished cerebral blood flow during AF,[227] which may contribute to the risk of cognitive decline & dementia.[228] Thus, abnormalities in cardiac volume (afferent) regulation in AF could serve as mechanism for this association of AF with dementia. Finally, AF is associated with the development of heart failure[229] and elimination of AF (rhythm treatment of AF) is associated with improved outcomes, including mortality.[205] This suggests that the mechanisms that produce and maintain AF could result in both heart failure and worsened outcomes from it. A shared abnormality such as loss of homeostatic feedback mechanism (owing to dysregulation of cardiac volume) could join these two conditions together, from a mechanism point of view and may be quite an important avenue to explore in further research.

1.3 Bi-directional relationship between AF & ANS: A potential contributor to "AF begets AF"

The clinical progression of AF – and the dictum "AF begets AF" is well understood.[24] There is overwhelming evidence that risk factors for AF may at least partially mediate their impact through autonomic dysfunction. Autonomic dysfunction may even potentiate risk factors such that cardiovascular disease, including AF may become much more likely in patients with more than one risk factor. Similarly, there is a strong link between autonomic dysfunction and the development of AF. Therefore, it is with substantial precedent that we hypothesise a bidirectional relationship between ANS dysfunction and the incidence of AF. Figure 1D

Dr Varun Malik, PhD thesis, 2018 - 2021

Figure 1D: AF & the ANS



The bidirectional relationship of AF and the autonomic nervous system.

Traditionally, the role of the ANS in the development of AF has been moulded from the concept that acute perturbations in autonomic tone can cause functional (reversible) abnormalities in atrial electrophysiologic properties that result in a "bout" of AF.[28]

As we have presented in prior sections, there is increasing evidence to support chronic changes in autonomic function (autonomic remodelling) at the level of the atria due to AF.[211, 220, 221] Animal studies have shown that heterogenous sympathetic denervation results in electrophysiological substrate development as well as substantially increased AF inducibility.[212] In a study of dogs, 6 weeks of pacing induced AF produced hyperinnervation of efferent sympathetic nerves.[211] Heterogeneity of sympathetic innervation was also seen, which correlated with electrophysiological remodelling (atrial refractoriness). Finally, propranolol blunted these effects at 6 weeks, whereas others have shown that acutely, ß-blockade does not. These data suggest that AF itself that promotes atrial autonomic remodelling.[211] Further evidence of longer term autonomic remodelling (> 6 weeks of pacing induced AF in dogs) comes from immunohistochemical assessment of the cardiac intrinsic ganglia, which has shown an increase in both sympathetic[220, 221] and parasympathetic neurons.[221] All of these changes can produce atrial electrophysiologic vulnerability to AF.

In patients with heart failure and chronic AF, assessment of single unit MSNA, which provides the advantage of detailed assessment of sympathetic activation per cardiac cycle and overcomes the limitations of multiunit MSNA recording during AF, has demonstrated an increase in sympathetic activity over heart failure patients in sinus rhythm.[204] More importantly, variability in the R-R interval in AF was found to be associated with changes in sympathetic nerve activity. Specifically, lengthening of the R-R interval (with associated reduction in diastolic pressure) results in greater single unit MSNA.[204] This strongly implicates

that AF itself is responsible for the increase in sympathetic activity. Although the precise mechanisms by which this occurs has not been extensively evaluated, a longer R-R interval could unload arterial baroreceptors; augmenting sympathetic activity.[23, 204] Indeed, Gould *et al*.[206] have shown impaired sympathetic responses to passive head-up tilt (which unloads baroreceptors) in patients with heart failure during AF in comparison to those in sinus rhythm. In patients with persistent AF referred for cardioversion, baroreflex abnormalities were corrected by restoring sinus rhythm.[203]

In this thesis, we have sought to study the function of the afferent or regulatory component of the ANS in patients with AF. In doing so, we may identify potential mechanisms contributing to some clinical sequelae of AF; such as an independently increased risk of falls and orthostatic intolerance in older adults,[226] diminished cerebral blood flow during AF,[227] which may contribute to the risk of cognitive decline & dementia[228] as well as heart failure.[229] Thus, we introduce an important the concept upon which the work presented in this thesis builds; that the ANS and AF likely share a bidirectional, positive-feedback relationship where the ANS (and dysfunction thereof) can trigger AF, and AF can promote alterations (and dysfunction) of the ANS.

1.4 Clinical implications and perspective for planned research

A thorough understanding of the important role that the ANS plays in the development of cardiovascular diseases, particularly AF, could present us more potentially effective methods to mitigate AF risk in susceptible individuals as well as halt its progression. Measurement of autonomic activity may provide a better marker of risk in individuals with one or more risk factors for AF. Strategies to manage risk factors could be refined to better modulate the ANS, such as individualised targets of weight loss using sympathetic activity as a guide, management of hypertension using agents that dampen down central sympathetic activity, advising particular kinds of physical exertion, or psychological techniques such as mindfulness or yoga[230] to reduce autonomic perturbations that could trigger AF. In patients with established AF, neuromodulation techniques, which have already shown to be useful in reducing the burden of AF,[23, 38] may also delay its progression. Indeed, neuromodulation can also be considered in those who are unable to manage their risk factors by lifestyle adjustments or during the period in which they are making changes.

Delineating the mechanisms responsible for afferent ANS dysfunction and its integration into higher centres to control overall autonomic activity warrants attention. This is an area that has been thus far neglected and may provide important insights into the progression of AF; specifically, whether this represents an additional mechanism of atrial remodelling and whether it could provide a mechanism that links anatomic to structural remodelling, and thus, explain the progression of AF where efferent tone could not. Finally afferent autonomic dysregulation could represent the mechanistic link between AF and its consequences such as heart failure,[205] falls and orthostatic intolerance,[226] cognitive decline and dementia.[228]

In this thesis, we shall examine the effects of AF on clinical features of autonomic dysfunction, explore afferent or regulatory function of the ANS, define the effect on the ANS of a variety of treatments for AF; cardioversion (to restore sinus rhythm), Pulmonary vein isolation (rhythm management strategy) and neuromodulation (using low-level tragus nerve stimulation). Finally, given the potential intersect we have defined between anatomic and structural remodelling, we shall examine the potential for structural cardiac remodelling in a known autonomic disorder.

Chapter 2: Atrial fibrillation is associated with syncope and falls in older adults: A systematic review and meta-analysis: clinical context to the concept of autonomic dysfunction due to Atrial Fibrillation

Background

The prevalence of atrial fibrillation (AF) climbs significantly in older adults with a steep rise after the age of 65.[231] It is becoming increasingly evident that AF is a significant burden to the global community and is associated with rising health care resource utilisation, hospitalisations and associated costs.[3, 232] Recent data indicates that hospital presentations for AF have surpassed that of other common cardiovascular conditions such as heart failure and myocardial infarction.[233] Apart from the long-established association between AF and heart failure, a recent large-scale global population registry (GARFIELD-AF) has unveiled an association between AF and increased all-cause mortality at 3.83 per 100 person-years over 2-year follow-up.[3] Notably, about half of the deaths in the GARFIELD-AF registry were not related to cardiovascular causes or malignancy, highlighting the many unknown associations between AF and other clinical conditions.

There has been increasing focus on the relationship between AF and cognitive impairment, dementia, fracture risk, falls and syncope.[234, 235 236, 237 238-240] Indeed, the association between AF and increased risk of falling in older adults would not be surprising given the haemodynamic effects of AF such as reduction in cardiac output and stroke volume, that may be responsible for symptoms such as dyspnoea, light headedness, and syncope. These may occur in the absence of poor ventricular rate control[241] and thus other mechanisms such as autonomic insufficiencies attributable to AF could contribute.[203, 206] Falls contribute

substantially to hospitalisations, are associated with fractures and can cause both significant morbidity and mortality in older adults.[242] Therefore, it is imperative that we closely examine this potential association to AF. Here, we undertook a systematic review and meta-analysis to answer the following question: Do older patients with AF have an increased risk of falls or syncope in comparison to those without AF?

Methods

This systematic review complies with the consensus statement outlined by the Meta-analysis of Observational Studies in Epidemiology (MOOSE) group.[243] (Table 2A) and has been registered in PROSPERO (registration number: CRD4201810721).

Table 2A: MOOSE Checklist

	Page	Comments
Reporting of background should include		
Problem definition	83	
Hypothesis statement	83	
Description of study outcomes	83,91	
Type of exposure or intervention used	90	
Type of study designs used	90	
Study population	91	*Additional material presented in Table 2Ca & b*
Reporting of search strategy should include		
Qualifications of searchers (e.g., librarians and investigators)	90	
Search strategy, including time period used in the synthesis and key words	89	*Additional material presented in Figure 2A*
Effort to include all available studies, including contact with authors	90,91	
Databases and registries searched	90	
Search software used, name and version, including special features used (e.g., explosion)	89	*Additional material presented in Figure 2A*
Use of hand searching (e.g., reference lists of obtained articles)	91	
List of citations located and those excluded, including justification	95	*Additional material presented in Figure 2A*
Method of addressing articles published in languages other than English	89	
Method of handling abstracts and unpublished studies	91	
Description of any contact with authors	-	*Not required*
Reporting of methods should include		
Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	91	*Additional material presented in Table 2Ca & b*

Rationale for the selection and coding of data (e.g., sound clinical principles or convenience)	91	
Documentation of how data were classified and coded (e.g., multiple raters, blinding and interrater reliability)	91	*Additional material presented in Table 2B*
Assessment of confounding (e.g., comparability of cases and controls in studies where appropriate)	91	*Additional material presented in Table 2B*
Assessment of study quality, including blinding of quality assessors, stratification, or regression on possible predictors of study results	91	*Additional material presented in Table 2B*
Assessment of heterogeneity	94-106	*Additional material presented in Figures 2A-F*
Description of statistical methods (e.g., complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	94	
Provision of appropriate tables and graphics	94-106	*Tables 2A, B, Ca &b, and Figures 2A-2H*
Reporting of results should include		
Graphic summarizing individual study estimates and overall estimate	94-106	*Figures 2A-G*
Table giving descriptive information for each study included	96-104	*Tables Ca &b*
Results of sensitivity testing (e.g., subgroup analysis)	108-111	*Figures 2B-F*
Indication of statistical uncertainty of findings	112-113, 115 & 120	*Figures 2B-F*
Reporting of discussion should include		
Quantitative assessment of bias (e.g., publication bias)	105-106, 112-113 & 115	*Tables 2B*
Justification for exclusion (e.g., exclusion of non-English language citations)	95	
Assessment of quality of included studies	112-113	*Tables 2B, 2Ca &b*
Reporting of conclusions should include		

Consideration of alternative explanations for observed results	116-121	
Generalization of the conclusions (e.g., appropriate for the data presented and within the domain of the literature review)	116-121	
Guidelines for future research	121	
Disclosure of funding source	9	

Transcribed from the original paper within the Support Unit for Research Evidence (SURE), Cardiff University, United Kingdom. February 2011.[243]

Search strategy

The scientific literature was searched from inception to 31 January 2019 using the CENTRAL (Cochrane Central Register of Controlled Trials), PubMed and EMBASE databases. The search terms consisted of a series of MeSH or tree headings and keywords relating 'AF', 'falls', 'syncope' or 'postural hypotension' and was created with the assistance of an experienced librarian. The PubMed search was: "Atrial Fibrillation[mh] OR Atrial Fibrillation[tiab]" AND "Orthostatic intolerance[mh] OR Orthostatic intolerance[tiab] OR postural syncope[tiab] OR syncope[mh] OR syncope[tiab] OR Accidental falls[mh] OR fall[tiab] OR falls[tiab] OR falling[tiab]". Figure 2A contains the exact search terms used for each database. Non-English articles were translated using an online service (Google LLC, California, United States). The search was undertaken by two independent investigators (VM and CG) who independently identified relevant studies, abstracted, and reviewed full texts where appropriate and assigned a risk of bias to the studies included in the analysis. Retrieved references were exported to the reference manager Endnote X8 (Clarivate Analytics, Philadelphia, United States); where duplicate citations were removed.

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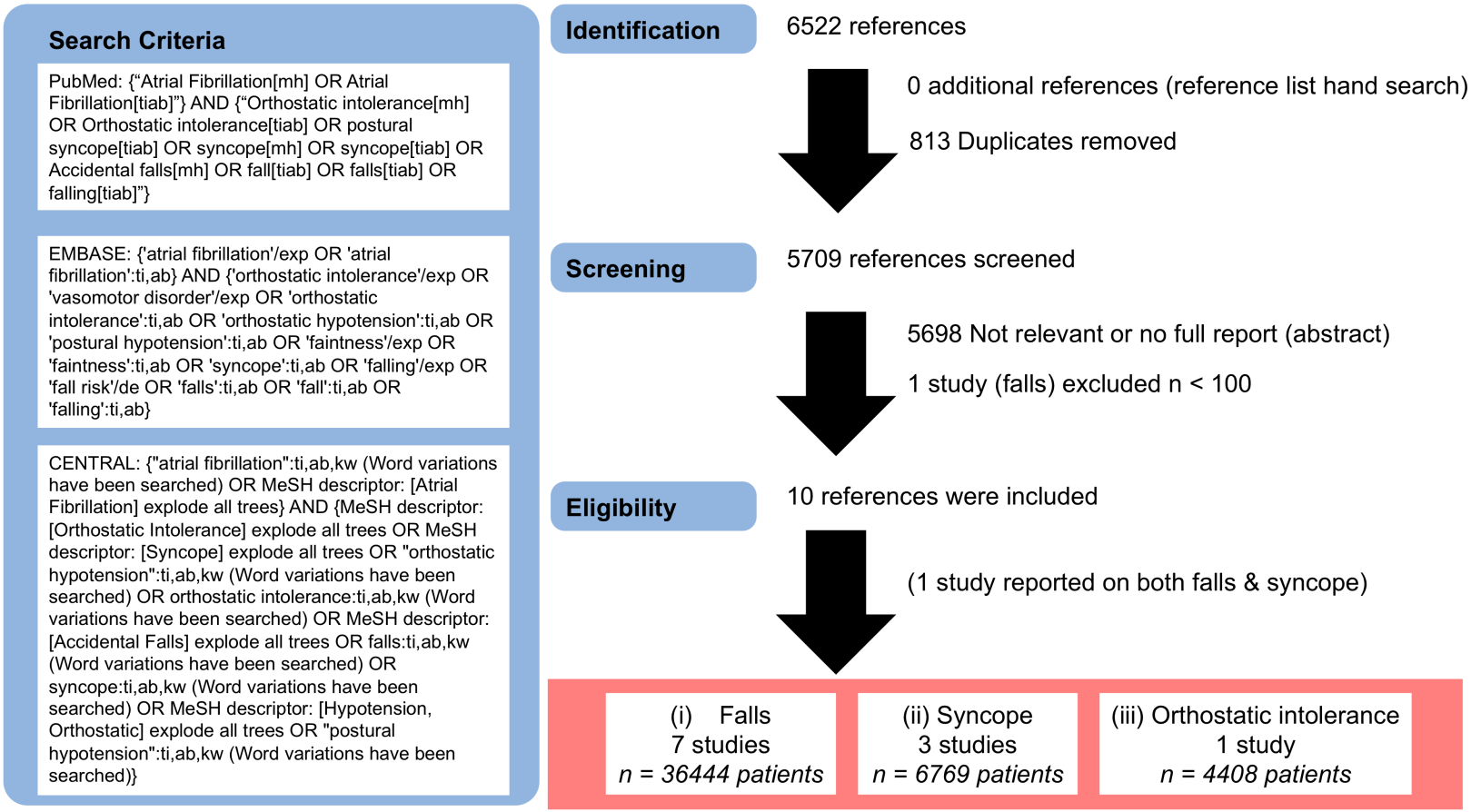
Figure 2A: Search criteria & PRISMA diagram



Search strategy and PRISMA flow diagram of the literature selection process

Inclusion and exclusion criteria

The articles of interest were those describing an association of AF with falls and syncope. Studies of both prospective and retrospective design examining the association of falls or syncope with AF with a reported odds ratio quantifying this relationship were included for review. Studies exploring the effect of falls or syncope on the development of AF were excluded. The reference list of relevant studies as well as reviews were hand searched in order to identify any relevant citations. Editorials, reviews, and case reports were excluded from the analysis. Any identified conference proceedings were not excluded. Falls were defined as "any falling event" in which a person inadvertently comes to rest on a lower level, such as the ground. Syncope was defined as a "faint", "blackout" or "loss of consciousness".

Study selection and data extraction

Data extracted from each study included: study design, number of participants, mean age of participants, date of data collection, ascertainment of AF, ascertainment of falls, syncope and orthostatic hypotension, inclusion/exclusion criteria, and results; including information regarding adjustment for covariates. Disagreements were resolved by consensus. For each study, we summarised the risk of bias using the Newcastle – Ottawa Scale (NOS)[244] and classified this as low, moderate or high. Table 2B Our outcomes were the association of AF to (i) falls; (ii) syncope and (iii) orthostatic intolerance.

Table 2B: Newcastle-Ottawa scale risk of bias assessment

Study	Selection	Comparability	Exposure: case-control, cross-sectional studies Outcome: Cohort studies	Overall risk of bias
FALLS				
Chen et al.[245]	-	-	★	**High**
O'Neal et al.[240]	★★★★	★★	★★★	Low
Jansen et al.[238]	★★★	★★	★★	Low - Moderate
Hung et al.[246]	★	★★	★★	Moderate
Sanders et al.[239]	★	★★	★★	Moderate
Wallace et al.[237]	★★★★	★★	★	Low - Moderate
Arita et al.[247]	★★	★★	★	Moderate
SYNCOPE				
Sule et al.[248]	★	★★	★	**High**
Jansen et al.[238]	★★★	★★	★★	Low - Moderate
Chen L et al.[249]	★★★★	★	★	Moderate
ORTHOSTATIC INTOLERANCE				
McNicholas et al.[250]	★★★	★★	★★	Low - Moderate

Statistical analysis

Continuous variables are presented as the mean ± standard deviation and categorical variables as number and percentage. Pooled analyses were performed where there were more than two studies addressing an association. The effect of reports with a high risk of bias on the overall results was assessed by *post-hoc analysis* excluding the study from the pooled analysis. The I^2 statistic and heterogeneity P-value were used as a measure of variability in observed effect estimates attributable to heterogeneity between the studies. For the pooled analysis, we entered the most adjusted Odds Ratios (OR) and 95% confidence intervals (CI) in a Dersimionian and Laird inverse variance random- effects model. OR from either a multivariate analysis, or a univariate analysis where a multivariate model was not constructed were used. Where possible, a subgroup analysis was performed in order to test the stability of the association despite differences in study design (exposure, outcome ascertainment). All analyses were undertaken using Review Manager Version 5.3 (Nordic Cochrane Center, Copenhagen, Denmark).

Results

Search and synthesis of the literature

We identified 7380 citations. After removing duplicates and screening for studies that were relevant to the topic, we identified 10 full reports that met the inclusion and exclusion criteria. The rate of chance -adjusted agreement was 0.9-1.0 between VM and CG. There were three small studies which reported the presence of Atrial fibrillation assessed using implantable loop recorders which were implanted for unexplained palpitations or syncope. None of these quantified any association with either syncope or falls and AF[251-253] Thus, a total of 10 references with 47 621 patients were included in the analysis – seven studies relating to falls[237-240, 245, 246, 254]; one of the studies reporting falls[238] also reported on syncope in the same patients. There were two additional studies relating to syncope.[248, 249] and one study relating to orthostatic intolerance.[250] Figure 2A

Ascertainment of AF in reported studies

The ascertainment of AF in the included studies varied; from a combination of electrocardiogram (ECG) data and patient history (documented AF from hospital records [246, 254], Medicare data[237] or self-report of a physician diagnosis of AF)[240] to patient history assessment (obtained from medical records) without ECG data[239] or from ECG data only.[238, 250] In the case of the four studies that utilised both patient history and ECG data; two studies had central reading centres[237, 240]. In one study, the authors adjudicated AF, themselves[246]. It was unclear how AF was ascertained in the study by Chen *et al*.[245] reporting on falls and in two syncope studies.[248, 249] Study characteristics are listed in Table 2Ca and Table 2Cb.

TABLE 2Ca: Studies included studies in the systematic review: study characteristics (Part 1)

Author (reference number), year	Mean age	Patient number	Study design Study population	Follow up	AF ascertainment	Outcome ascertainment
FALLS						
Chen et al.[245], 2011	80±10	408	Retrospective case-control; single centre. Review of in- hospital incident report database for falls *in aged care wards*. 3 groups identified: 1 in- hospital fall. ≥ 2 in- hospital falls and 3. Control sample from the same wards.	-	Unclear	Reported incidence in a hospital incident management system
Hung et al.[246], 2013	82±0.2	401	Cross-sectional; single centre Age ≥ 75; Admitted in-patients for geriatric syndromes. Completed comprehensive geriatric assessments	-	Review by study personnel of medical records and ECG	Documented in medical records or in a geriatric unit database
Jansen et al.[238], 2015	62±8	4888	Cross-sectional. Irish Longitudinal Study on Ageing (TILDA; a prospective cohort) Age ≥ 50; community *dwelling* older adults	-	10-minute ECG only	Self-reported
O'Neal et al.[240], 2015	65±9	24117	Cross-sectional *Community dwelling* residents No age criteria for inclusion	-	ECG (read at central reading centre; blinded physicians) and self-reported history.	Self-reported
Sanders et al.[239], 2012	80±8	442	Retrospective cohort; single centre	-	History from documented medical records	Physician adjudicated. Accidental falls defined as: slipping, tripping, stumbling;

			Emergency department presentations with fall as chief complaint. Presentations for syncope excluded. Non-accidental compared to accidental falls. Age ≥ 65.			catching feet or assistive devices on objects; twisting of joint/ fall due to activity). Non-accidental fall: absence of above criteria.
Wallace et al.[237], 2017	74±5	4258	Prospective cohort Cardiovascular health study longitudinal cohort (Random sample of Medicare beneficiaries from 4 united states communities). Age ≥ 65.	1991-1999 (mean 6.3 years)	Annual study ECG (central reading centre), hospital discharge diagnosis or physician diagnosis (Medicare data)	Self-reported
Arita et al.[254], 2018	70±12	1930	Prospective cohort, single centre Patients enrolled from a database of admissions to a cardiovascular hospital for any reason. Stratified into Age <75 and >75 years.	3 years (mean 1.5 years)	Baseline physician assessment of history, ECG, and symptoms.	Self-reported Correlation to admissions for falls related injuries
SYNCOPE						
Sule et al.[248], 2011	66±17	325	Prospective cohort; single centre Consecutive patients hospitalised with syncope	27 months	Unclear	Hospital records demonstrating re-presentation for syncope
Jansen et al.[238], 2015	62±8	4888	Cross-sectional. Irish Longitudinal Study on Ageing (TILDA; a prospective cohort). Age ≥ 50; community *dwelling* older adults	-	10-minute ECG only	Self-reported
Chen l et al.[249], 2000	67±15	1556	Nested case-control. Framingham (original) and offspring cohorts.	-	Unclear.	Physician verified patient report of any loss of consciousness

			No age criteria for inclusion. Syncope cases identified and verified. Age, sex, and examination period matched controls from the study cohort (nested).		History of AF from study	during study medical examination (fainting, seizure, stroke, and transient ischemic attack. Head trauma excluded). Repeat verification with review of study notes and available health records (office or hospital attendances)
ORTHOSTATIC INTOLERANCE						
McNicholas et al.[250], 2017	66±6	4408	Cross-sectional. Irish Longitudinal Study on Ageing (TILDA; a prospective cohort). Age ≥ 50; community *dwelling* older adults	-	10-minute ECG only	Beat-to-beat blood pressure measured during active stand lasting 110 s

TABLE 2Cb: Studies included studies in the systematic review: study characteristics (Part 2)

Study	Geographic Region	Covariates Adjusted For
FALLS		
Chen et al.[245]	Australia	**Unadjusted analysis**
O'Neal et al.[240]	Taiwan	Age, Basic Activities of Daily Living, Geriatric Depression Scale, Impaired TUG Test, Impaired FR test, Polypharmacy, Stroke, Calcium Channel Blockers (ace, β- blockers, diuretics, alpha – blockers excluded on the basis of univariate analysis; P>0.1), Benzodiazepine, Muscle relaxants
Jansen et al.[238]	Ireland	Age, Gender, Education, Gait speed, Depressive symptoms, Visual acuity, MMSE, Psychotropic drugs, CV drugs, History of stroke
Hung et al.[246]	United States	Age, Sex, Race, BMI, Cognitive impairment, Mobility impairment, Alcohol consumption, Exercise habits, Diabetes, Antihypertensive medications, Benzodiazepine use
Sanders et al.[239]	United States	Age, Hypertension, Any neurological disorder, Polypharmacy (>5 medications), Antihypertensive (other than β- blockers, digoxin, Calcium channel blockers & anti-arrhythmic drugs all of which were P>0.1 in univariate analysis) Also excluded psychotropics; P>0.1 in univariate analysis)
Wallace et al.[237]	United States	Age, Sex, Race, Clinic, Education, Physical activity level, Alcohol, Smoking, Baseline Body Mass Index, Antihypertensives, Bisphosphonates, Diabetes mellitus, Hypertension, Coronary heart disease, Incident stroke or heart failure
Arita et al.[247]	Japan	Age, Sex, Body Mass Index, Hypertension, Diabetes mellitus, prior stroke, heart failure, structural heart disease, Antihypertensives, diuretics, hypnotics, Chronic Kidney Disease, Anaemia
SYNCOPE		

Sule et al.[248]	United States	**Unadjusted analysis**
Jansen et al.[238]	Ireland	Age, Gender, Education, Gait speed, Depressive symptoms, Visual acuity, MMSE, Psychotropic drugs, CV drugs, History of stroke
Chen L et al.[249]	United States	**Unadjusted analysis** Negative (P = 0.23) in initial stepwise univariate regression analysis.
ORTHOSTATIC INTOLERANCE		
McNicholas et al.[250]	Ireland	Age, sex, baseline heart rate, education, mean systolic blood pressure, smoking, frailty, anti-hypertensive use, β-blockers, and self-reported cardiovascular conditions

The association between AF and falls

The total number of participants was 36 444 across 7 studies that reported the association of AF to falls. The mean age of all participants included was 72±10 years old. The proportion of males was 57%. There were five retrospective studies (three cross-sectional studies, one case-control and one cohort study) and two prospective cohort studies.[237, 254] In one, falls was a secondary analysis.[237] In the other prospective study by Arita *et al*.[254] the AF cohorts were divided into two groups (>75 and <75 years old). Significant co-morbidities included: hypertension (51%) with a high consequent use of antihypertensive medications (49%),[237, 239, 240, 254] coronary artery disease (19%) and diabetes mellitus (18%). There was also a high proportion of polypharmacy, defined as greater than 4 medications in three studies (67%).[239, 245, 246] The proportion of patients with heart failure was low (4%) in the five studies that reported this parameter.[238, 239, 245, 246, 254] The presence of cognitive impairment was only reported in two studies (6.2%).[240, 245] In two other studies,[238, 246] the combined average Mini-Mental State Examination was 26 (out of a maximum of 30).

Using pooled analysis, AF was found to be associated with an increased risk of falls (OR 1.19; 1.07-1.33 95% CI, P=*.001*; Figure 2B). Most studies, except the report from Chen *et al*.[245] adjusted for other risk factors for falls. The covariates used to adjust the odds ratio of the association of AF to falls are included in Table 2Cb. There was overall low-moderate heterogeneity (I^2 = 37%; P=*.11*). There was an overall moderate risk of bias in the included studies; one study had a high risk of bias, four studies had moderate bias and two had a low risk of bias. The study by Chen *et al*.[245] had a high risk of bias because the ascertainment of AF was unclear, as was its selection of controls and the authors did not adjust for covariates. Given our pre-specified plan to address high risk of bias; we performed a *post-hoc* analysis; which did not affect the association of AF to the risk of falls (OR 1.16; 1.06 –1.28 95% CI;

P=.*001*). Study heterogeneity remained non-significant (I^2=26%; P=.*21*). Figure 2C Subgroup analyses assessing the stability of this association demonstrated that the method of ascertainment of AF (strong; physician verified vs weak; Medicare data/review of health records only) did not affect the association. Figure 2D Neither subgroup analyses; Falls ascertainment (as a "snapshot" presentation to hospital with falls vs self-reported history of *any fall*; Figure 2E) nor the comparison of study type (prospective vs retrospective; Figure 2F) showed any differences in the association of AF with falls.

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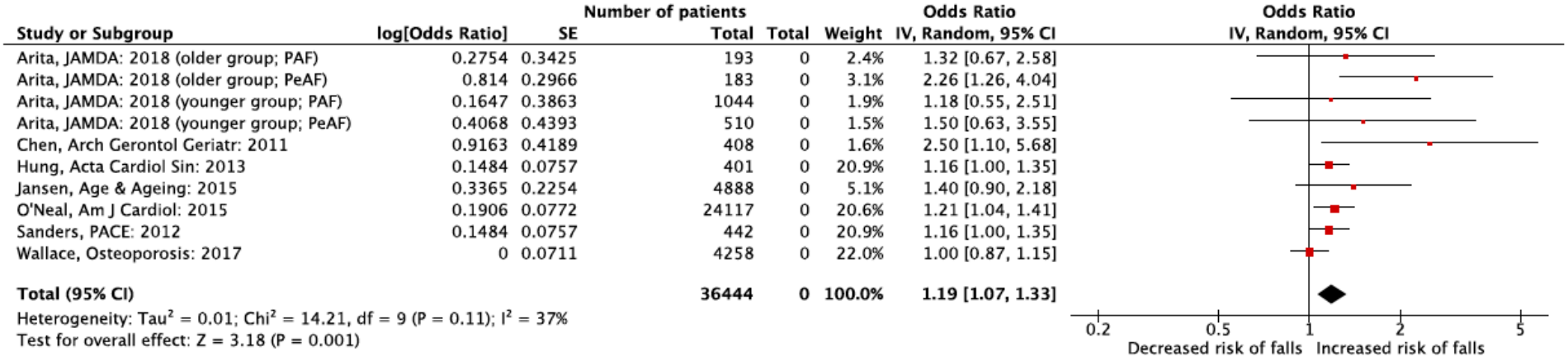
Figure 2B: Pooled analysis Forest plot – The association of AF with falls.



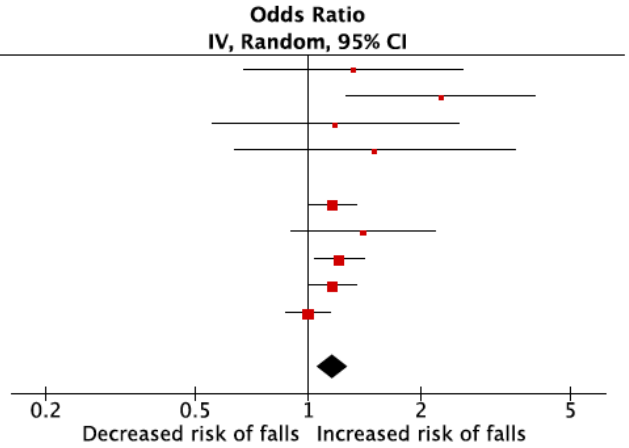
Arita et al. provides risk estimates in each cohort: older group (>75 years) with paroxysmal AF (PAF), older group (>75 years) with persistent AF (PeAF), younger group (<75 years) with PAF and younger group (<75 years) with PeAF. AF is associated with increased risk of falls (Odds Ratio 1.19 [1.07-1.33]; P=0.001). There is overall low-moderate heterogeneity (I^2=37%; P=0.11).

Figure 2C: *post-hoc* analysis – The association of AF with falls

Study or Subgroup	log[Odds Ratio]	SE	Number of patients Total	Total	Weight	Odds Ratio IV, Random, 95% CI
Arita, JAMDA: 2018 (older group; PAF)	0.2754	0.3425	193	0	1.9%	1.32 [0.67, 2.58]
Arita, JAMDA: 2018 (older group; PeAF)	0.814	0.2966	183	0	2.5%	2.26 [1.26, 4.04]
Arita, JAMDA: 2018 (younger group; PAF)	0.1647	0.3863	1044	0	1.5%	1.18 [0.55, 2.51]
Arita, JAMDA: 2018 (younger group; PeAF)	0.4068	0.4393	510	0	1.2%	1.50 [0.63, 3.55]
Chen, Arch Gerontol Geriatr: 2011	0.9163	0.4189	408	0	0.0%	2.50 [1.10, 5.68]
Hung, Acta Cardiol Sin: 2013	0.1484	0.0757	401	0	22.0%	1.16 [1.00, 1.35]
Jansen, Age & Ageing: 2015	0.3365	0.2254	4888	0	4.1%	1.40 [0.90, 2.18]
O'Neal, Am J Cardiol: 2015	0.1906	0.0772	24117	0	21.5%	1.21 [1.04, 1.41]
Sanders, PACE: 2012	0.1484	0.0757	442	0	22.0%	1.16 [1.00, 1.35]
Wallace, Osteoporosis: 2017	0	0.0711	4258	0	23.5%	1.00 [0.87, 1.15]
Total (95% CI)			**36036**	**0**	**100.0%**	**1.16 [1.06, 1.28]**

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 10.78$, df = 8 (P = 0.21); $I^2 = 26\%$

Test for overall effect: Z = 3.19 (P = 0.001)



Despite the removal of Chen *et al*.[245] (high risk of bias) from the analysis, the association persisted. Study heterogeneity remained low and not statistically significant (P=0.21).

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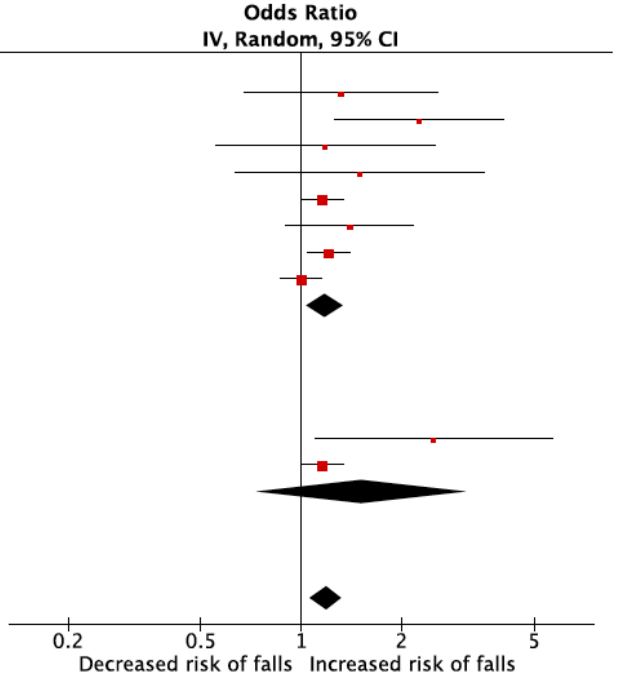
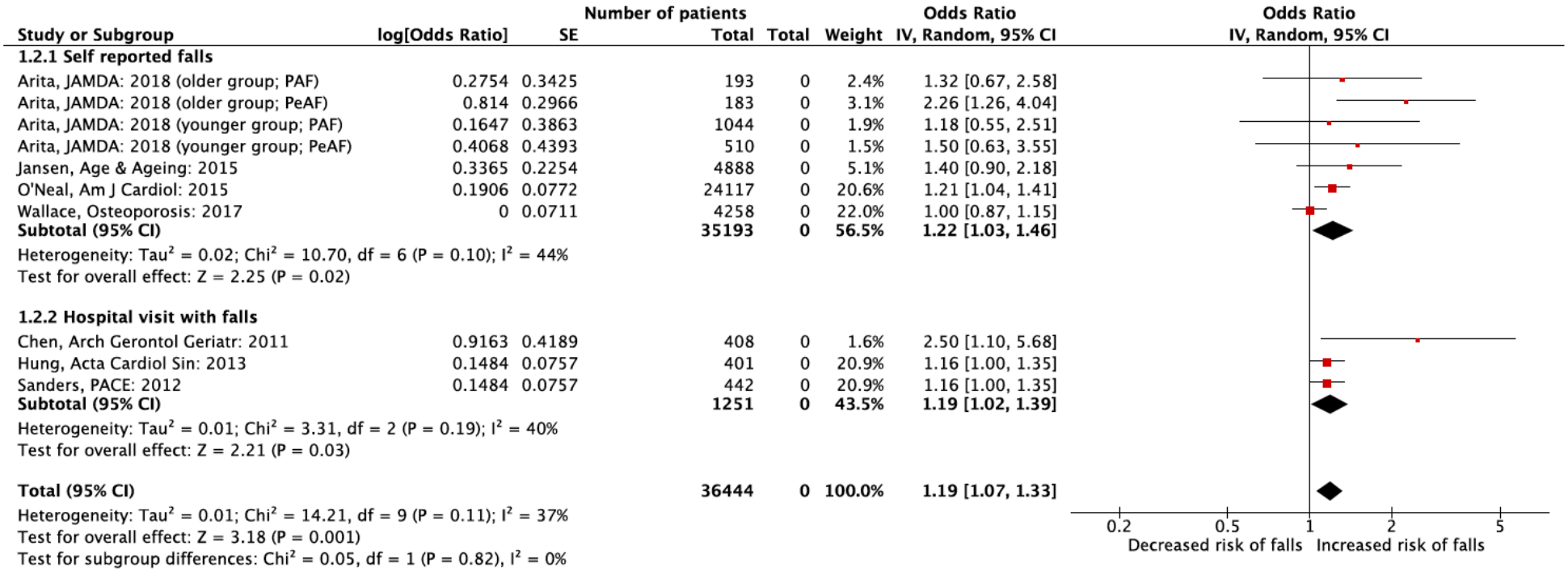
Figure 2D: Subgroup analysis 1; Strength of ascertainment of AF

Study or Subgroup	log[Odds Ratio]	SE	Number of patients Total	Total	Weight	Odds Ratio IV, Random, 95% CI
1.2.1 Strong AF ascertainment						
Arita, JAMDA: 2018 (older group; PAF)	0.2754	0.3425	193	0	2.4%	1.32 [0.67, 2.58]
Arita, JAMDA: 2018 (older group; PeAF)	0.814	0.2966	183	0	3.1%	2.26 [1.26, 4.04]
Arita, JAMDA: 2018 (younger group; PAF)	0.1647	0.3863	1044	0	1.9%	1.18 [0.55, 2.51]
Arita, JAMDA: 2018 (younger group; PeAF)	0.4068	0.4393	510	0	1.5%	1.50 [0.63, 3.55]
Hung, Acta Cardiol Sin: 2013	0.1484	0.0757	401	0	20.9%	1.16 [1.00, 1.35]
Jansen, Age & Ageing: 2015	0.3365	0.2254	4888	0	5.1%	1.40 [0.90, 2.18]
O'Neal, Am J Cardiol: 2015	0.1906	0.0772	24117	0	20.6%	1.21 [1.04, 1.41]
Wallace, Osteoporosis: 2017	0	0.0711	4258	0	22.0%	1.00 [0.87, 1.15]
Subtotal (95% CI)			**35594**	**0**	**77.4%**	**1.18 [1.04, 1.34]**
Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 10.75$, df = 7 (P = 0.15); $I^2 = 35\%$						
Test for overall effect: Z = 2.66 (P = 0.008)						
1.2.2 Weak AF ascertainment						
Chen, Arch Gerontol Geriatr: 2011	0.9163	0.4189	408	0	1.6%	2.50 [1.10, 5.68]
Sanders, PACE: 2012	0.1484	0.0757	442	0	20.9%	1.16 [1.00, 1.35]
Subtotal (95% CI)			**850**	**0**	**22.6%**	**1.52 [0.74, 3.13]**
Heterogeneity: $Tau^2 = 0.20$; $Chi^2 = 3.25$, df = 1 (P = 0.07); $I^2 = 69\%$						
Test for overall effect: Z = 1.15 (P = 0.25)						
Total (95% CI)			**36444**	**0**	**100.0%**	**1.19 [1.07, 1.33]**
Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 14.21$, df = 9 (P = 0.11); $I^2 = 37\%$						
Test for overall effect: Z = 3.18 (P = 0.001)						
Test for subgroup differences: $Chi^2 = 0.47$, df = 1 (P = 0.49), $I^2 = 0\%$						



The strength of the method of ascertainment was examined in this subgroup analysis. Strong methods of AF adjudication (1.2.1) *vs* weak (1.2.2) did not alter the overall association of AF with falls (P=0.49).

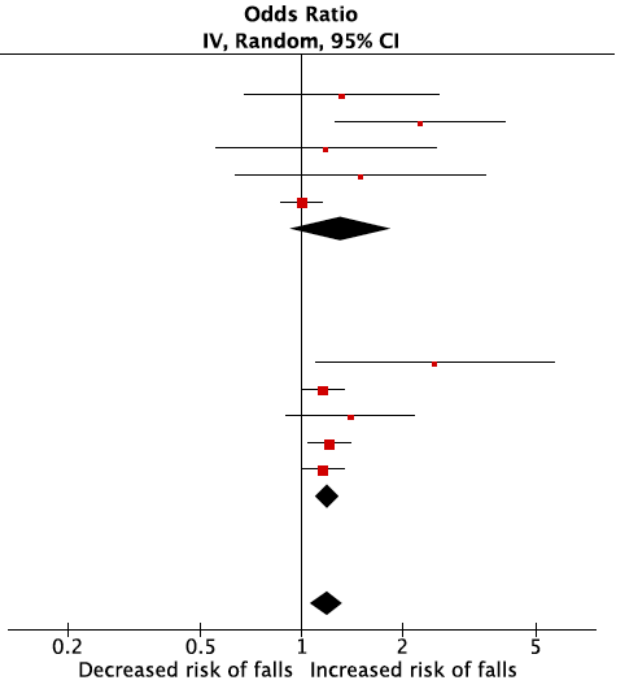
Figure 2E: Subgroup analysis 2; Ascertainment of falls (outcome)



The method of ascertainment of falls ascertainment (as a "snapshot" presentation to hospital with falls; 1.2.1 vs self-reported history of *any fall*; 1.2.2) did not alter the association of AF with falls.

Figure 2F: Subgroup analysis 3; the effect of prospective vs retrospective enrolment

Study or Subgroup	log[Odds Ratio]	SE	Number of patients Total	Total	Weight	Odds Ratio IV, Random, 95% CI
1.2.1 Prospective studies						
Arita, JAMDA: 2018 (older group; PAF)	0.2754	0.3425	193	0	2.4%	1.32 [0.67, 2.58]
Arita, JAMDA: 2018 (older group; PeAF)	0.814	0.2966	183	0	3.1%	2.26 [1.26, 4.04]
Arita, JAMDA: 2018 (younger group; PAF)	0.1647	0.3863	1044	0	1.9%	1.18 [0.55, 2.51]
Arita, JAMDA: 2018 (younger group; PeAF)	0.4068	0.4393	510	0	1.5%	1.50 [0.63, 3.55]
Wallace, Osteoporosis: 2017	0	0.0711	4258	0	22.0%	1.00 [0.87, 1.15]
Subtotal (95% CI)			**6188**	**0**	**30.9%**	**1.31 [0.93, 1.85]**
Heterogeneity: $Tau^2 = 0.07$; $Chi^2 = 8.26$, df = 4 (P = 0.08); $I^2 = 52\%$						
Test for overall effect: Z = 1.54 (P = 0.12)						
1.2.2 Retrospective studies						
Chen, Arch Gerontol Geriatr: 2011	0.9163	0.4189	408	0	1.6%	2.50 [1.10, 5.68]
Hung, Acta Cardiol Sin: 2013	0.1484	0.0757	401	0	20.9%	1.16 [1.00, 1.35]
Jansen, Age & Ageing: 2015	0.3365	0.2254	4888	0	5.1%	1.40 [0.90, 2.18]
O'Neal, Am J Cardiol: 2015	0.1906	0.0772	24117	0	20.6%	1.21 [1.04, 1.41]
Sanders, PACE: 2012	0.1484	0.0757	442	0	20.9%	1.16 [1.00, 1.35]
Subtotal (95% CI)			**30256**	**0**	**69.1%**	**1.19 [1.10, 1.30]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 3.93$, df = 4 (P = 0.42); $I^2 = 0\%$						
Test for overall effect: Z = 4.11 (P < 0.0001)						
Total (95% CI)			**36444**	**0**	**100.0%**	**1.19 [1.07, 1.33]**
Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 14.21$, df = 9 (P = 0.11); $I^2 = 37\%$						
Test for overall effect: Z = 3.18 (P = 0.001)						
Test for subgroup differences: $Chi^2 = 0.27$, df = 1 (P = 0.60), $I^2 = 0\%$						



Subgroup analyses examining study type (prospective; 1.2.1 vs retrospective; 1.2.2) did not showed any differences in the association of AF with falls (P=0.6).

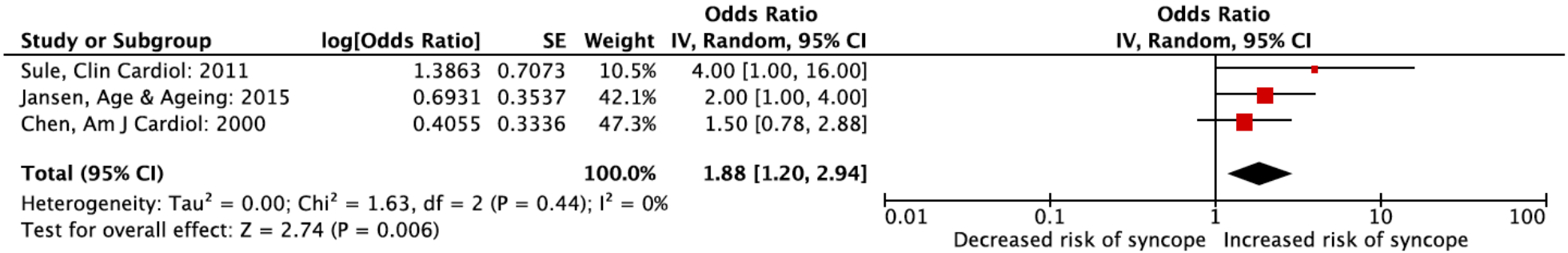
The association between AF and syncope

The number of participants (mean 65±3 years old) across three studies with data for syncope was 6769. The proportion of males was 44%. There was one case-control study, one cross-sectional observational study and one prospective cohort study from a single centre. Chen *et al*.[249] (case-control study) corroborated each report of syncope with hospital or office documentation, however they included loss of consciousness adjudicated to seizures, stroke, and transient ischaemic attacks in their analysis. Sule *et al*.[248] (prospective cohort study) reviewed medical records for 325 patients admitted with a physician diagnosis of syncope. Multiple aetiologies of syncope (whether or not they were associated with AF) were included in their analysis; vasovagal syncope (18%), documented brady-arrhythmia (9%), tachy-arrhythmia (11%), valvular heart disease related (3%), intracranial abnormalities (2%) and hypoglycaemia (1%) are examples. Baseline characteristics presented in Table 2Ca. Multivariates were adjusted for in Jansen *et al*.[238]; a large cross-sectional study of community dwellers. Table 2Cb It was also the only study to specifically assess whether AF was related to syncope. Chen *et al*.[249] performed a stepwise logistic analysis for variety of variables, including AF, which was not statistically significant.

Using pooled analysis, AF was found to be associated with an increased risk of syncope (OR 1.88; 1.20-2.94 95% CI, P=*.006*; Figure 2G) with low statistical heterogeneity (I^2=0%; P=*.44*). The risk of bias was moderate in Jansen *et al*.[238] and Chen L et al.[249] Sule *et al*.[248] had a high risk of bias owing to unclear ascertainment of AF, limitation to representation to hospital with syncope in a short time-frame (27-month follow-up), inclusion of other causes of syncope clearly not associated to AF and they did not adjust for covariates. See Table 2B for bias assessment. Exclusion of this study in a *post-hoc* analysis did not alter the association

identified from the pooled analysis (OR 1.72; 1.07-2.76 95% CI, P=*.03*) with low statistical heterogeneity (I^2=0%; P=*.6*)

Figure 2G: Pooled analysis Forest plot – The association of AF with syncope.



AF is associated with increased risk of syncope (Odds Ratio 1.88 [1.20-2.94]; P=0.006). There is overall low heterogeneity (I^2=0%; P=0.44).

The association between AF and orthostatic hypotension

There was only one study that reported AF was associated with orthostatic intolerance in 4408 community dwellers in Ireland (mean 66±6 years old).[250] The 101 (2.3%) patients with AF were older with higher proportion being male, higher rates of self-reported cardiovascular illness and medication use in comparison to those without AF. They found that AF was independently associated with both initial and classical orthostatic hypotension at 30 seconds of standing (OR 2.48 (1.59- 3.87 95% CI, P<*.001*) and 1.78 (1.07 – 2.94 95% CI, P=*.03*) respectively. This study contains moderate bias. Table 2B

Discussion

This systematic review and meta-analysis from more than 40,000 older individuals derived from a variety of clinical cohorts ranging from community participants to higher risk patients admitted to hospital for falls, identified AF as an independent risk factor for falls as well as syncope. Patients with AF often have multiple co-morbidities such as diabetes, hypertension and vascular (coronary and peripheral vascular) disease.[3] The rising prevalence of AF in the elderly mirrors the prevalence of other cardiovascular illness, co-morbidities and polypharmacy in this age group.[231] Therefore, falls and syncope in older adults with AF can be attributed to co-morbidities or to the presence of AF itself. Arita *et al*. found that persistent AF in an older cohort (i.e., higher burden of AF) was independently associated with the risk of falls.[254] Our pooled analyses reaffirm this finding with AF conferring an adjusted odds ratio for falls and syncope at 1.19 and 1.88 respectively.

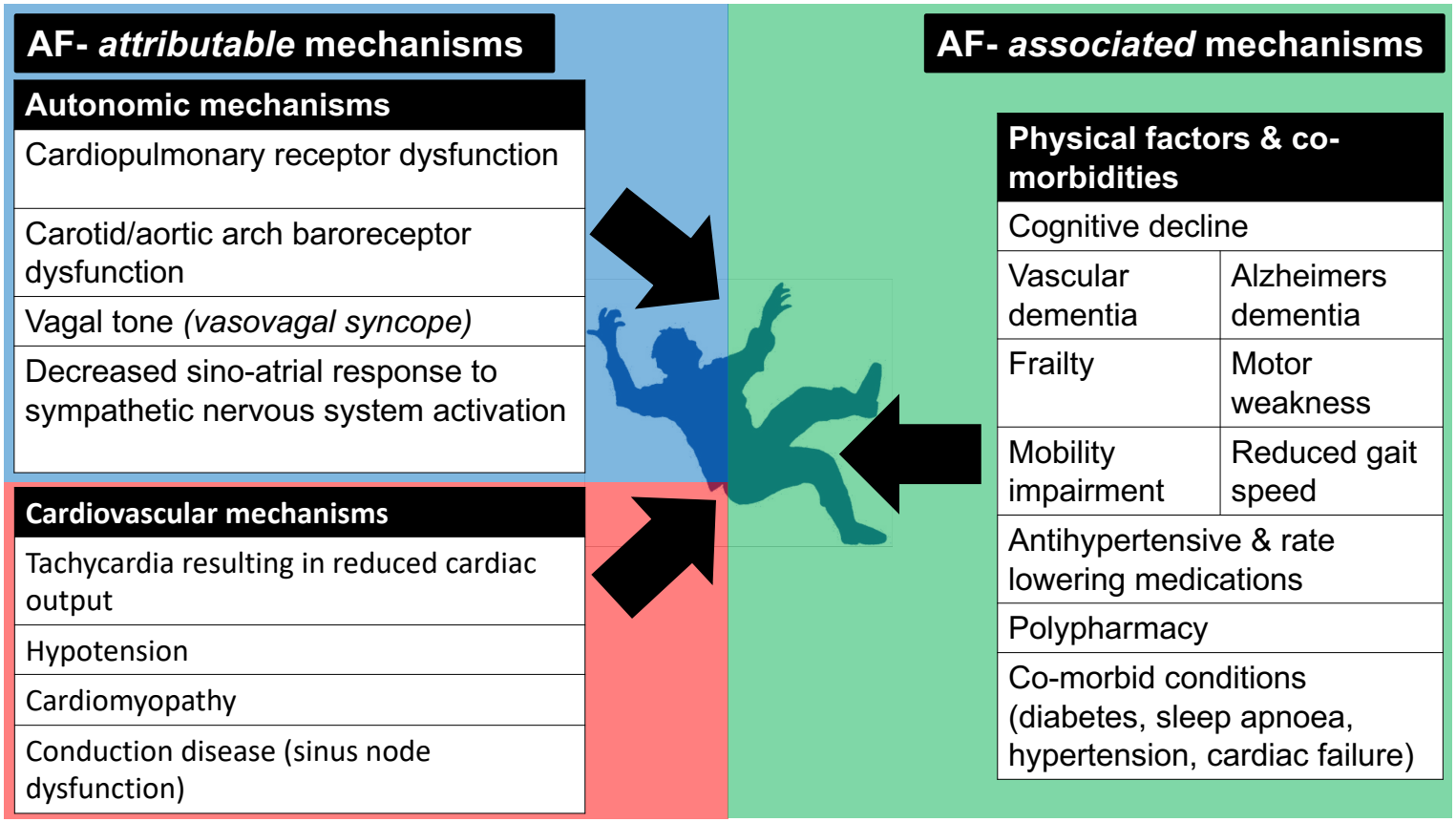
Our findings may in part explain the high proportion of deaths relating to AF in the GARFIELD-AF registry that are not attributable to cardiovascular causes or malignancy. Indeed, falls and syncope constitute a range of other associated conditions with AF that can result in increased morbidity and mortality. There are several mechanisms that may explain the associations of AF to falls and syncope in older patients with AF. These may be directly attributable to AF or related to associated conditions.

Falls and syncope: Mechanisms directly attributable to AF

The haemodynamic deficits of AF with rapid ventricular response and cardiomyopathy are well known and may contribute to underlying orthostatic intolerance.[255, 256] Further, autonomic dysfunction with abnormal reflex responses may also be responsible.[23, 175, 203] A

critical feature of cardiac autonomic influence is its tight control of postural reflexes. The autonomic reflex control of posture relies on intact afferent receptors, which respond to changes in cardiac volume, atrial stretch, and arterial pressure; central neural processing and autonomic efferent nerves[57, 257]. Cardiopulmonary receptors found in pulmonary vein – atrial junctions (interestingly co-located with the origin of the ectopic beats in AF) as well as arterial baroreceptors (located in the aortic arch and carotid bodies) are implicated in the normal response to standing as they respond to the decrease in arterial pressure and volume due to changes in posture and associated haemodynamic effects.[258] Dysfunction of both of these types of receptors could contribute.[259, 260] We have demonstrated autonomic dysfunction (reflex deficit to decrease in cardiac venous return) in patients with AF.[175] See Chapter 3 & Chapter 4. Brignole *et al*.[261] demonstrated that patients with paroxysmal AF presenting with syncope had abnormal reflex responses to head-up tilt and carotid sinus massage during both sinus rhythm and AF. The presence of orthostatic intolerance[250] together with improvement of cardiac output following cardioversion,[241] in patients with persistent AF and adequate rate-control provides further mechanistic explanations to the link between autonomic dysfunction due to AF and falls or syncope. Figure 2H; Central diagram

Figure 2H: Central diagram



Mechanisms for falls and syncope associated with AF can be classified according to those *directly attributable* or to those *associated with* AF. Autonomic mechanisms may be quite important to consider in elderly patients and AF as a mechanism responsible for the clinical associations described.

Falls and syncope: Mechanisms attributable to AF-associated conditions

Several AF-associated conditions may contribute to the increased risk of falls and syncope in this population. A reduction in physical and cognitive abilities in association with AF could in part contribute to falls and syncope with cognitive decline,[234, 235] reduced gait speed[262] and increased frailty;[263, 264] all associated with motor weakness. AF is also known to be associated with conduction system disease, predominantly sinus node dysfunction due to AF-related atrial fibrosis and myopathy.[265] Additionally, the high prevalence of polypharmacy (also associated with AF) may also be a contributor to increased falls or syncope. The antihypertensive and rate lowering effect of medications used to treat AF may also contribute, although most included studies have addressed these factors through multivariate analyses. Figure 2H; Central diagram

Limitations

One major limitation of this is work is the predominantly retrospective nature of the included studies. There were biases in both the falls and syncope studies that could potentially underestimate the effect of AF, due to the inadequate ascertainment of AF that only occurred concurrently at the time of falls or syncope or due to the ascertainment of asymptomatic AF by ECG measurement (which limits generalisability). Indeed, longer duration of AF monitoring would provide more accurate data. It is also possible that despite adjustment for co-morbidities in most of the included studies, these AF-associated conditions may play a greater role to our findings. Our study does not specifically assess the relative contribution of AF- attributable or AF-associated factors. The study by Arita *et al.*[254] was the only study which excluded the effect of structural heart disease. Therefore, the effect of co-existing valvular heart disease is not excluded. However, this seems less likely given the higher proportion of community dwellers rather than hospital patients. The observations of Arita *et al*.[254] that only persistent but not paroxysmal AF was independently associated with falls in older individuals implicate AF to play a key role. The results of the subgroup analyses strengthen the stability of this association. Also, our study is unable to differentiate the relative contribution of mechanisms such as rapid ventricular response rates, sinus node dysfunction, postural hypotension, or autonomic dysfunction. These will need to be explored in further detail. Finally, our findings remain limited to the demonstration of association rather than causality between AF and falls or syncope.

Concluding remarks

Clinical Implications

The identification of the long-term adverse effects of AF such as increased falls and syncope as demonstrated in this study, underscores the importance of improved rhythm management early in the course of AF, including aggressive modification of AF risk factors.[13] Improved understanding of the associations between falls, syncope and AF will be helpful when managing older individuals who are at heightened risk. Whether improved surveillance and pro-active medical management will reduce the risk and improve clinical outcomes remain to be determined.

Conclusion

This review demonstrates an independent association of AF with falls risk and syncope in older adults. There is a need for well-designed prospective studies to further delineate this association and to guide improved care of elderly patients with AF. Further, there is great need to identify causal mechanisms, and in particular, mechanistic studies evaluating the role of the autonomic nervous system. In the subsequent chapters (Chapter 3 & Chapter 4) we evaluate the role of cardiovascular volume and pressure regulating reflexes in patients with AF in order to define the concept of clinical autonomic dysfunction due to AF.

Chapter 3: Novel clinical evidence of autonomic dysfunction in patients with Atrial Fibrillation studied in sinus rhythm: A new clinical entity?

Background

The autonomic nervous system is implicated in the genesis of atrial fibrillation (AF) with robust experimental[39, 266-268] and clinical evidence.[40, 223] Indeed, cardiac ganglionic plexi are in close proximity to the sites where pulmonary veins enter the left atrium.[136] Experimentally, stimulation of autonomic nerves has been shown to elicit rapid firing triggered by early afterdepolarizations in pulmonary vein preparations, providing a possible mechanistic explanation for autonomic triggering of AF.[39] While triggering of AF by the autonomic nervous system has received much attention the converse that AF begets autonomic nervous system dysfunction has not been extensively studied in humans. Animal models of rapid atrial pacing-induced AF has been shown to increase both sympathetic and parasympathetic innervation of the atria.[211, 220, 221] In humans, Brignole *et al.*[269] demonstrated autonomic disturbances in patients with syncopal events associated with onset of AF. Further, there is case evidence that restoration of sinus rhythm can abolish incessant orthostatic hypotension in a patient with persistent AF.[270]

In normal individuals, lower body negative pressure (LBNP) decreases venous return to the heart, unloads cardiopulmonary receptors and results in compensatory systemic vasoconstriction.[97] Atrial stretch receptors, particularly cardiopulmonary receptors found in the pulmonary vein-left atrial junctions are critical mediators of such reflexes.[57, 97] Here, we hypothesize that AF induces autonomic dysfunction with abnormal reflex responses to decreased venous return. Therefore, we compared reflex cardiovascular responses to LBNP

in subjects with frequent episodes of paroxysmal atrial fibrillation (PAF) to age- and sex-matched healthy controls. This chapter presents data with regards to our first study examining volume-regulating (low-pressure) baroreceptor reflexes.

Methods

All participants provided written informed consent and the study was approved by the institutional human research ethics committees. This study involved two groups. The first group consisted of twenty patients who had symptomatic paroxysmal AF referred for consideration of pulmonary vein isolation (PVI) and were studied *prior* to PVI. These patients were compared to fourteen age and sex-matched healthy controls (Control group). The PAF patients were highly symptomatic, requiring anti-arrhythmic drug (AAD) therapy and were consecutively enrolled after having been referred to an electrophysiologist for consideration of catheter ablation. Patients who enrolled were selected to undergo PVI. Enrolled patients were free from cardiovascular (coronary artery disease, valvular heart disease or heart failure) and other confounding conditions (autonomic disorders, untreated hypertension, postural hypotension, diabetes mellitus or renal disease). Patients who had received amiodarone during the last six months were also excluded.

The control group was enrolled last, after analysing the age and sex of the patient cohorts. Participation was sought from hospital workers between the ages of thirty and seventy years after advertisement through hospital notice boards, flyers, and department presentations. The total number of participants in each five-year age category as well as the proportion of males and females was established for the AF group. Volunteers were consecutively enrolled and assigned to each five-year age group until similar numbers were achieved in each age-group.

Experimental protocol

The experimental protocol was performed in the fasting state (4 hours) with abstinence from caffeine, alcohol, and strenuous exercise in the preceding 24 hours. All AAD (including ß-

blockers) were withheld for five half- lives, symptoms allowing, prior to the study protocol. All testing was performed in a climate-controlled laboratory with room temperature of 22°C. All patients were in sinus rhythm during the testing protocol. Resting brachial blood pressure was checked in the supine position with a standard mercury sphygmomanometer. Continuous beat-to-beat recordings were obtained using finger photo plethysmography (Nexfin, BMEYE, Amsterdam, The Netherlands)[271] to derive:

Mean arterial pressure (MAP, *mmHg*);

Heart rate (HR, *bpm*);

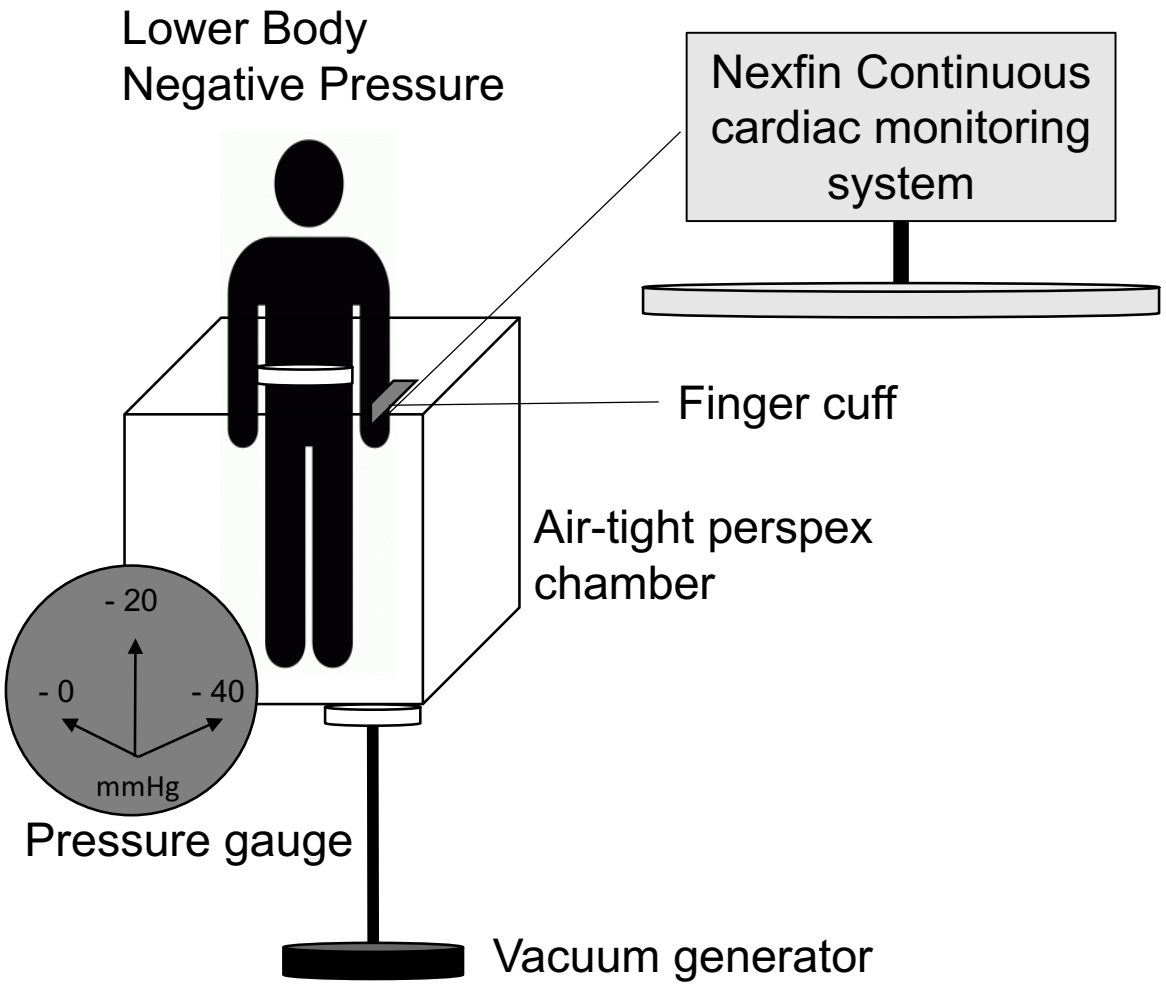
Stroke volume index (SV, mL/m^2);

Cardiac index (CI, $L/min/m^2$); and

Systemic vascular resistance index (SVRI, $dynes\ \ s*\ m^2/cm^5$).

Patients were then subjected to LBNP using a custom-made Perspex chamber placed over the subject's lower limbs with a seal at the iliac crest. A vacuum generator (Model UZ-930; Electrolux; Stockholm, Sweden) was attached to the chamber and connected to a voltage converter, which allowed graduated control of the vacuum intensity (Figure 3A). Thus, negative pressure could be precisely controlled to 0, -20 and -40 mmHg with the aid of an industrial pressure gauge (Ambit Instruments; Wetherill Park, NSW, Australia). All patients underwent a short 2-minute familiarization period with LBNP application followed by a resting period of 10 minutes prior to LBNP protocol commencement. The degree of LBNP intensity was randomly applied for each patient for 10 minutes at each level with a 5-minute break in between. Negative pressure was applied slowly over 30 seconds to minimize patient movement and discomfort.

Figure 3A: Lower Body Negative Pressure (LBNP) technique



Schematic depicting Lower Body Negative Pressure (LBNP) technique.

Statistical Analysis

Continuous patient variables were expressed as mean ± SD. Categorical variables were expressed as frequencies and percentages. The last two minutes of beat-to-beat recorded data for each level of negative pressure (0, -20 and -40 mmHg) was extracted and averaged (mean ± SEM). Comparisons within groups were made by repeated measures one-way analysis of variance (ANOVA) with the Dunnett's post-test analysis. Differences across groups were compared with ANOVA and the Bonferroni post-test analysis. Statistical analysis was performed using GraphPad Prism (version 6.0, California, USA). Statistical significance was set at $P<0.05$.

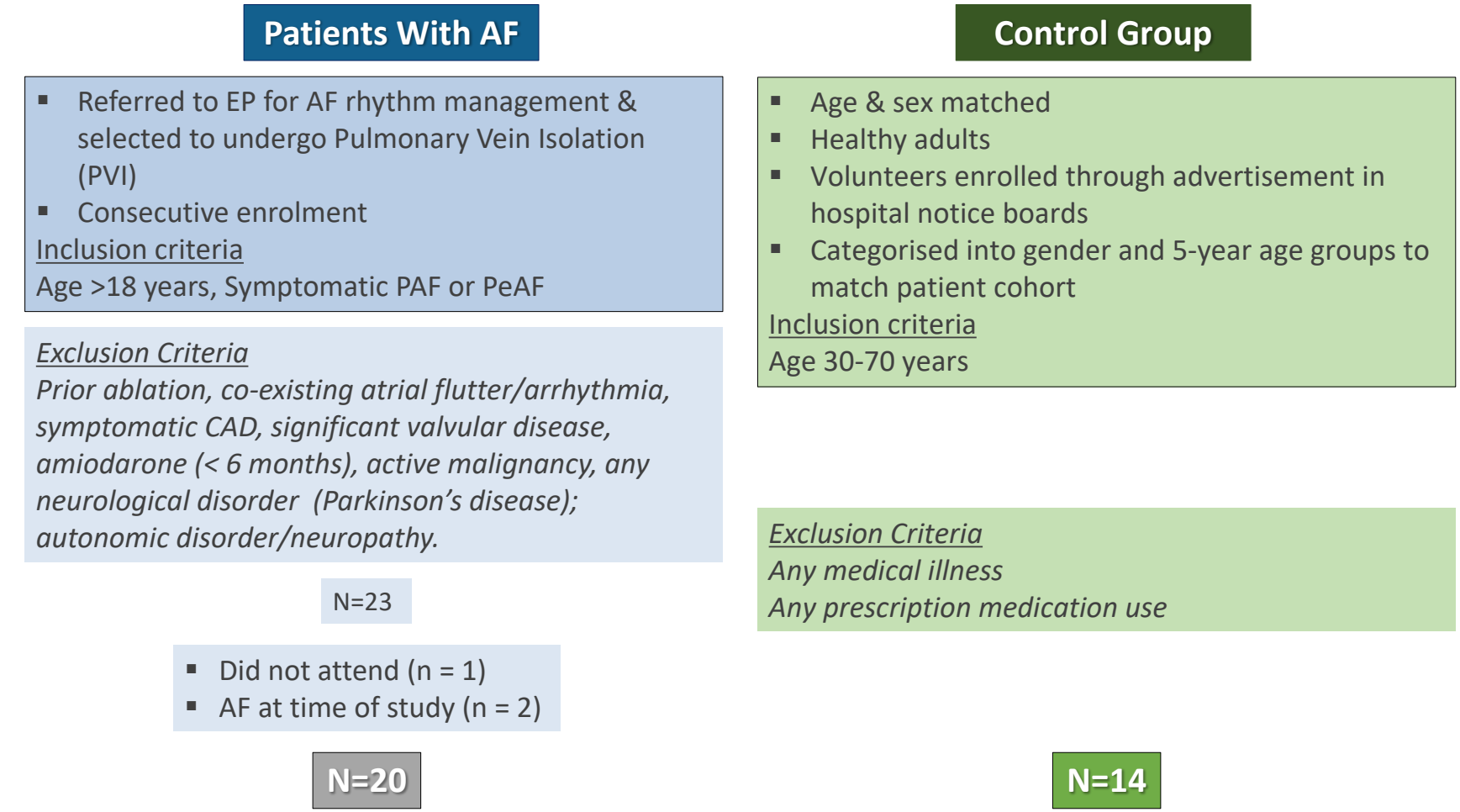
Results

Patient Recruitment and Baseline Characteristics

Figure 3B presents the CONSORT diagram of the patient groups. In the PAF group, 23 patients with symptomatic AF, referred to an electrophysiologist for consideration of PVI were consecutively enrolled after having been assessed for eligibility. 3 patients did not undergo autonomic testing and therefore 20 patients were included. Fourteen patients (of 16 volunteers) were enrolled in the control group. Subjects were age- and sex-matched between groups (all $p>0.05$). The overall mean age was 57±8.5 years, 70% males. The baseline characteristics of the included patients are presented in Table 3A (below).

Dr Varun Malik, PhD thesis, 2018 - 2021

Figure 3B: CONSORT Diagram



CONSORT Diagram. Enrolment flow chart, including patient recruitment, inclusion, and exclusion criteria for each group

Table 3A: Baseline Characteristics

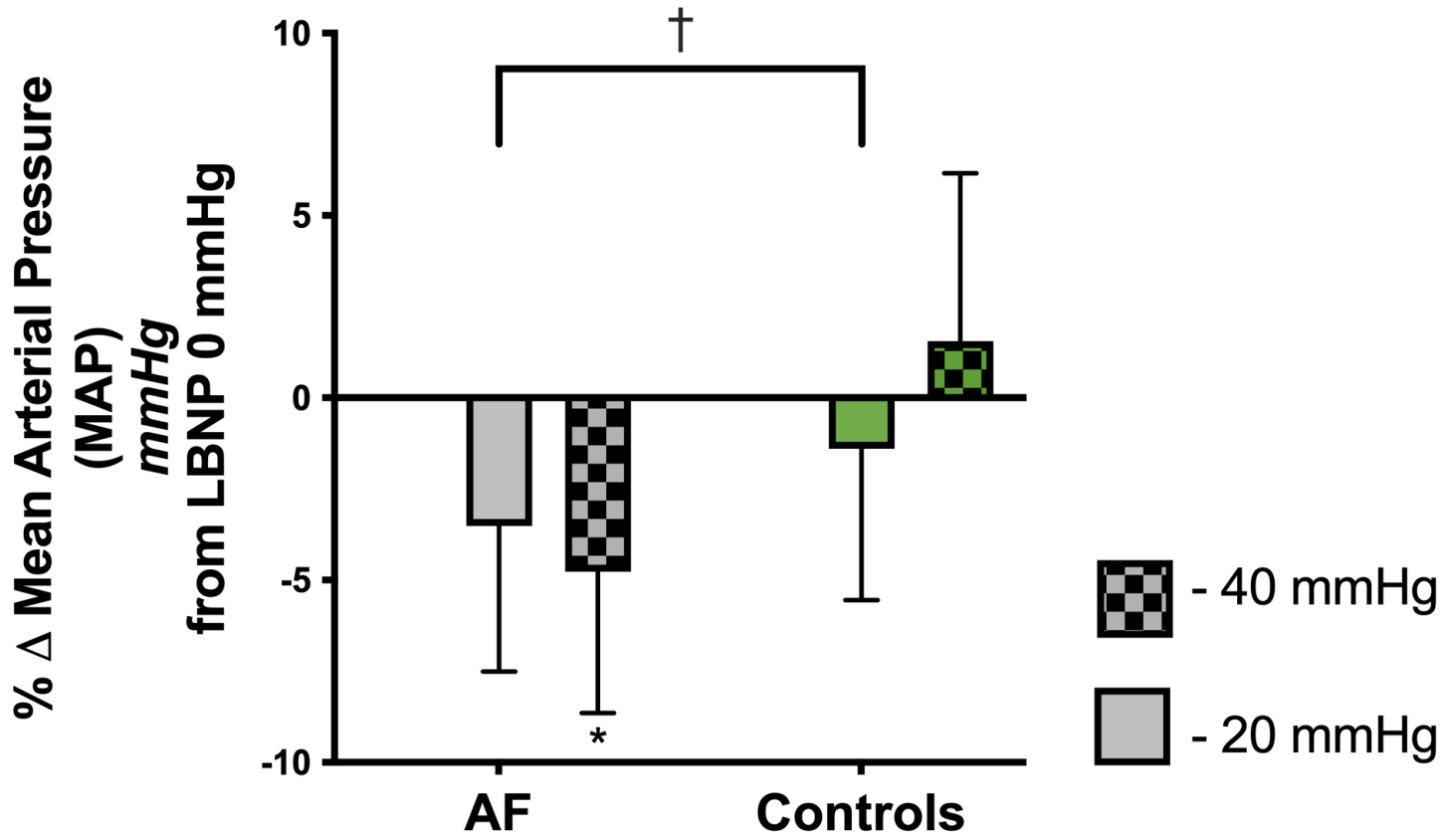
	PAF (n=20)	Controls (n=14)	p-value
Age (years)	58±10	55±5	0.38
Males, n (%)	13 (65)	9 (64)	0.35
Mean body mass index, kg/m^2	30.3±5	25.6±4	0.02*
Hypertension n (%)	8 (57)	0 (0)	-
Patients not on any AAD, n (%)	4 (20)	14 (100)	-
ß-blockers (except Sotalol), n (%)	7 (35)	0 (0)	-
Sotalol, n (%)	8 (40)	0 (0)	-
Amiodarone, n (%)	0 (0)	0 (0)	-
Flecainide, n (%)	6 (30)	0 (0)	-
Digoxin, n (%)	1 (5)	0 (0)	-
Calcium channel blocker, n (%)	0 (0)	0 (0)	-

One-way ANOVA (Bonferroni post- test) for continuous variables or students-*t* as appropriate: Between group statistical differences: *p<0.05. Categorical variables; χ^2 test or Fishers exact test *p<0.05. AAD: Anti-arrhythmic drugs.

Hemodynamic responses to LBNP: Mean Arterial Pressure

Baseline MAP was similar across groups: 97±3 mmHg in the control group and 100±3 mmHg in the PAF group (Table 3B). In control subjects, MAP was maintained at both -20 and -40 mmHg LNBP (Figure 3C, p=0.4). In the PAF group, the decrease in MAP was not significant at -20 mmHg, however there was a statistically significant decrease at -40 mmHg (Figure 3C, $p<0.05$). The MAP response to LBNP was different in the PAF group (p=0.04) in comparison to control subjects.

Figure 3C: Mean Arterial Pressure Responses



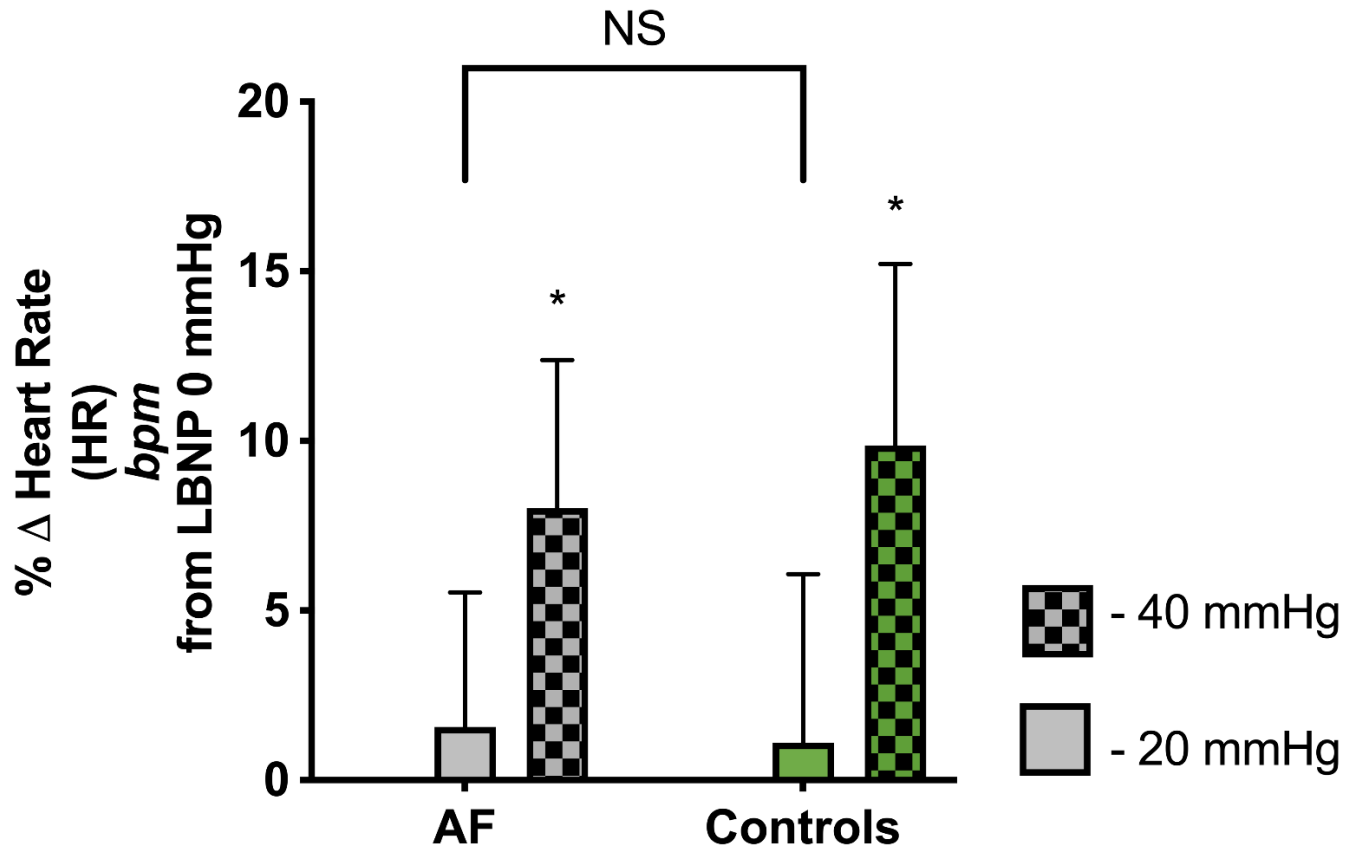
Percentage change in MAP with LBNP (-20 and -40 mmHg) for each group expressed as mean±SEM.

*Statistical difference in comparison to baseline at 0 mmH*g* LBNP ($p<0.05$). †Indicates statistically significant change in response to LBNP between groups ($p<0.05$). NS: non-significant.

Hemodynamic responses to LBNP: Heart Rate

Baseline HR was similar between groups (61±2 vs. 57±2 in the control vs. PAF groups respectively, p=0.4; Table 3B). HR did not change at -20 mmHg LNBP, however at - 40 mmHg LBNP there was significant increase in HR consistently in both groups. There was no statistical difference in the HR response to LBNP between groups (Figure 3D).

Figure 3D: Heart Rate Responses



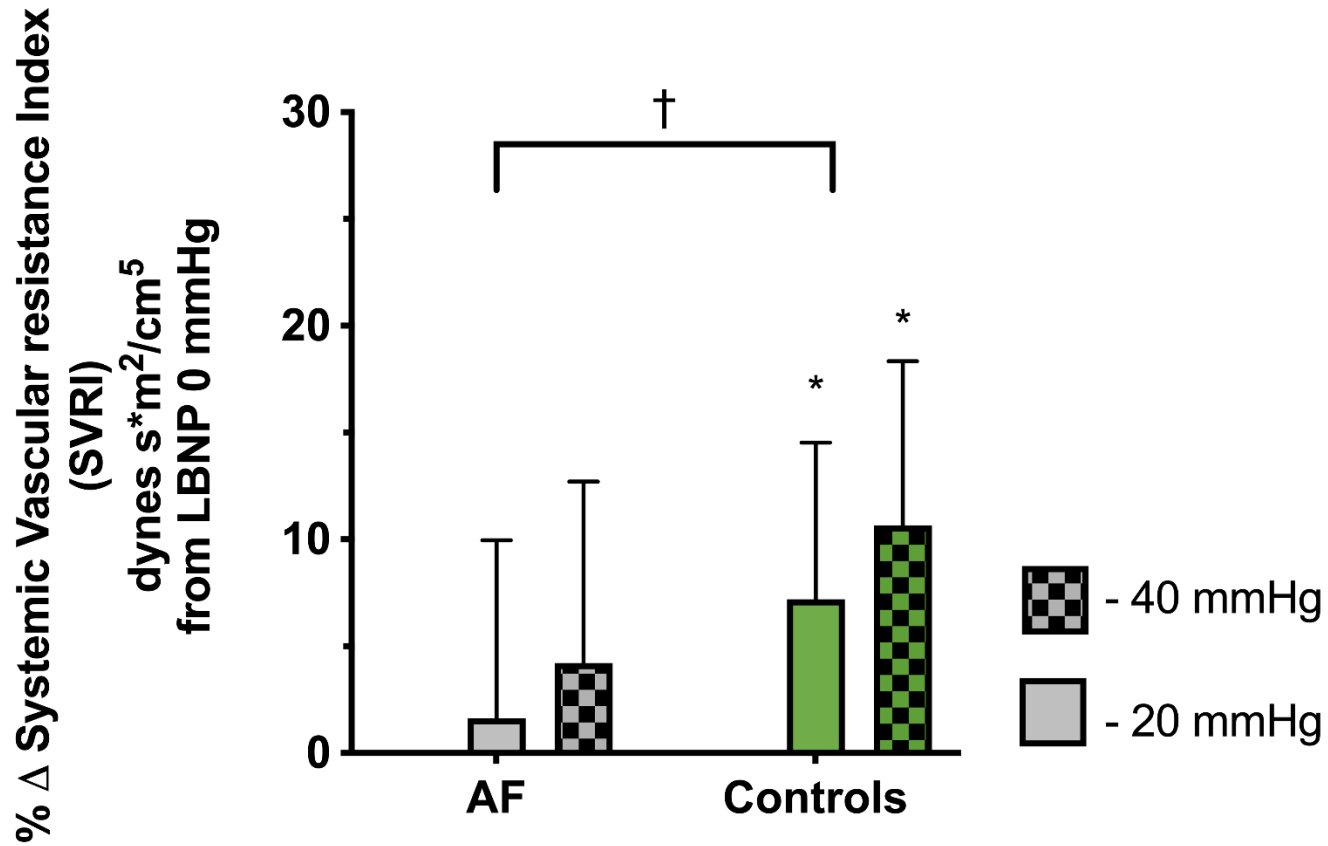
Percentage change in mean HR with LBNP (-20 and -40 mmHg) for each group expressed as mean±SEM.

*Statistical difference in comparison to baseline at 0 mmHg LBNP ($p<0.05$). †Indicates statistically significant change in response to LBNP between groups ($p<0.05$). NS: non-significant.

Hemodynamic responses to LBNP: Systemic Vascular Resistance

In controls, there was a significant increase in SVRI at both -20 and -40 mmHg LBNP (Figure 3E, both $p<0.01$). In contrast, there was no SVRI response to LBNP at either -20 or -40 mmHg in patients with PAF (both $p>0.05$). Overall SVRI response to LNBP was different between the PAF and control groups (Figure 3E, $p=0.04$).

Figure 3E: Systemic Vascular Resistance Index Responses



Percentage change in mean SVRI with LBNP (-20 and -40 mmHg) for each group expressed as mean±SEM.

*Statistical difference in comparison to baseline at 0 mmH*g* LBNP ($p<0.05$). †Indicates statistically significant change in response to LBNP between groups ($p<0.05$). NS: non-significant.

Hemodynamic responses to LBNP: Cardiac Index and Stroke Volume Index

Table 3B shows the CI and SVI at baseline and after application of -20 and -40 mmHg LBNP. CI and SVI were similar at baseline and changed similarly in both groups following application of LBNP at -20 and -40 mmHg. Changes induced by LBNP were statistically significant within each group, but responses were not different between groups (all $p>0.05$).

Table 3B: Cardiac Index and Stroke Volume Index

	LBNP (mmHg)	PAF (n=20)	Controls (n=14)	p-value
Mean arterial pressure (*mmHg*)	0	100 ± 3	97± 3	0.04†
	-20	97 ± 3	96 ± 3	
	-40	96 ± 3*	99 ± 3	
Heart rate (*bpm*)	0	57 ± 2	61 ± 2	NS
	-20	58 ± 1	61 ± 2	
	-40	61 ± 2***	67 ± 2**	
Systemic Vascular Resistance Index (dynes s* m2/cm5)	0	3131 ± 198	2720 ± 132	0.04†
	-20	3182 ± 166	2916 ± 140**	
	-40	3263 ± 167	3010 ± 150**	
Cardiac Index ($L/min/m^2$)	0	2.70 ± 0.12	2.89 ± 0.09	NS
	-20	2.51 ± 0.09**	2.69 ± 0.08**	
	-40	2.43 ± 0.09***	2.62 ± 0.09**	
Stroke Volume Index ($mL/min/m^2$)	0	47.9 ± 1.6	45.3 ± 2.1	NS
	-20	43.8 ± 1.5***	41.8 ± 2.1***	
	-40	39.8 ± 1.5***	37.2 ± 2.5***	

Within group differences: * p< 0.05, ** p< 0.01, *** p< 0.001. Between group differences: †Indicates statistically significant change in response to LBNP between groups (p<0.05). NS: non-significant.

Discussion

This study evaluated the effects of AF on the autonomic nervous system by comparing cardiac reflex response to LBNP in patients with symptomatic PAF to healthy control subjects. Our principal findings are as follows: Reflex (vasoconstrictor) responses to LBNP were attenuated in patients with PAF, suggesting that autonomic dysfunction in patients with AF persists beyond a bout of the arrhythmia. The above changes were seen in the absence of differences in HR, CI, and SV response to LNBP.

Abnormal Cardiac Reflex Responses to LBNP due to AF

LBNP is an established technique[96] used to study reflex responses to decreased blood volume. LBNP simulates mild to moderate hypovolemia by displacing 400-550 mL to 500-1000 mL of volume at -20 and -40 mmHg respectively.[272] The application of LBNP at these pressures produces a progressive decrease in venous return and consequent changes in cardiac output in keeping with the Frank-Starling effect.[273] The efferent response to LBNP is primarily a sympathetically mediated peripheral vasoconstriction in order to maintain MAP.[57, 97] Receptors at veno-atrial junctions are thought to be critical in mediating this homeostatic reflex. Arterial receptors are not engaged unless blood pressure falls. HR changes from LBNP are generally the result of interference from other reflexes and therefore not helpful in the evaluation of the reflex response to LBNP.[274]

In this study, LBNP lowered both SVI and CI to a similar extent in both groups, indicating that an equivalent stimulus was applied in each group. Blood pressure was maintained at both levels of LBNP in the control subjects but not in the AF group, where MAP fell progressively. While no differences were seen in HR response, clear differences were

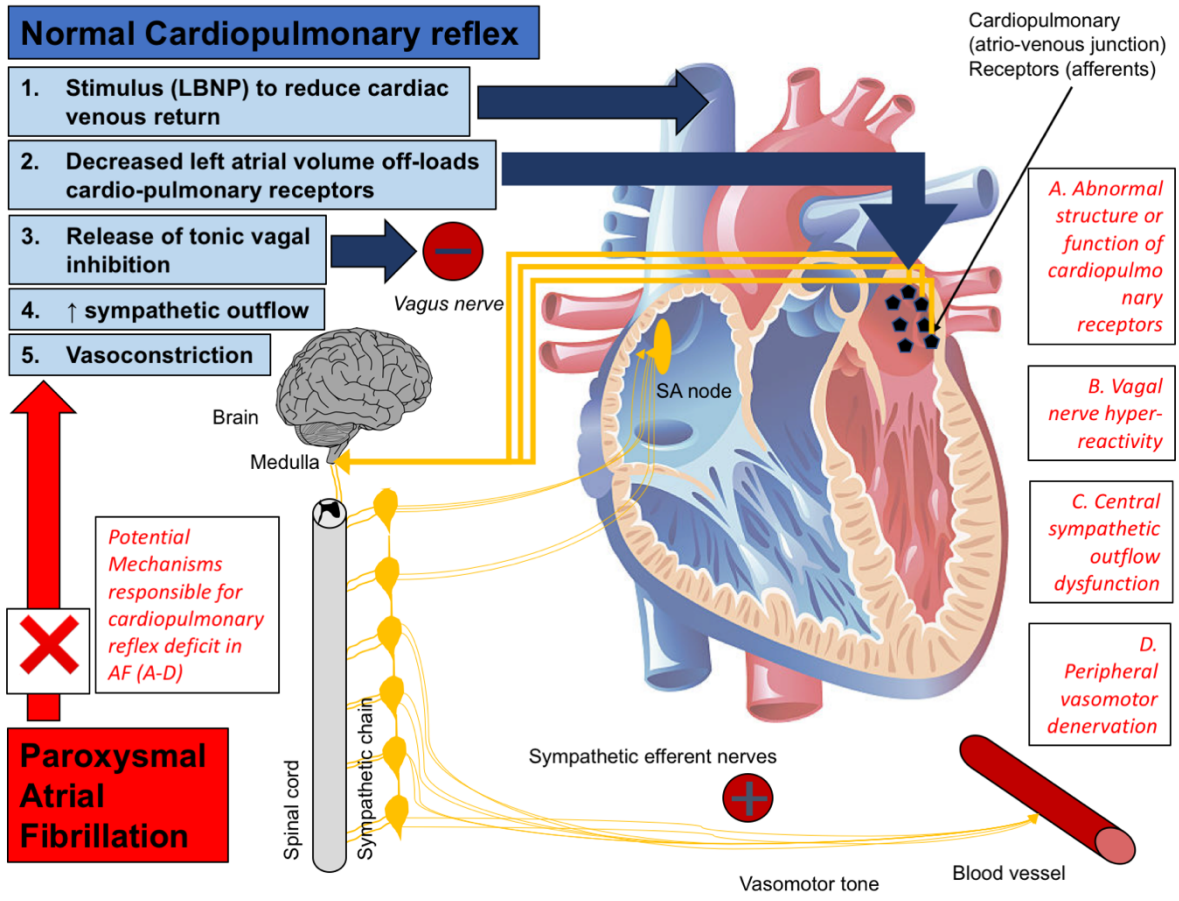
observed between groups in the reflex vasoconstrictor responses elicited by LBNP. This indicates a clear reflex deficit in subjects with PAF studied in normal sinus rhythm. Specifically, no significant vasoconstrictor response was seen in PAF subjects at either level of LNBP.

Our finding of impaired cardiac reflex response due to AF affirmed previous findings by Brignole *et al*.[269] whereby disturbances of the autonomic nervous system was thought to be responsible for syncope in patients with PAF. Further, our results may also explain the enhanced susceptibility to autonomic provocation seen in patients with lone PAF.[275] Persisting autonomic changes as a result of AF have not previously been described. Thus, our study adds to prior observations by demonstrating reflex changes that are not the immediate response to AF but rather changes in reflex function that persist beyond a bout of PAF.

Potential Mechanisms

Changes in efferent innervation of the atria have been reported after induction of AF in dogs.[211, 220, 221] Both structural and functional changes in atrial autonomic innervation may be mechanistically responsible for the abnormal reflex changes we have observed in our PAF patients. The normal reflex response to LBNP is a complex reflex arc that relies on intact afferent receptors, vagus nerve traffic, inputs to the medulla and the sympathetic outflow from the spinal cord, which together mediate peripheral vasoconstriction in response to decreased venous return to the heart (Figure 3F: Central Diagram).

Figure 3F: Central Diagram



A schematic of the neural components responsible for normal reflex control of blood pressure and heart rate in responses to LBNP. PAF results in a reflex deficit (attenuation of vasomotor response to LBNP). This diagram incorporates a sketch of the heart and the brain that have been modified from clipart provided by www.openclipart.org and www.pixabay.com, respectively. These images have been placed in the public domain, have had their copyright waived as part of the creative commons zero 1.0 public domains license and can be modified and reproduced without permission (https://creativecommons.org/publicdomain/zero/1.0). No other components of this diagram have been copied or modified from other sources.

Our findings may result from an abnormality of any one of these; however, we hypothesise that aberrations within the left atrium are principally responsible for our findings, and we therefore postulate either atrial deafferentation or functional deficits of the afferent low-pressure volume-sensing receptors as possible explanations for our findings. Figure 3F: Central Diagram.

Limitations

There were limitations to our study. Our patients were highly symptomatic from AF, requiring rate slowing and anti-arrhythmic drugs therapy that might interfere with the HR component of the response; not attributed to atrial cardiopulmonary receptor unloading from LBNP.[274] However, β-blockade has been shown to not have any impact on vasoconstrictor responses to LBNP [276], which are mediated by α- adrenergic receptors. These medications may also decrease resting blood pressure however there was no differences seen in resting MAP between the groups. Further, we did not see any differences in the resting HR and HR increase induced by LBNP among the groups.

The PAF group was heavier than control subjects and therefore obesity may represent a potential confounder. The presence of hypertension, which, itself is a risk factor for AF, may influence responses to LBNP; although this occurs only in the context of untreated hypertension with severe left ventricular hypertrophy.[54] There were no patients with either untreated hypertension or severe left ventricular hypertrophy. Last, we did not measure MAP invasively as the protocol was conducted in all subjects in the ambulatory setting.

Concluding remarks

Clinical implications

Our observations may have important clinical implications. Autonomic dysfunction may be associated with orthostatic intolerance in those with AF, particularly in the elderly, which could provide a mechanism for the increased risk of falls and syncope in this cohort of patients with AF (Chapter 2). In addition, autonomic dysfunction caused by AF could compound the neuro-hormonal dysregulation seen in heart failure, a common co-existing morbidity in AF individuals.

The progression of intermittent AF through to a more permanent form (AF begets AF) occurs through atrial remodelling; a process which relies on a number of both anatomical (structural) and physiological (functional) components; of which autonomic dysfunction may be one. Taken together, the presence of these clinical conditions in individuals with AF may warrant more aggressive rhythm control strategy to improve outcomes. Further work is needed to evaluate the role that AF itself plays, whether burden is important in these reflex deficits and the potential reversibility of these reflex deficits. In subsequent chapters we will evaluate these reflexes during AF (Chapter 4) and further explore the effect of restoring rhythm using cardioversion (Chapter 5), rhythm control interventions using catheter ablation in Chapter 6 and finally, we shall assess the effect of low-level tragus stimulation (a novel neuromodulation technique[38, 277] that in some individuals can reduce the burden of AF (Chapter 7).

Conclusions

The abnormal vasoconstrictor response to LBNP seen in PAF patients provides novel clinical evidence of autonomic dysfunction that persists during sinus rhythm. Therefore, not only does autonomic disturbance predisposes to AF, it may also be a consequence of AF, leading to a feedback loop that could partially contribute to the well-known dictum of 'AF begets AF'.

Chapter 4: Abnormal cardiac volume-sensitive reflex in the presence of Atrial Fibrillation: implications on atrial remodelling

Background

Atrial fibrillation (AF) is progressive[8] with experimental evidence revealing that the arrhythmia itself propagates its maintenance.[24] The role of the autonomic nervous system (ANS) in the initiation of AF is well described.[23, 28] A bidirectional relationship likely exists between the ANS and AF[31] with a number of studies demonstrating changes in atrial autonomic innervation due to AF.[211, 212, 220, 221, 225] However, extension of the classical experimental work by Wijffels et al.[24] addressing the potential reasons underlying AF progression has not shown efferent autonomic tone as a cause.[25] Chronic (intermittent) low-level vagal (tragus) nerve stimulation (LLTS) reduces AF burden in some individuals[38], persisting beyond the stimulus, indicating that ANS remodelling may yet play a role in AF maintenance. Thus, whilst there is electrophysiologic vulnerability to AF produced from changes in the ANS,[225] a link between autonomic dysfunction and atrial remodelling contributory to AF is unclear.

Although significant effort has been placed in the role of the efferent limb of the cardiac ANS in producing electrical AF substrate,[28] much less is known about the cardiac afferent (reflex or regulatory) limb of the ANS. Veno-atrial junctions in the heart (including pulmonary vein-atrial tissue) contain stretch-driven, low-pressure (cardiopulmonary) baroreceptors that respond to changes in cardiac volume; modulating central sympathetic activity to maintain cardiovascular homeostasis.[57, 175] Abnormalities of the cardiopulmonary reflex have been identified in heart failure[55], hypertensive left ventricular hypertrophy[54] and from alcohol.[189]

Here, we hypothesized that the presence of AF results in dysfunction of cardiovascular autonomic reflexes. To evaluate this, we undertook a thorough evaluation of clinical autonomic reflex tests in patients with AF either *during* AF or *during* SR and compared their responses to healthy participants.

Methods

Study population

The study comprised consecutively enrolled patients referred to the Centre for Heart Rhythm Disorders with paroxysmal or persistent AF. The following were exclusions: amiodarone (preceding 6 months); active malignancy; symptomatic coronary artery disease; significant valvular disease; neurological disorders (Parkinson's disease, autonomic disorders, neuropathy); prior ablations; other arrhythmias; inability to enter a lower body chamber (frail or >120kg); or inability to withhold anti-arrhythmic/anti-hypertensives.

We studied AF patients in 2 groups: during sinus rhythm, SR (in-SR) or during AF (in-AF) according to their rhythm at the time of testing. For a reference group, we also enrolled age and sex-matched control participants (1:2), who were either healthy volunteers recruited by advertisement, or referred for evaluation of atypical chest pain or general cardiac check-up, and without symptoms of syncope, echocardiographic structural heart disease, heart rhythm or conduction disorders. In cases of chest pain, negative functional testing was required. We stratified clinical enrollment into 5-year age and gender groups and enrolled one control case per two in each patient group to achieve age and gender matching. Risk factors for cardiac disease; hypertension, obesity and dyslipidemia were permissible. Exclusions also applied to the control group.

All participants provided written informed consent and the study was approved by the University of Adelaide human research ethics committee. This study was prospectively registered with the Australian New Zealand Clinical trials registry (ACTRN12619000186156).

Patient preparation

All patients withheld alcohol (24 hours) and caffeine (48 hours), refraining from vigorous exercise (48-hours) prior to the study. All anti-arrhythmic (rate and rhythm controlling) and anti-hypertensive medications were withheld for 5 half-lives. Patients fasted for 4 hours allowing water *ad libitum*.

Autonomic testing was performed in a climate-controlled facility (22°C). We collected baseline demographics; risk factors; cardiac chamber measurements (echocardiography); and baseline serum for High-sensitivity CRP (Hs-CRP).

Autonomic reflex testing protocol

A standardized clinical autonomic testing protocol was performed in the same order. After a patient training period (reflex maneuvers explained and proficiency tested) and a 5-minute rest (semi-recumbent), we performed 3 well-established clinical autonomic tests:

1. Isometric Handgrip (*somato-sympathethic)* reflex (IHR);
2. Valsalva (*baroreceptor)* reflex;
3. Lower Body Negative Pressure, LBNP (*cardiac afferent, volume-sensitive, low-pressure baroreceptor)* reflex elicited by decreased cardiac volume.

The first 2 were performed semi-recumbent and LBNP, supine. We obtained beat-beat recordings of Heart rate (HR), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and Mean Arterial Pressure (MAP) using finger photoplethysmography (Human NIBP Nano, ADInstruments, Sydney, Australia) including a 5-minute baseline.

Submaximal isometric handgrip reflex (IHR)

IHR is a pressor reflex elicited by motor stretch of skeletal muscle (somato-sympathetic). Efferent sympathetic tone is increased; together with increases in heart rate (HR) and BP. [111] Muscle mechanosensitive and chemosensitive afferent nerves are activated; which transmit through the dorsal root to the rostral ventrolateral medulla (RVLM) where they activate efferent sympathetic neurons innervating the heart and blood vessels (vasomotor neurons) as well as adrenoceptors, achieving a pressor response which can be measured using beat-to-beat finger photoplethysmography.[111, 117] Although the reflex pathways are between peripheral somatic nerve afferents and central efferent sympathetic outflow; it can be modulated by baroreceptors (afferent pressure sensitive nerves found either in the carotid body and aortic arch – arterial high pressure baroreceptors[278] or in cardiac veno-atrial junctions, including pulmonary vein-atrial tissue – volume-sensitive, low pressure baroreceptors).[97] Figure 4A.

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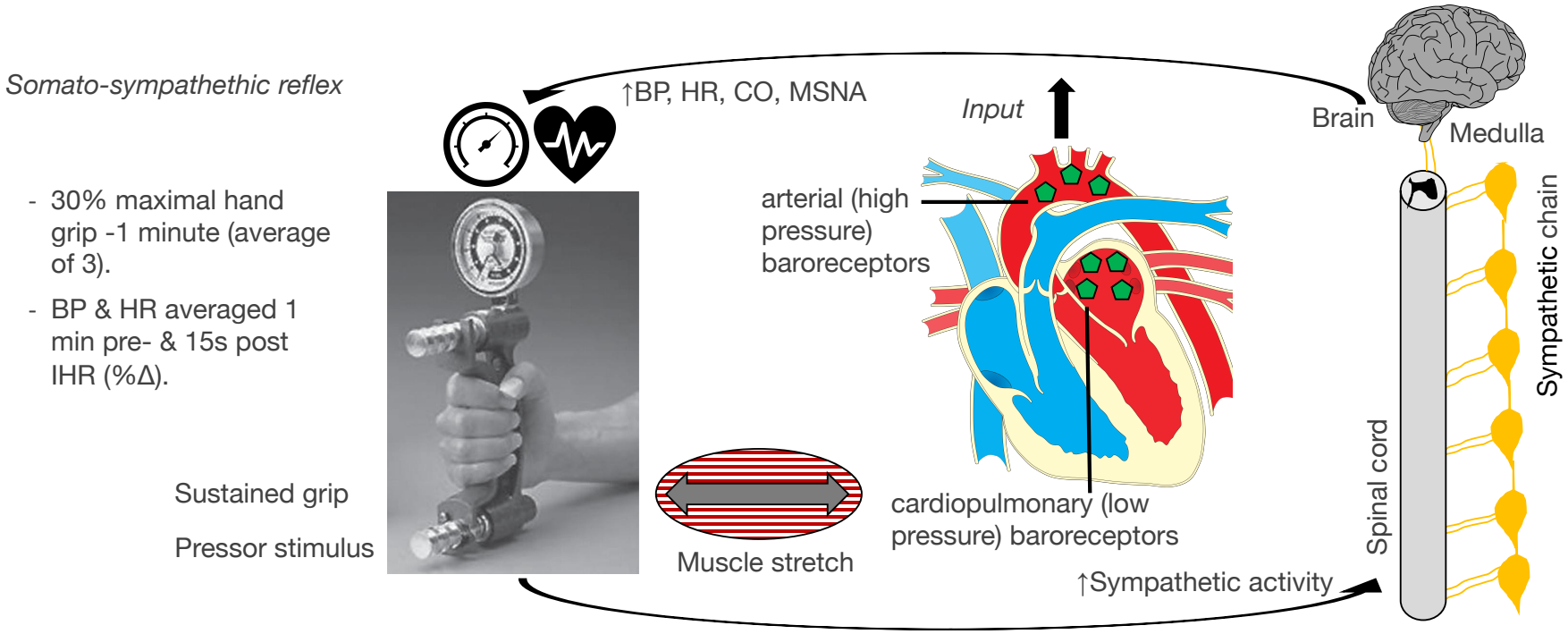
Figure 4A: Submaximal isometric handgrip reflex



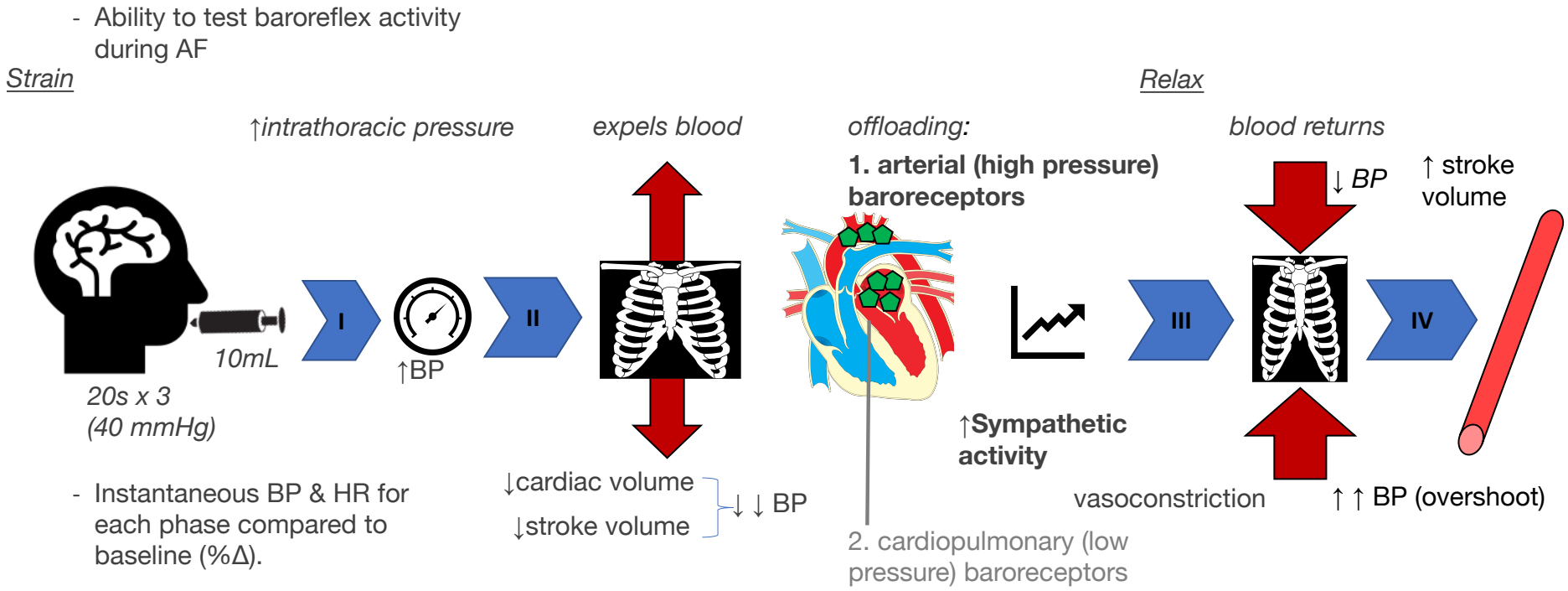
IHR or somato-sympathetic reflex is initiated by handgrip (30% maximal handgrip strength for 1 minute) stimulates central efferent sympathetic activity which increases cardiac output (CO); Blood Pressure (BP), Heart rate (HR) and Muscle Sympathetic Nerve Activity (MSNA). This reflex is modulated by both arterial (high-pressure) and cardiopulmonary (low-pressure) baroreceptors.

We determined the maximal handgrip with 3 attempts: using a Jamar smart hand dynamometer (Patterson Medical Ltd, Illinois, USA), calculated 30% and asked the patient to maintain this submaximal grip (with feedback) for 1 minute. After ensuring familiarization we performed 3 IHR, 3-minutes apart, ensuring adherence. We averaged each recording for 1-minute pre-IHR and for the final 15s of IHR, expressed this as percentage change and averaged the triplicates.

Valsalva manoeuvre

The Valsalva reflex tests predominantly high-pressure baroreceptor function. There are four phases; two mechanical and the other two involve autonomic reflexes, contributing to homeostasis. Briefly, the reflex is initiated by intrathoracic pressure increase, expelling blood from the thorax (transiently increasing BP). Continued strain then results in decreased cardiac volume due to the impediment of blood returning to the thorax; in turn, leading to decreased stroke volume and arterial pressure. The first autonomic phase increases efferent sympathetic outflow due to both low BP (majority of stimulus) and volume from high- and low-pressure baroreceptors (specifically, baroreceptors are offloaded; decreasing sympathetic restraint). This results in measurable increases in HR and BP (peripheral vasoconstriction). During relaxation, the mechanical impediment to cardiac filling is removed and a BP overshoot occurs owing to a vasoconstricted state. The final autonomic phase involves decreases in sympathetic activity to baseline. Beat-to-beat data has significantly aided our understanding of this reflex.[90] Figure 4B.

Figure 4B: Valsalva manoeuvre



There are four phases in the Valsalva manoeuvre: two are mechanical and the other two are reactive (autonomic phases). This reflex has two parts. A strain part and relaxation part. BP, Blood Pressure and HR, Heart Rate. Arterial baroreceptor function can be grossly evaluated even in the presence of AF.

We performed the Valsalva maneuvers three times using a 10mL syringe, into which the patient forcefully expired for 20s, forming a buccal seal at the hub, to produce enough pressure to thrust the plunger from its barrel. This generates approximately 40 mmHg pressure.[279, 280] According to prior reports, we determined instantaneous HR, SBP, DBP and MAP for each phase defined by the following parameters: Maximal MAP after onset of straining (Phase I); Lowest MAP during strain (Phase II_E, end of first mechanical phase); Next MAP peak, prior to cessation of strain (Phase II_L, first autonomic phase); Lowest dip in MAP following cessation of strain (Phase III, last mechanical phase) and the maximal MAP overshoot, within 20s of the maneuver (Phase IV). Each instantaneous recording was compared to the preceding baseline value and expressed as a percentage difference. Finally, we averaged the 3 Valsalva responses.

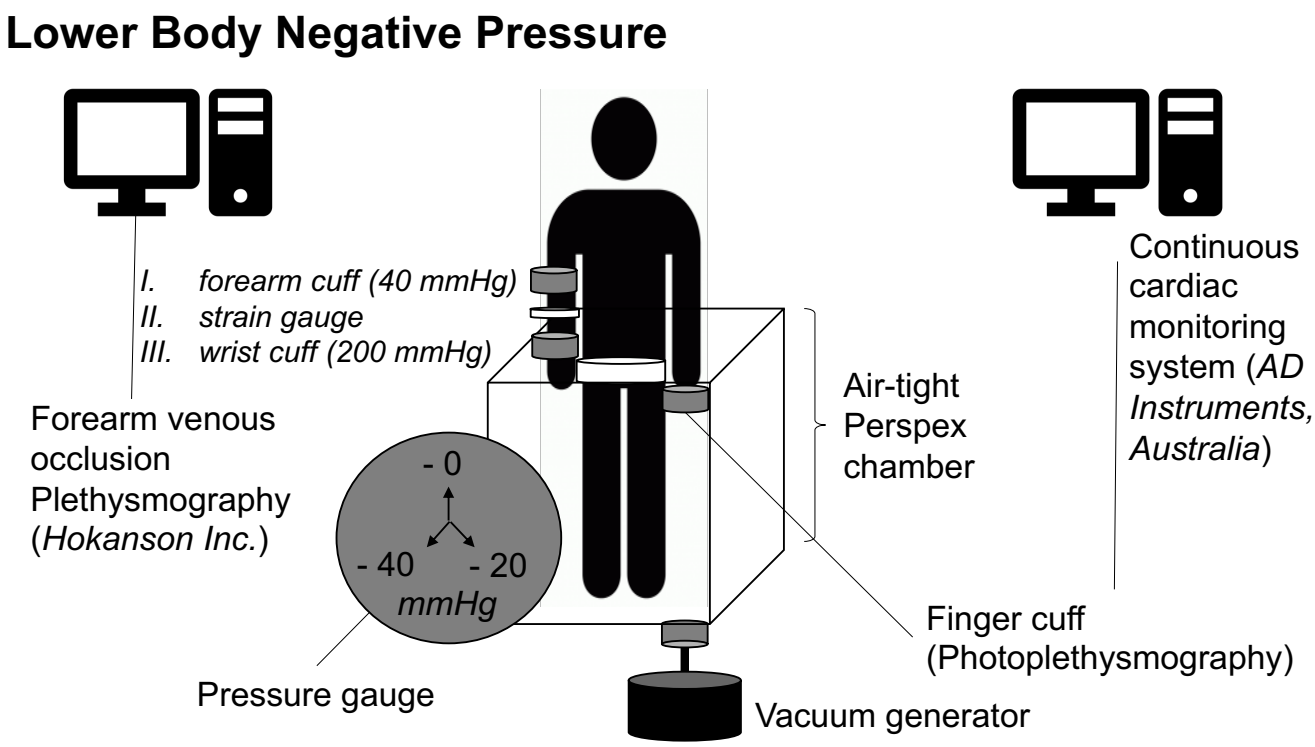
Lower Body Negative Pressure (LBNP)

LBNP is a unique method for testing autonomic reflexes in response to central hypovolaemia; first developed to determine the cardiovascular effects of weightlessness in astronauts.[96, 281] The first known usage of LBNP was in 1834 by Junod; who used negative pressure to create a dry operating field for surgery.[282] A by-product of this was that negative pressure caused syncope – useful to provide "surgical anaesthesia". Over a period of 7 decades, there have been an extensive number of studies establishing its physiological effects in healthy controls,[96] as well as in number of cardiovascular disease states; such as heart failure[55], hypertension[54, 283], shock[272] and cardiac transplantation.[56, 57] Thus, LBNP is a well-established method for testing autonomic reflexes in response to central hypovolemia. [54-57, 96, 272, 283] LBNP induces a fluid shift from the upper to lower body compartment, by applying negative pressure, resulting in vasoconstriction to maintain BP in healthy adults. LBNP does not elicit other reflexes from postural change (the majority of the effect is due to the change

in the position of the carotid sinus relative to the heart, however the initiation of otolithic or weight loading reflexes can also occur due to alterations in posture). Moreover, depending on the level of negative pressure, one can test the differing effects of low-pressure vs high pressure baroreceptors. At low levels, despite decreased cardiac volume, minimal changes in arterial pressure occur, whereas at high levels (simulating hemorrhage); BP decreases, offloading high pressure baroreceptors (also sensitive to posture).[96] Therefore, low-level LBNP mostly tests volume-regulating cardiopulmonary reflexes.

The LBNP apparatus is previously described[175] and detailed in Figure 4C (schematic) and Figure 4D (photograph). During LBNP we collected continuous hemodynamic data and Forearm Blood Flow (FBF) using venous occlusion plethysmography. This is a very well-validated technique to measure the vasoconstriction response to LBNP.[54, 55, 189, 284] FBF (*inversely* proportional to vascular resistance) was measured *thrice* during the final minute of each LBNP level and averaged. FBF was coded to allow blinded interpretation of the slopes, minimizing measurement bias. We also expressed vasomotor tone in terms of forearm vascular conductance (FVC =100 x FBF/MAP).[57] Hemodynamics for the final 2 minutes of each LBNP level was averaged, compared to 0 mmHg, and expressed as percentage difference. Figure 4E depicts the reflex response to LBNP as well as the technique used and interpretation of venous occlusion plethysmography.

Figure 4C: Lower Body Negative Pressure (LBNP) apparatus (schematic)



We performed LBNP using a custom- made chamber placed over the lower limbs with a neoprene seal at the iliac crest. A vacuum generator was attached to the chamber and connected to a voltage converter to control intrachamber pressure, to 0; -20; and -40 mmHg. Patients underwent a short 2-min familiarization period with LBNP application followed by a resting period of 10 min prior to LBNP commencement. LBNP intensity applied in a randomized order for 5 minutes at each level (0; -20 and -40 mmHg) with a 5-minute break in between. Negative pressure to the lower limbs was applied slowly over 30s to minimize patient movement and discomfort. During LBNP we collected continuous hemodynamic data (using finger photoplethysmography) as well as Forearm Blood Flow (FBF) using venous occlusion plethysmography

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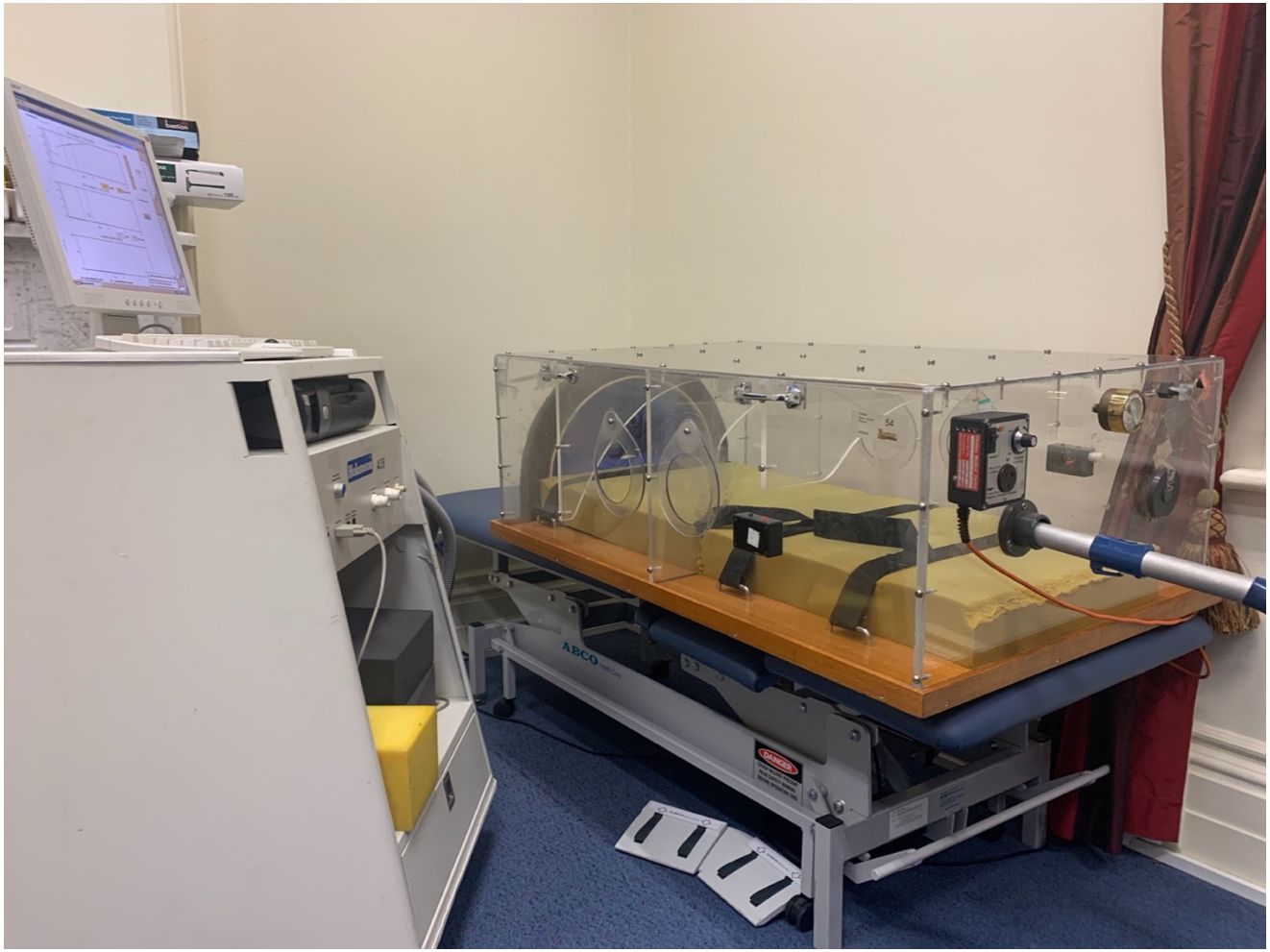
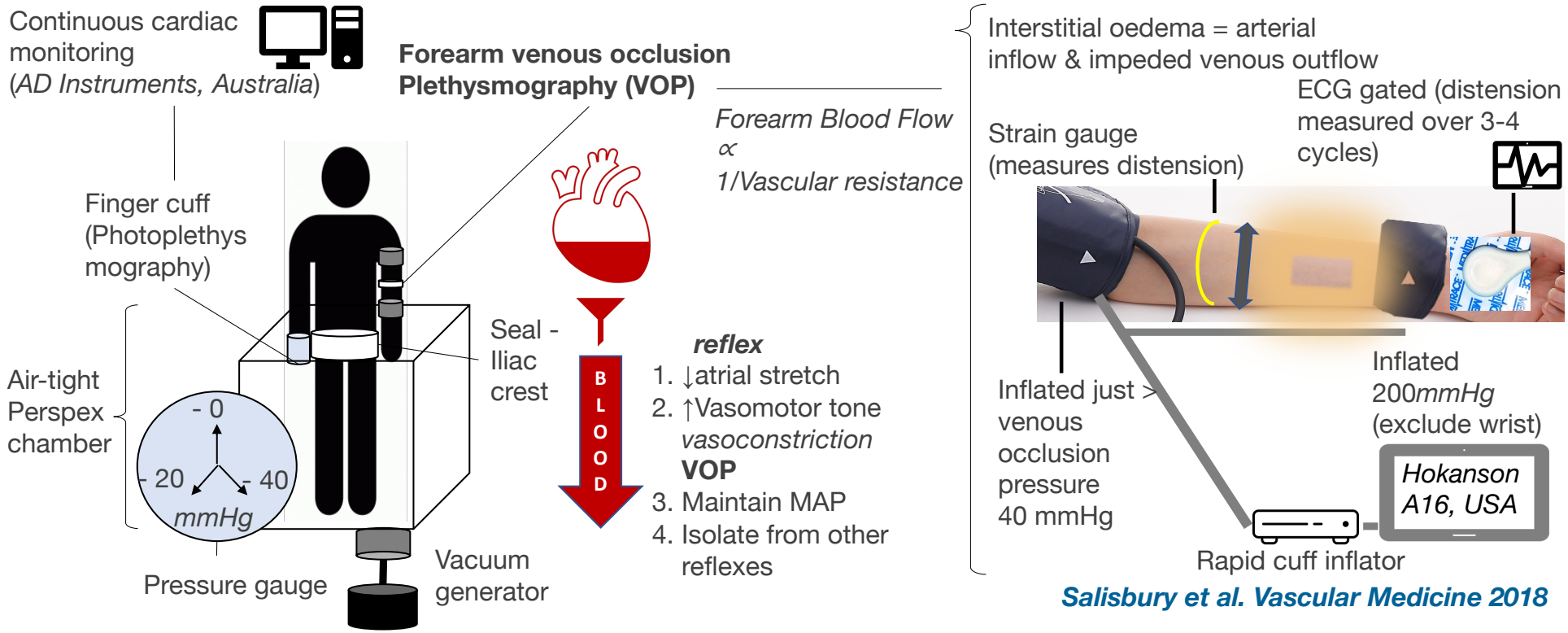
Figure 4D: Lower Body Negative Pressure (LBNP) apparatus (photograph)



A photograph depicting the LBNP apparatus (right) and the venous occlusion plethysmograph (left and to the fore of the photograph)

Figure 4E: LBNP reflex & venous occlusion plethysmography technique



Decreased cardiac volume decreases atrial stretch. A vasomotor (vasoconstriction) reflex to maintain MAP, Mean Arterial Pressure, is elicited. Vasomotor tone can be assessed using venous occlusion plethysmography (A16, Hokanson, Washington, USA); which measures FBF, Forearm Blood Flow, the arterial inflow of blood. FBF∝1/vascular resistance. ECG, electrocardiogram.

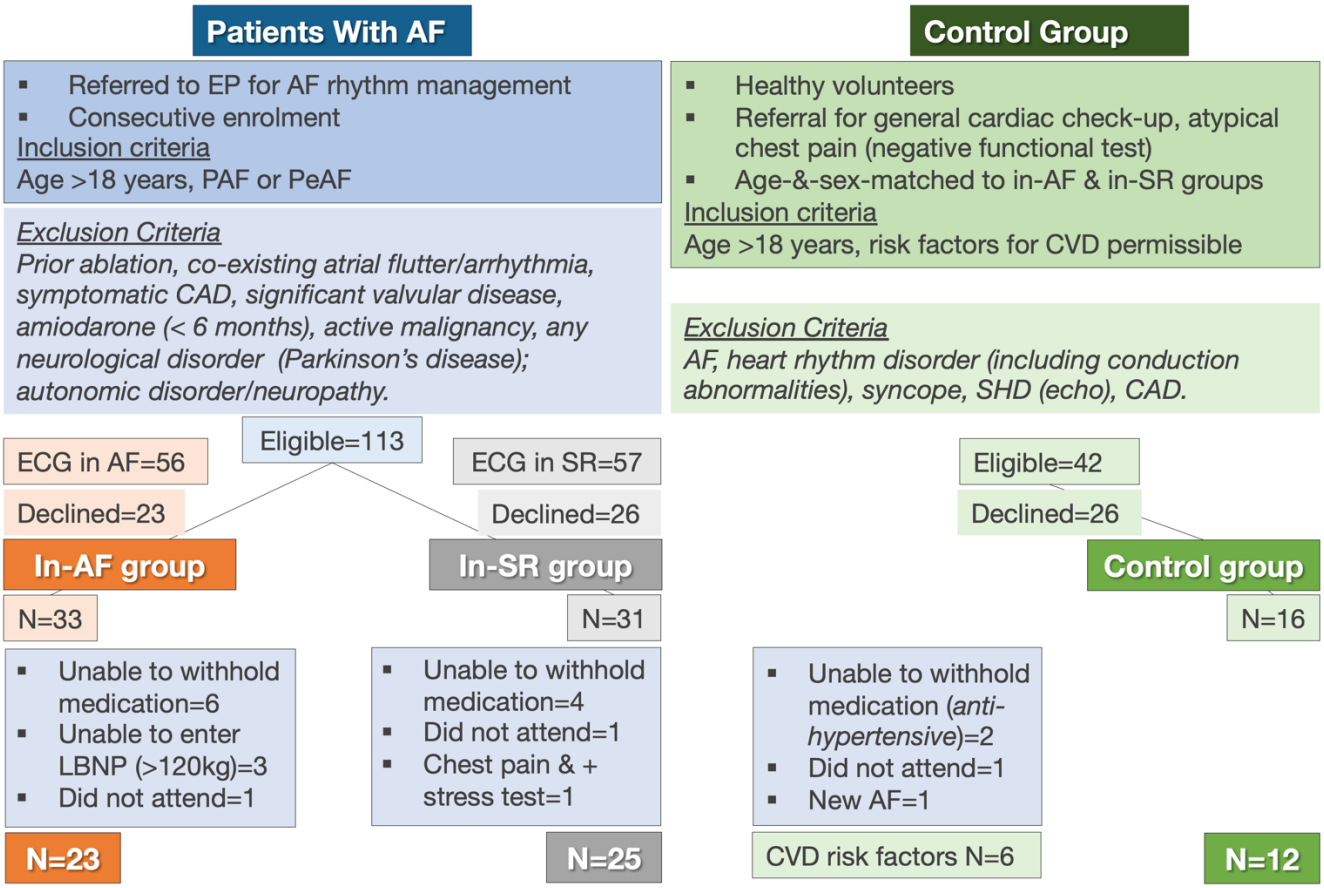
Statistical analysis

Continuous parameters were expressed as mean ± SEM. Categorical variables were expressed as frequencies and percentages and compared using Fisher's exact test. Normality was checked using the Shapiro-Wilk test. Within group (pre and post values) for each autonomic test were compared using paired t-tests (normally distributed data) or Wilcoxon signed rank test (non-normally distributed data) as appropriate. Differences between the groups were compared using Kruskal–Wallis test with multiple comparison using Dunn's test. To identify whether other confounding factors were responsible for the differences that we identified between groups, we used multiple linear regression adjusting for age, Body Mass Index (BMI), Left Ventricular Ejection Fraction (LVEF), Left Atrial Volume Indexed to body surface area (LAVI) and hemodynamic baseline. Statistical analysis was performed using STATA 16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC) and GraphPad Prism (version 9.02, California, USA). Statistical significance was set at $P < 0.05$.

Results

Forty-eight patients with AF were enrolled: n=23 in the in-AF group and n=25 in the in-SR group together with 12 matched controls. Details of the screening and recruitment for the study are presented in the **CONSORT diagram:** Figure 4F. The 3 groups were well matched for age (61±2, 59±3, 55±4, respectively; P=0.5) and gender (83% male, P=0.9). Hypertension, the most common risk factor across the groups was also matched (57%, 56% and 50%, respectively; P=0.1), as were dyslipidemia and vascular disease. Resting MAP and echocardiographic interventricular septal thickness were also similar (P=0.7). There were very low levels of Hs-CRP in all groups. The in-AF group had higher left atrial volume and resting HR than both in-SR and control groups. Baseline characteristics are presented in Table 4A. None of the parameters in the multiple regression analysis (age, BMI, LVEF or LAVI) were statistically significant.

Figure 4F: CONSORT diagram



CONSORT diagram – study enrolment. 3 groups enrolled. 2 patient groups (according to rhythm) and an age, sex, BMI & risk factor-matched control group.

Table 4A: Baseline characteristics

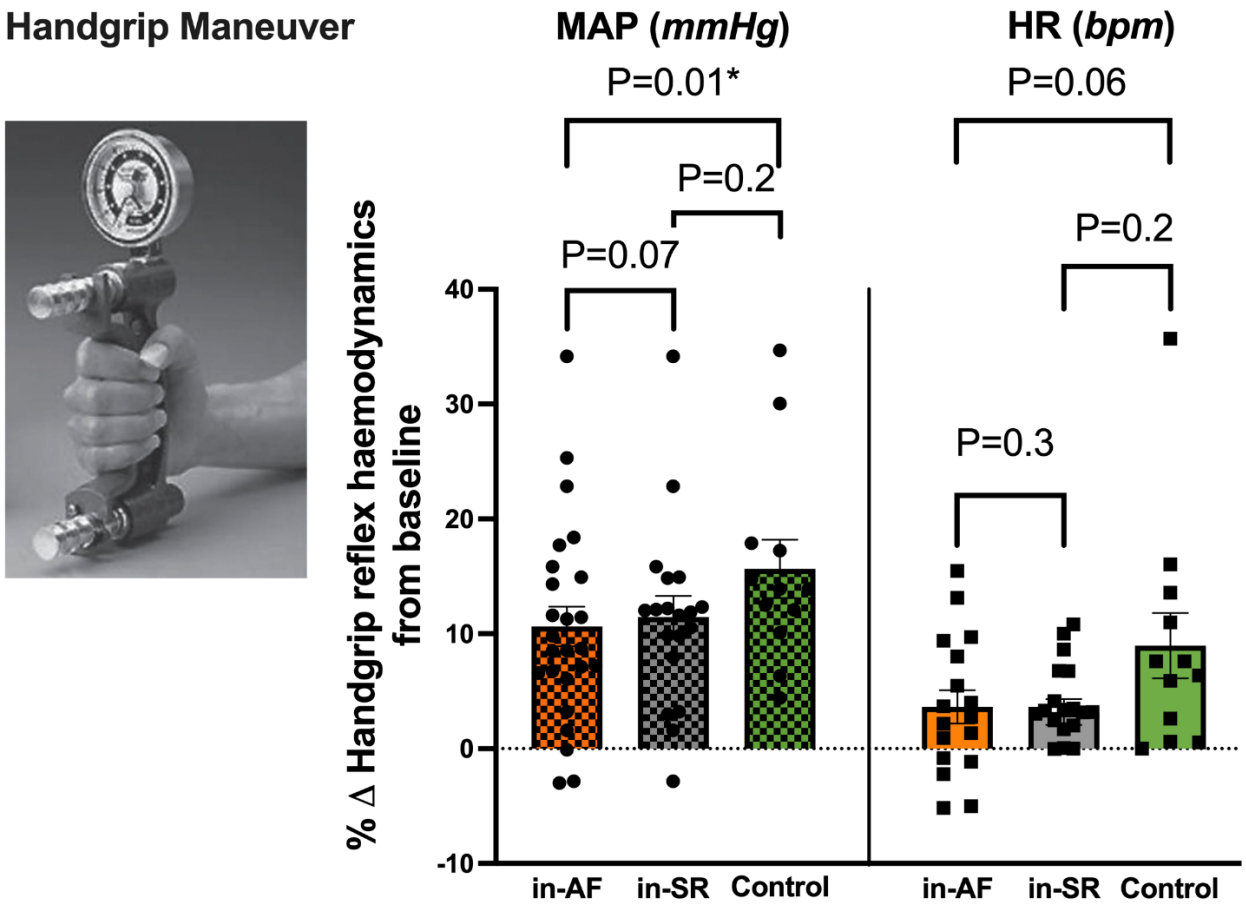
Baseline Characteristics	In-AF n=23	In-SR n=25	P Value	Controls n=12	P Value *vs in-AF*	P Value *vs in-SR*
Demographics & resting values						
Age	61±2	59±3	0.6	55±4	0.4	0.5
Males; n(%)	22(96)	20(80)	0.2	8(75)	0.1	0.99
BMI (kg/m^2)	29±1	27±1	0.3	27±1	0.4	0.99
Resting MAP	95±5	90±3	0.99	94±2	0.99	0.99
Resting HR	88±3	60±2	<0.001*	68±3	<0.001*	0.001*
Serum Hs-CRP (mg/L)	2.92±0.81	1.45±0.28	0.2	1.53±0.41	0.7	0.99
Echocardiographic parameters						
LVEF (%)	57±2	64±1	0.006*	66±1	0.005*	0.99
LAVI (ml/m^2)	40±2	33±2	0.01*	29±1	0.0005*	0.4
IVSd (cm)	1.05±0.03	0.96±0.03	0.2	0.97±0.04	0.99	0.4
Risk factors						
Hypertension, n (%)	13(57)	14(56)	0.99	6(50)	0.5	0.2
Dyslipidaemia	5(22)	5(12)	0.99	1(8)	0.6	0.6
Vascular disease	2(8)	1(4)	0.99	1(8)	0.99	0.99
OSA	2(9)	3(12)	0.99	0(0)	0.5	0.5
Alcohol excess	6(26)	6(24)	0.99	0(0)	0.07	0.1
Medications						
ß-blockers (except Sotalol), n(%)	6(35)	7(28)	0.7	0(0)	-	
Sotalol, n(%)	7(41)	5(20)	0.17	0(0)	-	
Flecainide, n(%)	1(6)	6(24)	0.2	0(0)	-	
Central CCB, n(%)	2(12)	2(8)	0.99	0(0)	-	
Digoxin, n(%)	0(0)	0(0)	-	0(0)	-	
ARB/ACEi	12(52)	9(36)	0.6	4(33)	0.5	0.99
Dihydropyridine CCB, n(%)	0(0)	0(0)	0.99	2(17)	0.1	0.09
Thiazide diuretic	1(4)	2(8)	0.99	0(0)	0.99	0.99

BMI, Body Mass Index; MAP, Mean arterial pressure; HR, Heart rate; LVEF, Left ventricular ejection fraction; LAVI, Left atrial volume indexed to body surface area; IVSd, Interventricular septal diameter during diastole; OSA, Obstructive sleep apnoea; CCB, Calcium channel blocker; ARB, Angiotensin receptor blocker; ACEi, Angiotensin-converting enzyme inhibitor; Hs-CRP, High-sensitivity C-reactive Protein. *P<0.05

Submaximal isometric handgrip reflex

IHR resulted in increases in MAP, SBP, DBP and HR in all groups (P<0.01). The in-AF group had an attenuated MAP response (+10.6±2%) in comparison to controls (+15.7±3%); P=0.01. The in-SR group response (+11.5±2%); was not different to controls (P=0.2). Although the increase in HR was numerically smaller in both in-AF (+3.6±1%) and in-SR (+3.2±1%) than in controls (+9±3%); this did not reach statistical significance (P=0.06 & P=0.2, respectively). Figure 4G. There were no differences in DBP between groups (P=0.6). Raw data are presented in Table 4B.

Figure 4G: Hemodynamic responses to Isometric Handgrip Reflex (IHR).

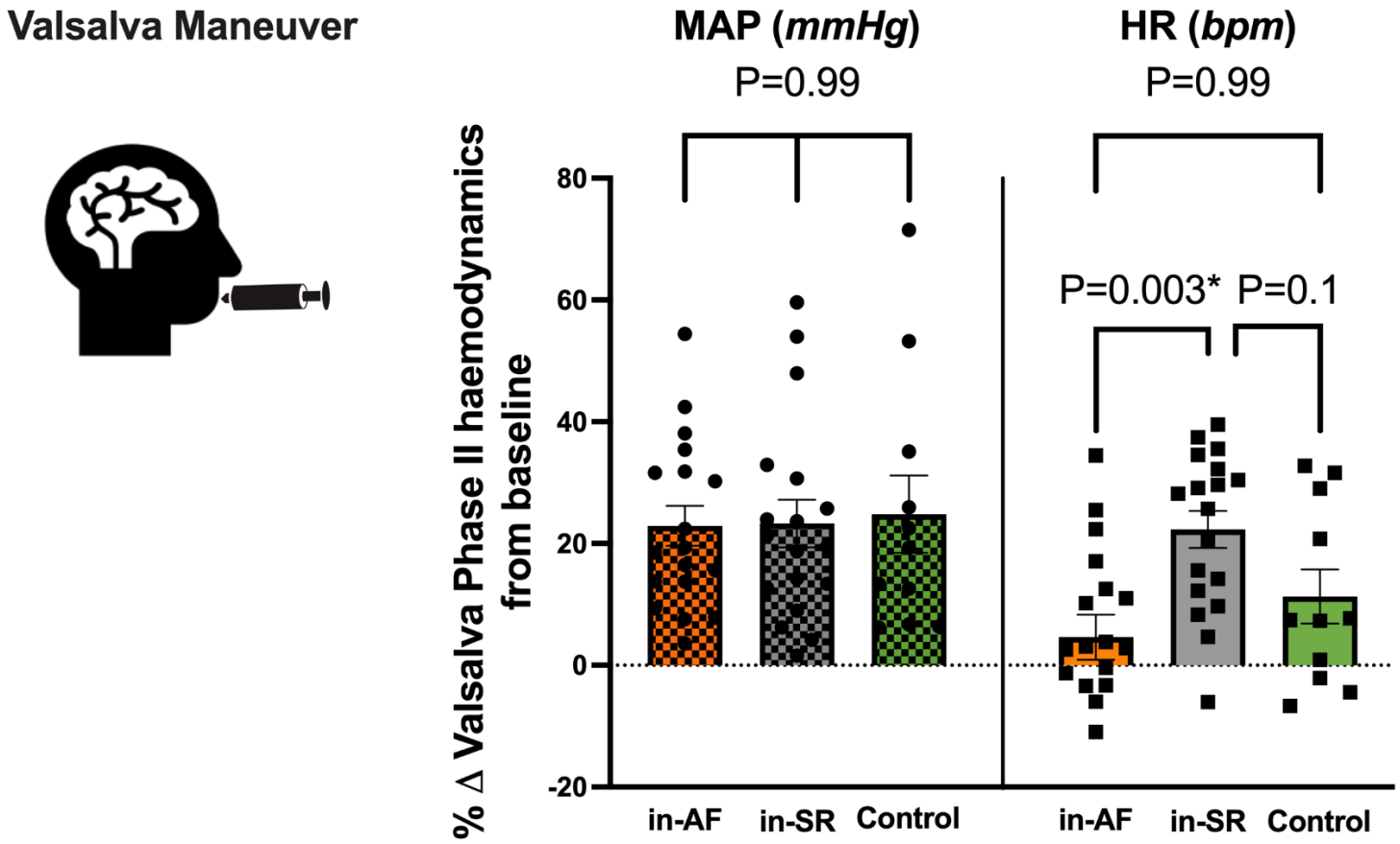


MAP (left) & HR (right) responses in the in-AF, in-SR, and control groups. MAP and HR increased in all groups; however, the in-AF group had an attenuated MAP response. *P<0.05.

Valsalva reflex

Valsalva responses were generally preserved in both in-AF and in-SR groups; with no differences in autonomic phases (II and IV) between groups. During phase II, there was a +25%±6 increase in MAP in controls, +23±4% in-SR and +23±3% in-AF groups (P=0.99). HR responses were attenuated in-AF (+4.6±4%; P=0.003) in comparison to in-SR (+22.3±3%) but not statistically significant compared to controls (+11.3±4%); P=0.99. Figure 4H. During phase IV, there was an overshoot of both MAP and HR in all groups with no difference between groups. Table 4B**.**

Figure 4H: Hemodynamic responses to the Valsalva maneuver



Phase II sympathetic response. MAP was maintained in the control group (normal). In the two study groups, in-SR, it was preserved, however in the in-AF group, MAP was not maintained. Results expressed as percentage difference (mean ± SEM). *P<0.05.

Table 4B: Hemodynamic responses to IHR & Valsalva maneuvers

	During AF		During SR		P Value	Controls		P Value	
IHR	Pre-HG	HG	Pre-HG	HG		Pre-HG	HG	*vs AF*	*vs SR*
MAP	91±5	99±5	87±4	96±4	0.07	93±2	108±4	0.01*	0.2
SBP	124±6	134±5	128±7	143±7	0.02*	135±3	153±6	0.2	0.2
DBP	77±4	83±4	67±3	75±3	0.2	75±3	85±3	0.2	0.4
HR	83±3	87±4	61±2	63±3	0.3	68±3	74±3	0.06	0.2
Valsalva II	II_{EARLY}	II_{LATE}	II_{EARLY}	II_{LATE}		II_{EARLY}	II_{LATE}		
MAP	97±5	118±7	90±5	111±8	0.99	99±5	122±9	0.99	0.99
SBP	124±6	145±8	116±7	136±10	0.99	133±11	151±10	0.99	0.99
DBP	91±5	103±5	83±4	98±7	0.99	92±6	104±9	0.99	0.99
HR	78±4	82±4	76±3	89±4	0.003*	85±5	92±7	0.99	0.1
Valsalva IV	$IV_{BASELINE}$	$IV_{OVERSHOOT}$	$IV_{BASELINE}$	$IV_{OVERSHOOT}$		$IV_{BASELINE}$	$IV_{OVERSHOOT}$		
MAP	95±5	116±6	89±3	119±7	0.99	94±3	114±5	0.99	0.3
SBP	126±6	159±7	128±5	178±10	0.99	135±5	168±10	0.8	0.2
DBP	80±4	89±5	70±3	85±5	0.6	75±2	87±4	0.99	0.3
HR	88±3	97±4	60±2	73±5	0.99	68±3	78±4	0.99	0.99

Data±SEM. Differences between the groups are represented by P values in the adjacent columns. MAP, Mean Arterial Pressure (mmHg); SBP, Systolic Blood Pressure (mmHg); DBP, Diastolic Blood Pressure (mmHg); HR, Heart rate (bpm). Between group comparisons using ANOVA (Kruskal–Wallis test with multiple comparison using Dunn's test). P<0.05*.

Lower Body Negative Pressure reflex

The hemodynamic response to LBNP differed significantly between all groups. In controls, MAP was maintained at all levels of LBNP. Control participants exhibited a notable (normal) vasoconstrictor response as demonstrated by a -48.7±5% decrease in FBF and -49.2±5% decrease in FVC. In contrast, the in-AF group demonstrated a decrease in MAP (-4.8%; P=0.008, in comparison to control group) with evidence of a dysfunctional vasoconstrictor response (+11.5±6% FBF/+20.7±8% FVC; P<0.001 and P<0.001). Figures 4I, 4J, 4K. In the in-SR group, MAP was maintained however the vasoconstriction response to LBNP was attenuated (-11.9±9% FBF; P=0.002 and -11.2±9% FVC; P=0.002) in comparison to controls. For the In-SR group, FBF/FVC response were also different to the in-AF group (P=0.005 and P=0.002, respectively). HR increased similarly both in-SR and controls (+15.7±3% and +15.6±3%; P=0.4). In contrast, HR in-AF decreased in response to LBNP (-2.1±2%) which was statistically different to both in-SR group and controls (both; P<0.001). Figure 4L and Table 4C.

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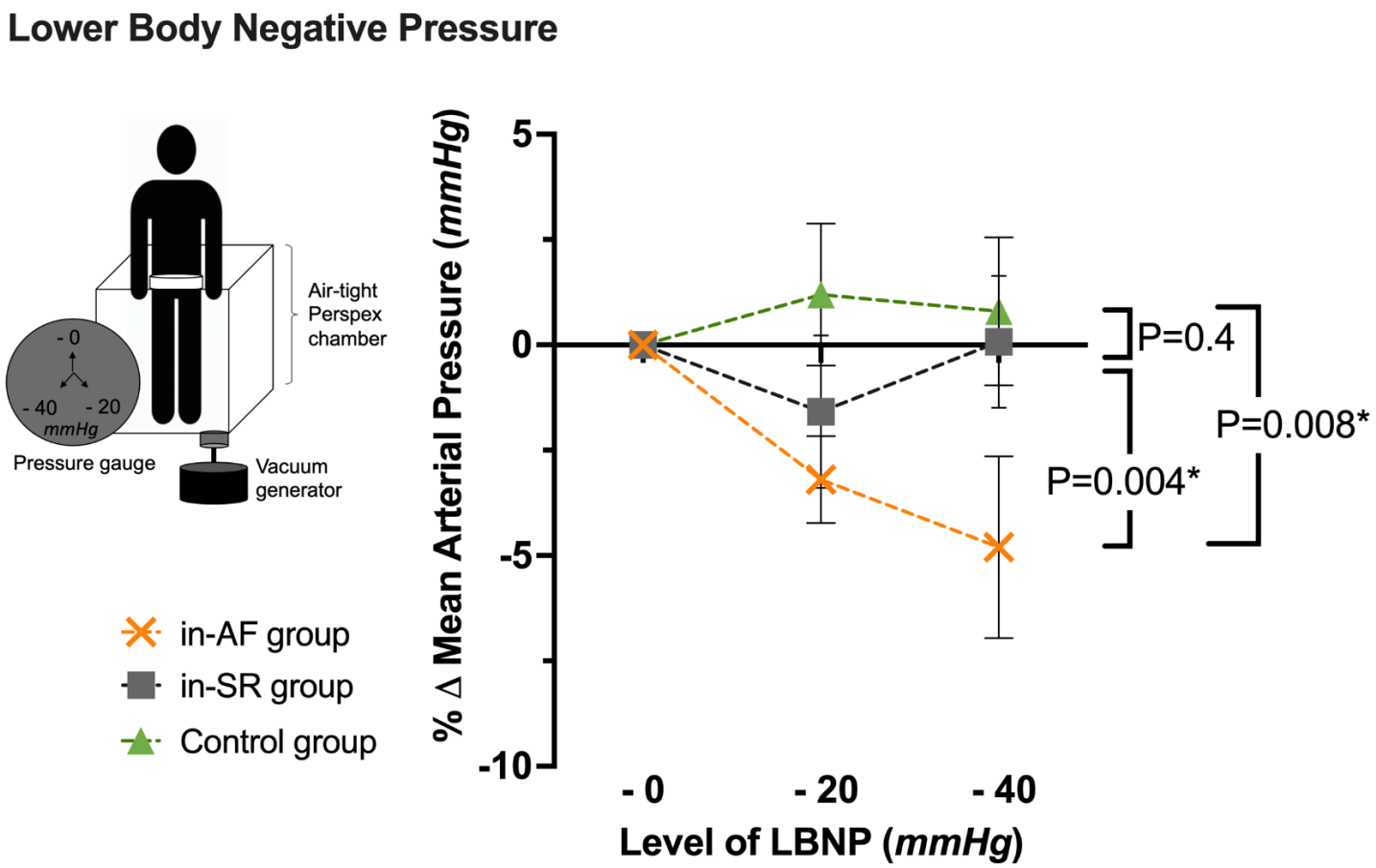
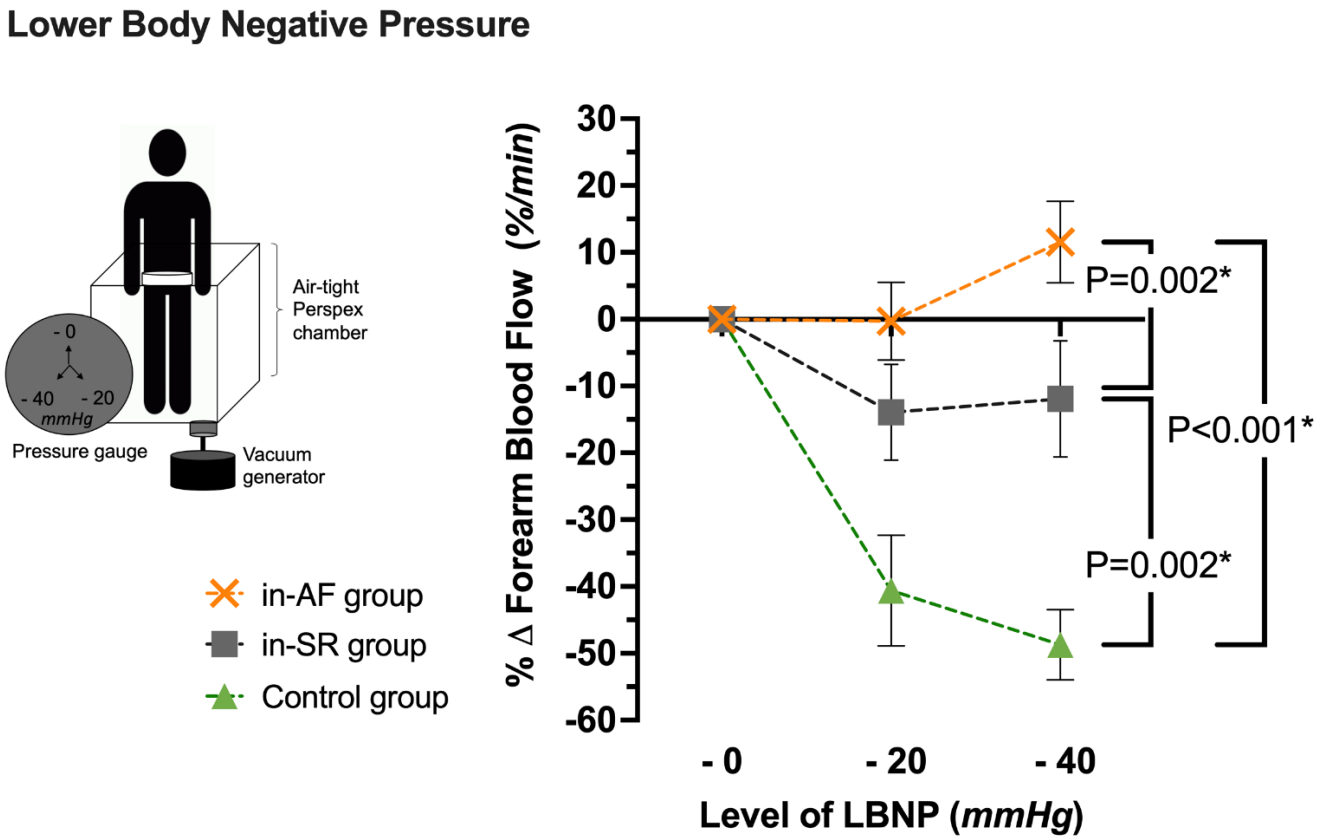
Figure 4I: Hemodynamic responses to low-level Lower Body Negative Pressure (LBNP): MAP responses in the in-AF, in-SR, and control groups.



MAP was maintained in the control group (normal). In the two study groups, in-SR, it was preserved, however in the in-AF group, MAP was not maintained. Results expressed as percentage difference (mean ± SEM). *P<0.05.

Figure 4J: Hemodynamic responses to low-level Lower Body Negative Pressure (LBNP): FBF responses in the in-AF, in-SR, and control groups.



A decrease in FBF (vasoconstriction) was seen as expected in controls; whereas in the 2 study groups responses were clearly different: in-SR, FBF response was *diminished*, and in-AF; it was *dysfunctional*. Results expressed as percentage difference (mean ± SEM). *P<0.05.

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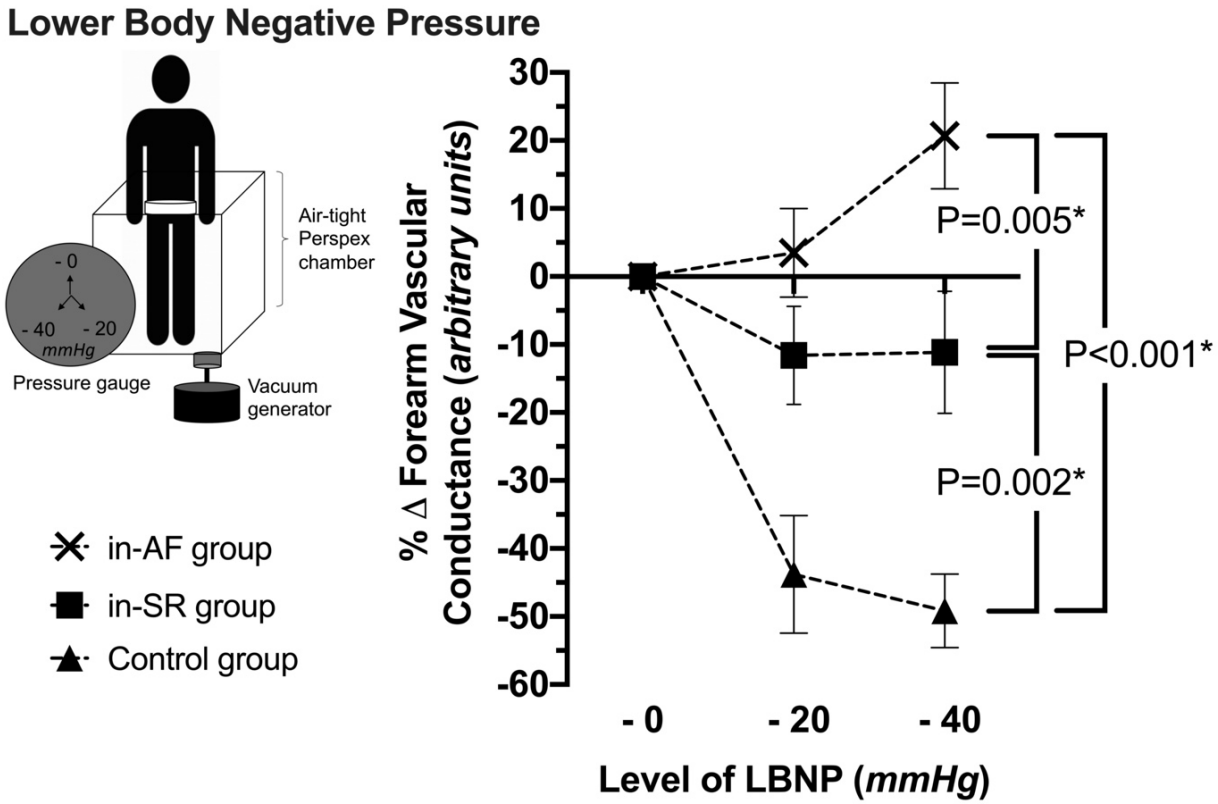
Figure 4K: FVC responses to LBNP between in-AF, in-SR & control groups



A decrease in Forearm Vascular Conductance; FVC (vasoconstriction) was seen as expected in controls; whereas in the 2 study groups responses were clearly different: in-SR, FBF response was *diminished*, and in-AF; it was *dysfunctional*. Results expressed as percentage difference (mean ± SEM). *P<0.05.

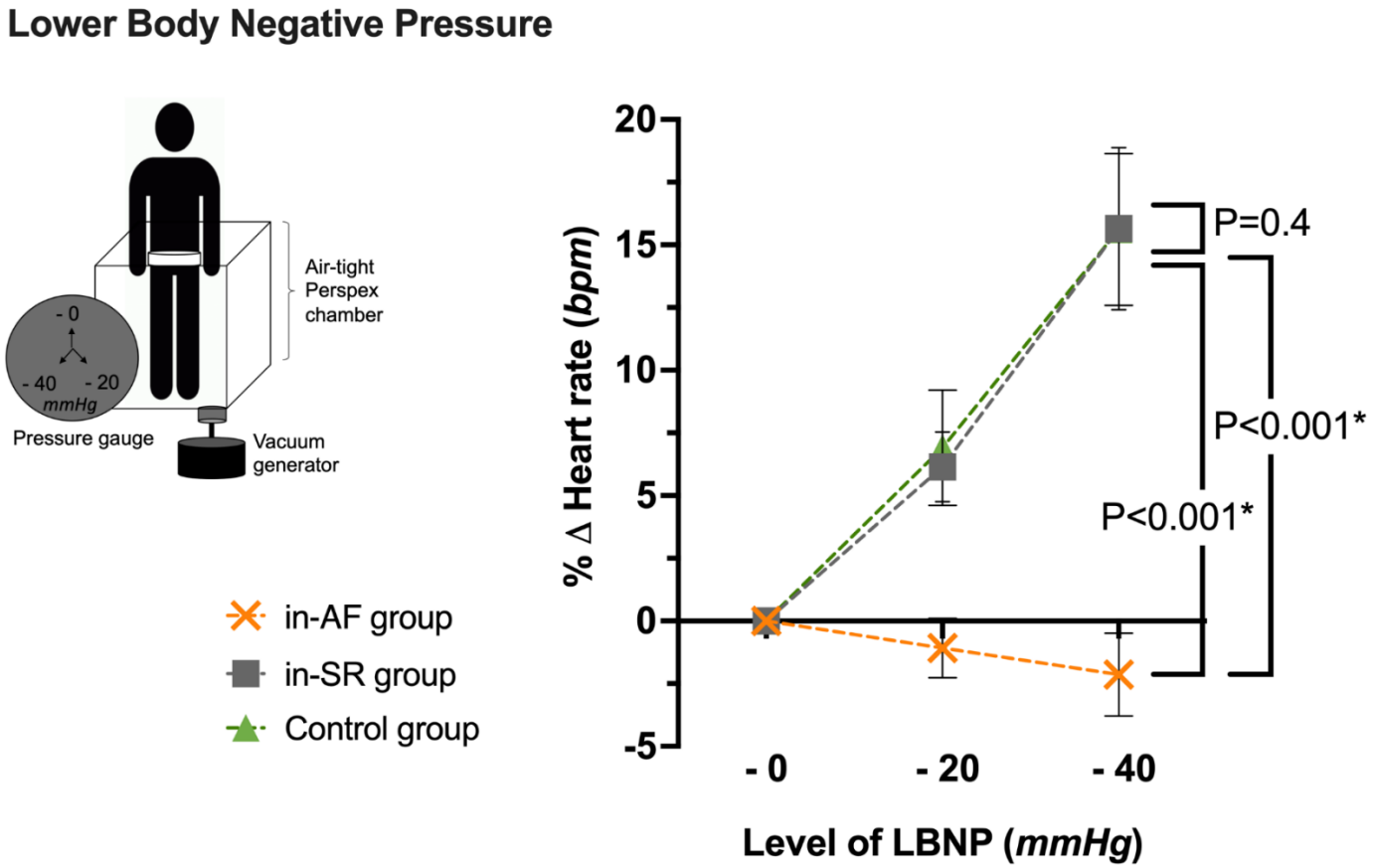
Table 4C: Hemodynamic responses to LBNP

	During AF			During SR			P Value	Controls			P Value	
LBNP Level	0	-20	-40	0	-20	-40		0	-20	-40	*vs AF*	*vs SR*
MAP	100±5	97±5	95±5	90±3	89±4	90±4	0.004*	91±2	92±2	91±2	0.008*	0.4
SBP	136±6	132±6	129±7	130±5	126±6	126±6	0.09	137±3	130±3	129±3	0.5	0.1
DBP	84±4	82±4	81±4	70±3	72±3	74±3	<0.001*	73±3	73±2	74±2	0.02*	0.2
HR	85±3	84±3	83±3	57±2	61±2	65±2	<0.001*	60±2	64±2	70±3	<0.001*	0.4
FBF	1.71±0.2	1.64±0.1	1.79±0.1	1.69±0.1	1.32±0.1	1.35±0.1	0.002*	1.39±0.2	0.88±0.2	0.73±0.1	<0.001*	0.002*
FVC	1.83±0.2	1.80±0.2	2.00±0.2	1.99±0.2	1.62±0.2	1.58±0.2	0.005*	1.36±0.1	0.77±0.1	0.72±0.1	<0.001*	0.002*

Data±SEM. Differences between the groups are represented by P values in the adjacent columns. MAP, Mean Arterial Pressure (mmHg); SBP, Systolic Blood Pressure (mmHg); DBP, Diastolic Blood Pressure (mmHg); HR, Heart rate (bpm); FBF, Forearm Blood Flow (%/min); FVC, Forearm vascular conductance (100*FBF/MAP, arbitrary units). Between group comparisons (at – 40 mmHg LBNP) using ANOVA (Kruskal–Wallis test with multiple comparison using Dunn's test). $P<0.05$.

Dr Varun Malik, PhD thesis, 2018 - 2021

Figure 4L: Hemodynamic responses to low-level Lower Body Negative Pressure (LBNP): HR responses in the in-AF, in-SR, and control groups.



HR increased in the control group (normal) and in-SR. However, in-AF, the HR response was attenuated. Results expressed as percentage difference (mean ± SEM). *P<0.05.

Discussion

This study, through a series of autonomic reflex tests in AF patients, evaluated whether the presence of AF results in afferent (regulatory) ANS dysfunction. The principal findings were:

1. The volume-sensitive reflex, elicited by LBNP effect on low-pressure (cardiopulmonary) baroreceptors is diminished in AF patients in-SR, compared with an age, sex and risk factor-matched control group.
2. LBNP reflex, tested during AF, is dysfunctional, whereby there is an absence of vasoconstriction (perhaps a paradoxical vasodilatation) and associated with this is an impaired maintenance of MAP.
3. Valsalva reflex, predominantly testing high-pressure (BP-sensitive) baroreceptors was preserved in all groups. IHR (which tests both baroreceptors) was present in all groups; albeit attenuated in-AF compared to controls.

Impaired cardiovascular autonomic regulation of blood volume in AF

Previous studies exploring AF and ANS have mostly examined efferent autonomic input to the heart.[23, 28, 31]. AF results in atrial sympathetic and parasympathetic hyperinnervation and heterogeneity increasing AF vulnerability. [211, 220, 221, 225] AF also increases central sympathetic activity under resting conditions.[82] Here, we explored the effect of AF on cardiovascular regulatory (afferent) reflexes. Each of the stimuli, testing the integrity of afferent input, have differing effects on the two main baroreceptor sub-types: volume-regulating low-pressure (cardiopulmonary) baroreceptors and blood pressure-regulating (arterial) high-pressure baroreceptors. We demonstrate dysfunctional volume-regulating baroreceptor reflexes due to AF.

Impairment of normal cardiovascular volume homeostasis using LBNP has been identified in structural cardiac diseases such as heart failure[55] and hypertension with left ventricular hypertrophy.[54] Alcohol, an important risk factor for AF, has a similar effect.[189] In a canine chronic heart failure model, structural change (reduced compliance) was associated with volume-sensitive left atrial receptor remodelling.[108] Thus, our findings may have important mechanistic implications for the prognosis of AF, as contributory to progressive atrial dilatation (remodelling), itself associated with AF progression.[285]

Autonomic remodelling of volume- regulating reflexes in SR

We reaffirm findings of abnormalities in the LBNP reflex in-SR [175], strengthening the concept of autonomic remodelling in AF patients perhaps due to secondary changes in plasticity, memory [52, 286] and set-point resetting.[278] The principal difference between the prior cohort[175] presented in Chapter 3 and in-SR group here was that the former consisted of selected, highly symptomatic AF patients, undergoing catheter ablation, whereas here, all AF patients were eligible.

Abnormal cardio neural control: inferring the site of neurologic abnormality

We can make several inferences regarding the location of the neurologic abnormality, which might occur anywhere along the reflex arc. Preserved Valsalva (high-pressure, arterial baroreceptor function) and attenuated IHR (both baroreceptors[111]), in contrast with dysfunctional LBNP response (low-pressure cardiopulmonary baroreceptor dysfunction) implicate low-pressure (cardiopulmonary) baroreceptors; either at the level of the receptor itself or its associated central regulatory input. The principal effect of the efferent limbs of Valsalva, IHR and LBNP are increased central sympathetic outflow and pressor effect.[90, 111] Although Valsalva and IHR increase sympathetic efferent input to the sinoatrial node (low-

level LBNP minimally); HR interpretation is constrained by AF (AV nodal contribution). The ability to produce a pressor response in-AF remained intact (Valsalva). Thus, the abnormality is unlikely from inadequate efferent outflow.

Incremental LBNP results in a shift from low-pressure receptor-predominating to other responses maintaining MAP[96], possibly explaining minor MAP decreases despite dysfunctional vasoconstriction in-AF. However, abnormalities in *peripheral efferent sympathetic nerve-endings (blood vessels)* are not excluded. In interpreting LBNP FBF response and the potential site of the neurologic abnormality, the use of Low-Level Tragus Nerve Stimulation (LLTS), a novel potential treatment for AF[38], could be helpful, especially if a change (reversal) of the abnormality is detected. It would not be helpful in this endeavour if there was no effect of LLTS. This sub study is presented in Chapter 7.

The challenge of evaluating autonomic function during AF

Assessment of ANS function during AF is challenging. Subtle baroreceptor sensitivity or heart rate variability are not feasible. Prior work has implied abnormal baroreceptor function in AF from measurements after cardioversion.[203]

There is great need for techniques to measure ANS function during AF. Venous occlusion plethysmography with LBNP to estimate vasomotor tone presents a unique solution. It is simple to perform and interpret, non-invasive and unaffected by heart rate[284] or irregularity (multiple cardiac cycles measured). Non-invasive measures of cardiac output and vascular resistance[175], derived from peripheral arterial wave forms are unreliable in AF. The issue of the potential for measurement error in AF in our study will be further assessed in future sub

studies assessing whether these reflex deficits are reversible with restoration of cardioversion (Chapter 5 and neuromodulation with LLTS; Chapter 7).

Limitations

Patient-related factors (circadian pattern, mental stress, antihypertensive/arrhythmic medications, and execution) could influence ANS measurements. Testing conserved reflexes provides consistency. To minimize measurement bias, we ensured adherence, excluded failed attempts, measured in triplicate and double-blinded LBNP levels. Medications were stringently withheld; however, highly symptomatic patients would be under-represented. Venous occlusion plethysmography is well-established and reproducible.[96, 284] Strain gauge positioning can influence results, thus we maintained one position throughout. Although resting HR was higher in-AF; this would not affect LBNP vasomotor response. [96, 284] Interpretation of HR responses to IHR and Valsalva is limited by sample size and AF. We performed IHR for only one minute, although this duration allowed us to ensure adherence. We have not invasively measured central venous pressure; however, we have previously shown appropriate stroke volume decrease with LBNP.[175]

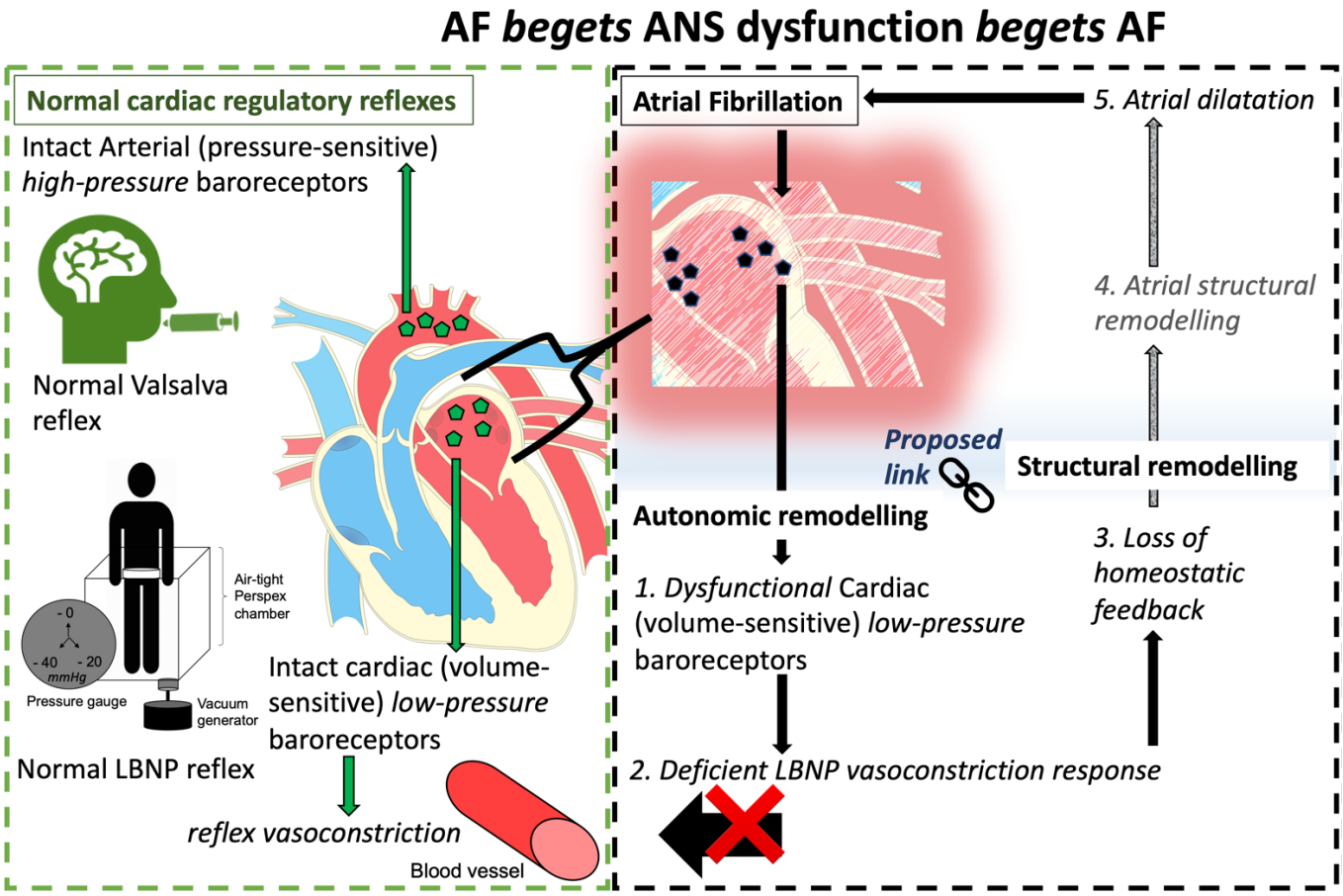
There was naturally some heterogeneity of AF severity in the two groups: in-AF more likely persistent (unpredictability of capturing a paroxysm). Thus, we planned to repeat tests in the in-AF group post cardioversion (Chapter 5). Studies to assess relative differences in cardiovascular reflex remodelling (best conducted in SR) in paroxysmal versus persistent AF are underway (Chapter 9).

Concluding remarks

Clinical implications

We have demonstrated abnormal autonomic regulation of cardiac volume due to AF, which we hypothesize, may result in progressive atrial dysfunction, eventually contributing to atrial dilatation and structural remodelling. Thus, the reflex abnormalities we have identified could link autonomic with structural remodelling as presented in Figure 4M. Further, these could, in-part, help explain AF-associated conditions such as falls, syncope, orthostatic intolerance and cognitive impairment in older adults[226] that we have presented in Chapter 2. What is needed is an assessment of reversibility as we have planned in further sub studies either using cardioversion, Chapter 5; catheter ablation of AF, Chapter 6 or LLTS; Chapter 7.

Figure 4M: Proposed link between autonomic remodelling and anatomic remodelling. "AF begets ANS dysfunction & ANS dysfunction begets AF".



Blood pressure and volume regulation rely on intact arterial (high pressure) and cardiopulmonary (low pressure) baroreflexes (*green).* AF results in cardiac afferent (volume-sensitive) baroreceptor reflex dysfunction, leading to loss of homeostatic feedback, potentially contributing to atrial dilatation and AF progression. Thus, we propose that cardiac afferent (volume-sensitive) baroreflex dysfunction represents a potential *link* between autonomic & structural remodelling (*black box*).

Conclusions

Cardiac volume-sensitive reflexes are diminished in patients with a history of AF and deficient during AF. Therefore, afferent dysfunction may represent a mechanistic link between autonomic and structural remodelling, responsible for both AF progression; "AF begets AF" and heart failure.

Chapter 5: Cardioversion reverses abnormalities of the cardiac volume-sensitive reflex due to Atrial Fibrillation: impact of rhythm

Background

The presence of AF produces increases in sympathetic nerve activity at baseline as assessed by muscle (left peroneal) microneurography.[82] This increase is mimicked by producing irregular atrial pacing and the degree of irregularity of pacing is associated with incremental ramping of sympathetic efferent activity to muscle.[82, 202] It is thought that the input to the high pressure (arterial) pressure baroreceptors due to long-coupled beats offloading these receptors produces heightened sympathetic nerve activity.[82] In addition to muscle sympathetic nerve activity increases, others have identified increases in skin sympathetic nerve activity preceding and at the termination of AF.[287] In Chapter 3 and Chapter 4 we have identified novel alterations in the ability to recognise and adapt to changes in blood volume. These autonomic reflexes are critical to maintain homeostasis and in older patients could contribute to the development of orthostatic intolerance, falls and syncope (Chapter 2) and as we have discussed in Chapter 4 could also promote the progression of AF and explain associations such as the development of heart failure. Indeed, persistent AF was associated with orthostatic intolerance (orthostasis relies on adequate cardiovascular autonomic function).[250]

It appears from the experimental data we have presented in Chapter 4 that there is a background level of abnormality (remodelling) in the response to Lower Body Negative Pressure (LBNP) and that there is a dysfunctional LBNP response when this is measured in

the presence of AF. However, the location of the abnormality as well as its reversibility is not known.

Prior work in terms of the assessment of autonomic function has been limited to its assessment in sinus rhythm, owing to the challenges of autonomic measurements during AF. The evaluation of vasomotor response to LBNP overcomes these challenges, which is why we employed this method in the analysis of autonomic reflexes during AF in comparison with sinus rhythm. In one study, high-pressure (arterial) baroreceptor function was measured immediately after cardioversion.[3] They showed a decrease in baroreceptor sensitivity in patients with persistent AF immediately after electrical cardioversion to restore SR. This work is limited by the assuming the effect of AF, where measurements were performed in SR. Nevertheless, this study does indicate that restoration of rhythm using cardioversion can influence autonomic function in patients with AF.

In this study, we examined an array of autonomic reflexes in a cohort of patients with AF on two occasions. First, prior to cardioversion, and second, at least 6 weeks after cardioversion (in sinus rhythm). Any improvement in the reflex deficits identified would add significant strength to our hypothesis that these abnormalities are indeed due to the presence of AF.

Methods

Study population, patient preparation & autonomic testing protocol

The study comprised consecutively enrolled patients referred to the Centre for Heart Rhythm Disorders with paroxysmal or persistent AF. The following were exclusions: amiodarone (preceding 6 months); active malignancy; symptomatic coronary artery disease; significant valvular disease; neurological disorders (Parkinson's disease, autonomic disorders, neuropathy); prior ablations; other arrhythmias; inability to enter a lower body chamber (frail or >120kg); or inability to withhold anti-arrhythmic/anti-hypertensives.

Patients with AF, scheduled to undergo cardioversion were studied in AF and then were contacted for a second visit at least 6 weeks after their cardioversion to restore normal rhythm. If they accepted, and were in sinus rhythm, they had to withhold medications as per the prior protocol and maintain the same preparatory measures and the same autonomic reflex tests were performed in the same laboratory, in the same order.

All participants provided written informed consent and the study was approved by the University of Adelaide human research ethics committee. This study was prospectively registered with the Australian New Zealand Clinical trials registry (ACTRN12619000186156).

Haemodynamic parameters, Systolic blood pressure (SBP); Diastolic pressure (DBP); Mean Arterial Pressure (MAP); Heart rate (HR) were collected continuously. Additional LBNP parameters including Forearm blood flow (FBF) and derived Forearm vascular resistance

(FVC) were assessed in an identical manner to the experiment performed and described in Chapter 4.

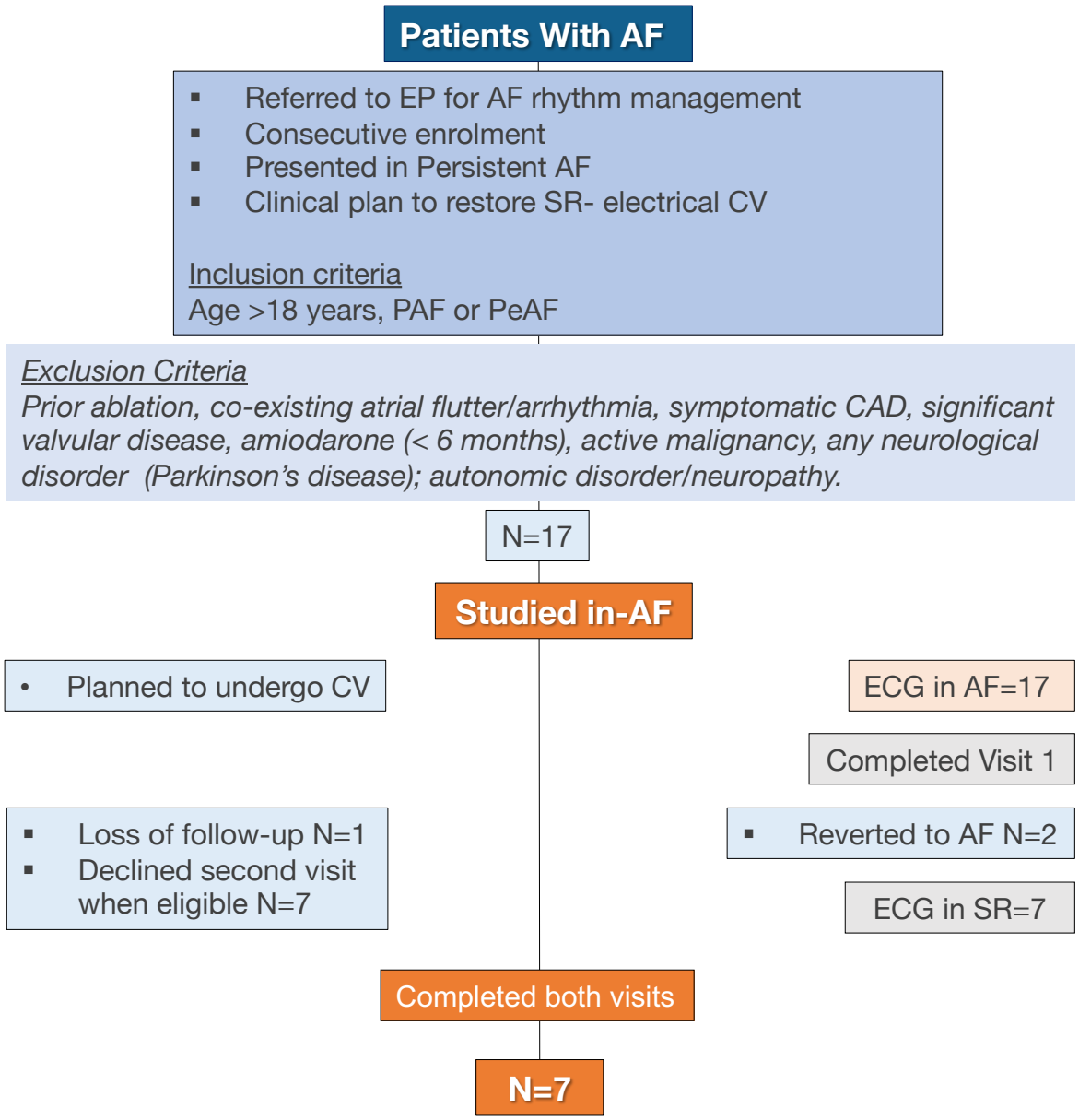
Statistical analysis

Continuous parameters were expressed as mean ± SEM. Categorical variables were expressed as frequencies and percentages and compared using Fishers exact test. Normality was checked and data compared using the students t-test or its non-parametric alternate. Data (pre vs. post) were compared using paired t-tests (normally distributed data) or Wilcoxon signed rank test (non-normally distributed data) as appropriate. Statistical analysis was performed using STATA 16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC) and GraphPad Prism (version 9.02, California, USA). Statistical significance was set at $P < 0.05$.

Results

We identified and recruited 17 patients for their first visit prior to scheduled cardioversion. Of these patients, who were studied during AF; 7 consented and returned for a second study visit >6 weeks after cardioversion. Mean age of the patients who performed both a pre and post CV visit was 62±4 years (100% male) and mean BMI was 26.5±1 kg/m^2. Transthoracic echocardiographic parameters at rest and at enrolment (prior to CV) were as follows; left ventricular ejection fraction 66±2%; left atrial volume (indexed to body surface area) 38.7±2.4 ml/m^2; interventricular septal thickness during diastole 1.08±0.08cm. Figure 5A: **CONSORT diagram**.

Figure 5A: CONSORT diagram



Consort diagram of patient recruitment and establishment of a cohort with completed pre- and post-CV (> 6weeks post cardioversion) visits, where CV restored sinus rhythm (SR).

The presence of cardiovascular risk factors included hypertension (n=2); dyslipidaemia (n=1); obstructive sleep apnoea (n=2). In this cohort, alcohol excess was not identified as a contributing risk factor. Medication usage was as follows; ß-blockers (except sotalol; n=4); sotalol (n=2); flecainide (n=2; one was started within the study period however it was withheld for 5-half lives as above); centrally acting calcium channel blockers (n=2); angiotensin converting enzyme-inhibitors or angiotensin receptor blockers (n=3); thiazide diuretics (n=1); digoxin (n=0).

Resting MAP and HR were 95±6 mmHg and 85±2 bpm respectively in the CV cohort. The resting MAP was 95±5 mmHg and resting HR was 88±3 bpm in the original group of AF patients who were studied in-AF (n=23; previous Chapter 4). Thus, the CV cohort resting haemodynamic parameters were very similar to the overall in-AF group presented earlier.

Effect of restoring SR using CV on Valsalva reflex response

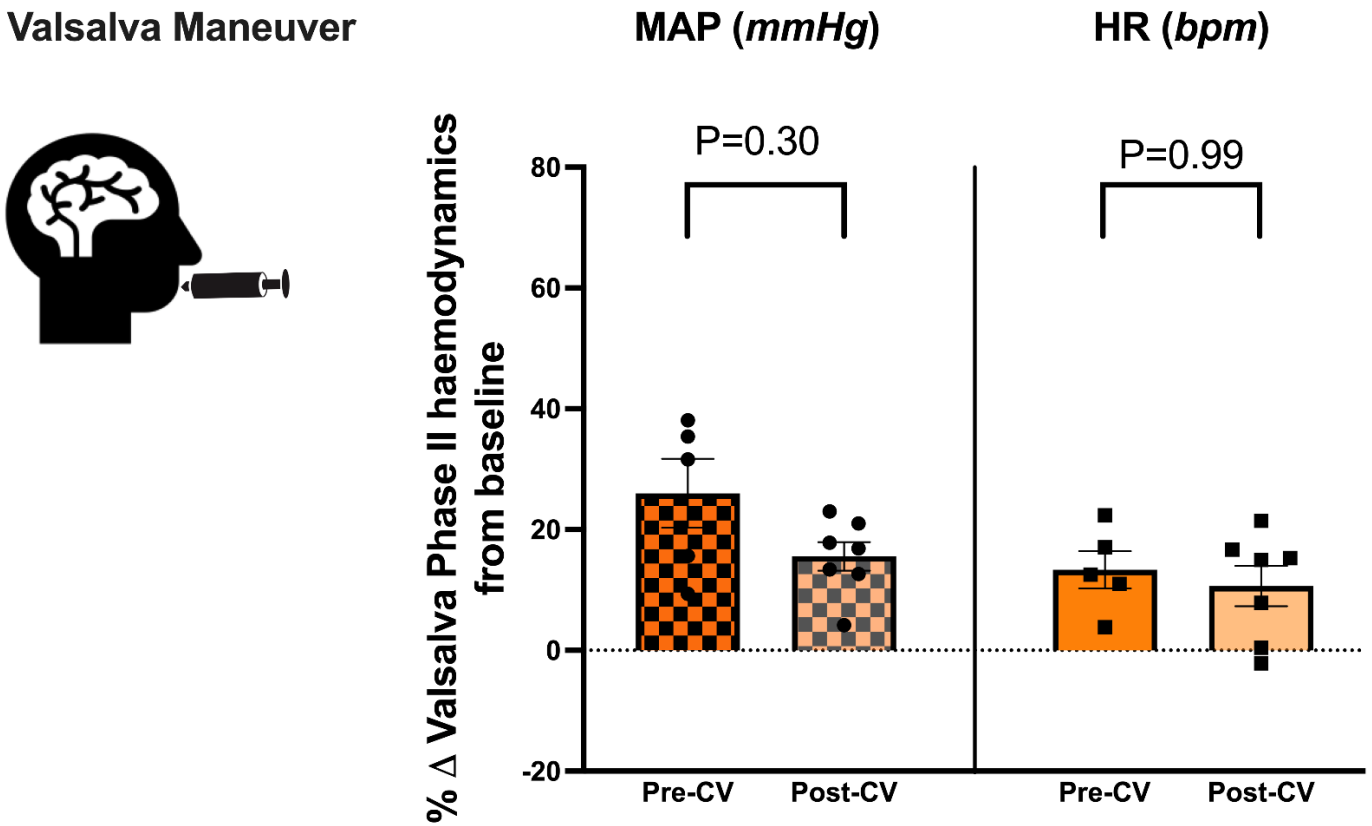
Haemodynamic data at each of the two autonomic phases – II and IV are presented in Table 5A. During phase II, pre-CV MAP increased by +26±6% and post-CV MAP increased by 18±4%. There was no statistical difference between time-points (P=0.3). HR also increased equivalently between time points (P=0.99). HR increased +13±3% and 12±4% pre- and post-CV respectively. Figure 5B. During phase IV; there was an overshoot of both MAP and HR at both time-points (P=0.3 & P=0.8, respectively) as presented in Table 5A.

Table 5A: Haemodynamic responses to Valsalva Pre & Post CV

	Pre CV (n=7)		Post CV (n=7)		P Value
Valsalva II	II_{EARLY}	II_{LATE}	II_{EARLY}	II_{LATE}	
MAP	105±9	130±8	97±10	103±10	0.3
SBP	133±10	160±15	116±13	128±14	0.4
DBP	100±9	114±7	84±8	93±9	0.4
HR	77±3	89±5	67±4	74±5	0.99
Valsalva IV	$IV_{BASELINE}$	$IV_{OVERSHOOT}$	$IV_{BASELINE}$	$IV_{OVERSHOOT}$	
MAP	97±8	128±6	81±5	116±10	0.3
SBP	131±14	177±9	125±11	138±10	0.06
DBP	80±6	97±9	61±4	84±9	0.99
HR	84±2	100±1	58±3	70±7	0.8

Data±SEM. Differences between time points are represented by P values in the adjacent column. MAP, Mean Arterial Pressure (mmHg); SBP, Systolic Blood Pressure (mmHg); DBP, Diastolic Blood Pressure (mmHg); HR, Heart rate (bpm). Valsalva phases were compared to corresponding baseline value prior to Valsalva and were expressed as percentage change. Between group comparisons for Valsalva for each autonomic phase: $II_{LATE} - II_{EARLY}$ or $IV_{OVERSHOOT} - IV_{BASELINE}$ were compared between time points (Pre vs Post-CV; $P<0.05$).

Figure 5B: MAP & HR responses to Valsalva Pre & Post CV



Data±SEM. MAP, Mean Arterial Pressure (mmHg) & HR, Heart rate (bpm). Valsalva phases were compared to corresponding baseline value prior to Valsalva and were expressed as percentage change. Pre and post comparisons for Valsalva for Phase II (autonomic phase: $II_{LATE} - II_{EARLY}$; $P<0.05$).

Effect of restoring SR using CV on Submaximal isometric handgrip reflex (IHR)

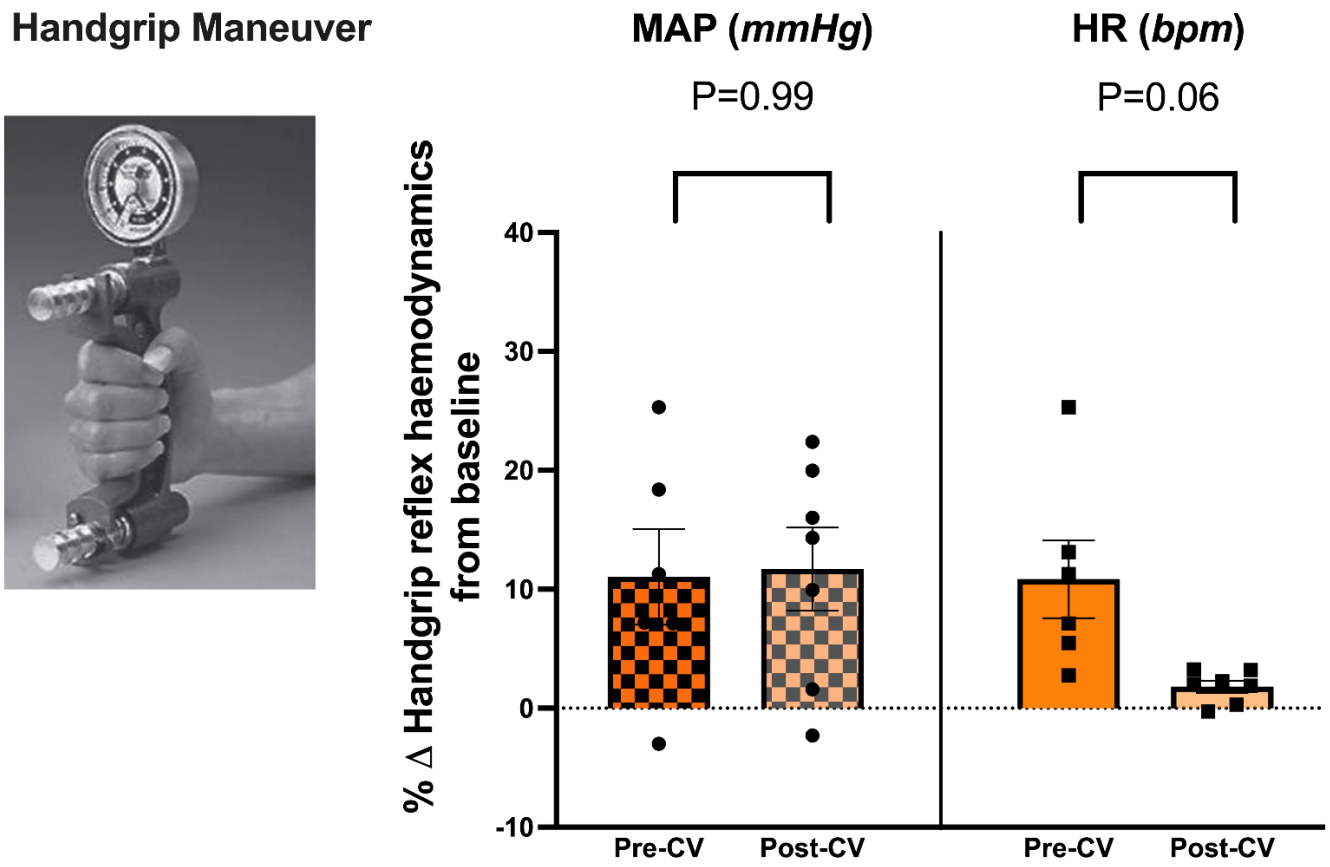
IHR increased MAP similarly both pre-CV and post-CV (+11±4% and +10±4; P=0.99). HR response to IHR was not significant between time points (P=0.06). Although, HR increased pre-CV in response to IHR (+11±3%), HR response to IHR was not as robust post-CV (+2±1%). Table 5B contains haemodynamic responses to IHR. The attenuated response of MAP to IHR detected in comparison to controls in Chapter 4 does not appear to improve after cardioversion. Figure 5C.

Table 5B: Haemodynamic responses to IHR Pre & Post CV

	Pre CV (n=7)		Post CV (n=7)		P Value
IHR	Pre-IHR	Post-IHR	Pre-IHR	Post-IHR	
MAP	99±7	109±6	84±5	93±7	0.99
SBP	133±12	148±10	129±11	141±12	0.8
DBP	82±5	91±5	63±4	70±5	0.99
HR	85±2	90±3	59±3	60±4	0.06

Data±SEM. Differences between time points are represented by P values in the adjacent column. MAP, Mean Arterial Pressure (mmHg); SBP, Systolic Blood Pressure (mmHg); DBP, Diastolic Blood Pressure (mmHg); HR, Heart rate (bpm); $P<0.05$.

Figure 5C: MAP & HR responses to IHR Pre & Post CV



Data±SEM. MAP, Mean Arterial Pressure (mmHg) & HR, Heart rate (bpm). IHR haemodynamic responses were compared to corresponding baseline value prior to IHR and were expressed as percentage change. Pre and post comparisons for IHR; $P<0.05$.

Effect of restoring SR using CV on LBNP responses

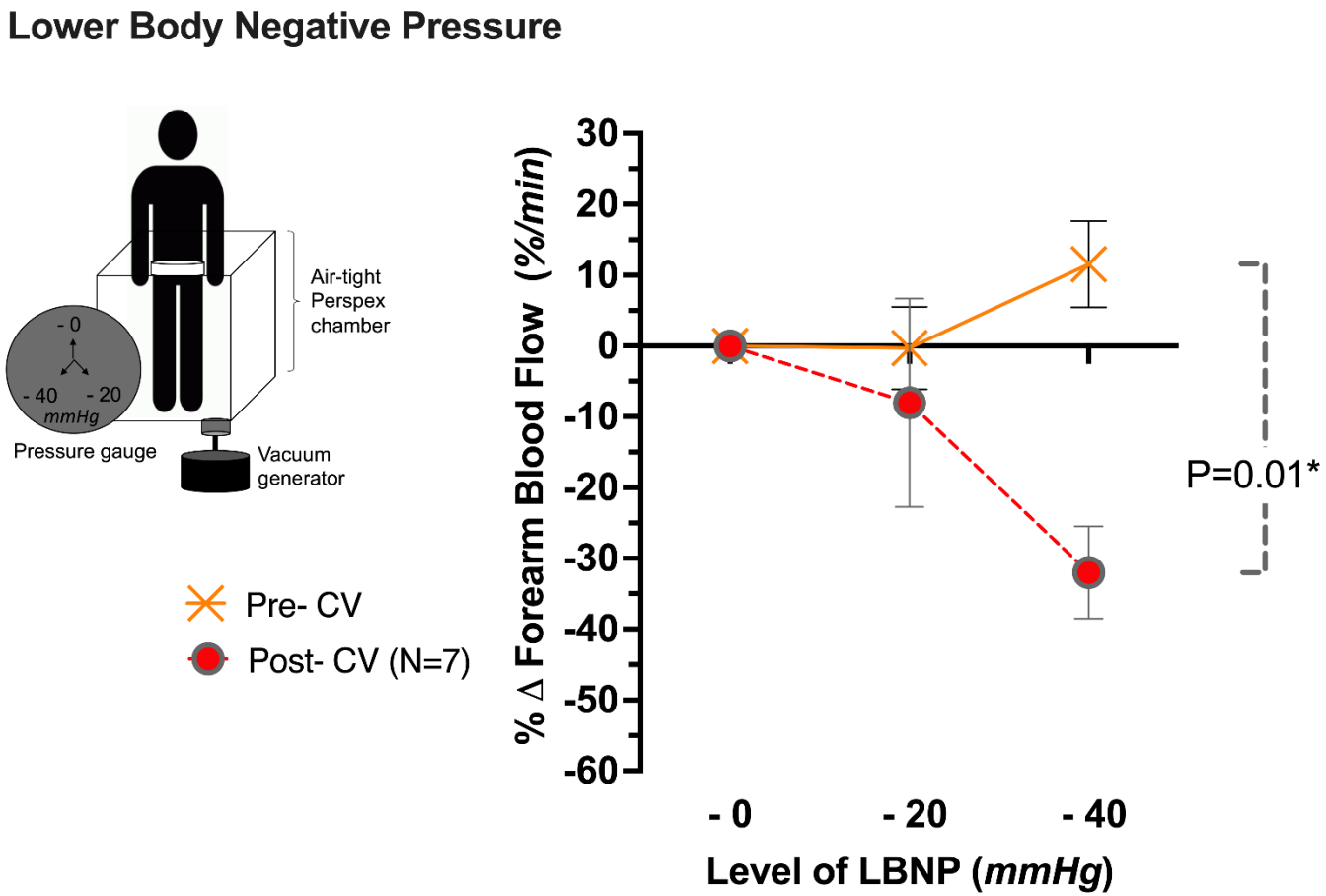
Haemodynamic parameters in response to LBNP at each level (-0 mmHg, -20 mmHg and -40 mmHg) pre and post CV are presented in Table 5C. LBNP (-40 mmHg) resulted in a decrease in MAP pre-CV (-7.2±5%) and post CV (-4±2%). HR did not increase substantially pre-CV (+3±3%). Post-CV HR increased (+12±3%). There was no statistical difference identified in the MAP or HR response between visits (P=0.6 and P=0.2 respectively). Pre-CV FBF increased (+17±10) whereas post-CV it decreased substantially (-32±7%). The statistical comparison of FBF response pre and post CV was statistically significant (P=0.01). This FBF decrease remained attenuated as seen in the in-SR cohort of the earlier study presented in Chapter 4 where FBF decrease was -11.9±9%. Figure 5D. Likewise, FVC increased pre-CV (+25±13%) whereas it decreased post-CV (-29±6%). The comparison of FVC between the two time points was also statistically significant (P=0.02).

Table 5C: LBNP responses to LBNP Pre & Post CV

	Pre CV (n=7)			Post CV (n=7)			P Value
LBNP Level	0	-20	-40	0	-20	-40	
MAP	101±7	97±6	93±6	91±5	89±4	87±5	0.6
SBP	134±10	128±11	125±12	134±10	129±8	124±10	0.9
DBP	84±5	83±4	79±4	69±3	69±3	70±3	0.2
HR	82±2	84±2	84±3	53±4	55±3	59±3	0.2
FBF	1.79±0.3	1.75±0.2	1.97±0.2	1.80±0.2	1.57±0.2	1.25±0.2	0.01*
FVC	1.83±0.3	1.77±0.3	2.13±0.2	2.03±0.3	1.80±0.2	1.47±0.3	0.02*

Data±SEM. Differences between the groups are represented by P values in the adjacent column. MAP, Mean Arterial Pressure (mmHg); SBP, Systolic Blood Pressure (mmHg); DBP, Diastolic Blood Pressure (mmHg); HR, Heart rate (bpm); FBF, Forearm Blood Flow (%/min); FVC, Forearm vascular conductance (100*FBF/MAP, arbitrary units). $P<0.05$

Figure 5D: FBF response to LBNP Pre- & Post-CV



Data±SEM. Forearm Blood Flow (FBF) response to LBNP – 20mmHg and – 40 mmHg negative pressure. Expressed as percentage change. Pre and post CV comparisons; P<0.05.

Discussion

Our principal findings in this Chapter are:

1. The dysfunctional (deficient) vasoconstriction response to LBNP (estimated by forearm blood flow) Pre-CV is partially reversible after cardioversion (restoration of sinus rhythm). and the improved reflex is at a similar level to AF patients who were studied in sinus rhythm in the prior chapter.
2. Valsalva responses reflect preserved function in the presence of AF; there is no difference post-CV.
3. There is no difference post-CV in terms of haemodynamic responses to IHR.

These findings strongly suggest that the abnormalities in the volume-handling reflexes we have identified in Chapter 4 is most likely due to the presence of AF. In the prior chapter, we had discussed the natural heterogeneity of AF severity in the two patient groups: with the in-AF more likely persistent due to the unpredictability of capturing a paroxysm. Thus, in repeating these autonomic tests in the cardioversion cohort; we have significantly aided our hypothesis testing in terms of the causal role that the presence of AF plays in these deficits.

These identified deficits have not previously been described in AF and as discussed in Chapter 1, the focus of prior work in this area has been to assess the electrophysiologic effects and vulnerability of AF to autonomic efferent stimulation. In Chapter 4 we have described the responses to autonomic cardiovascular stressors in patients with AF studied in AF or studied in sinus rhythm, comparing their responses to a reference group. Having

identified these afferent or regulatory abnormalities, we have naturally progressed to the next phase within this chapter, which is looking at the effect of treating AF. Treatment of AF is multifactorial from a clinical point of view, however from a purely rhythm-related point of view; one could either slow ventricular response during AF (rate control), cardiovert either with electrical shock applied to the heart as was done in this cohort or offer anti-arrhythmic medications to chemically cardiovert patients. These medications can be used to prevent AF paroxysms as well. Indeed, the following chapter (Chapter 6) we shall further this by assessing whether catheter based treatments to electrically isolate the posterior left atrium from the rest of the heart using radiofrequency thermal energy (which is where the majority of AF originates)[11] could alter the function of the afferent volume-regulating reflexes that are elicited by LBNP in AF either by; a. resulting in destruction of the afferent receptors that are co-located in this atrial tissue or b. by modulating the function of these receptors that we have now identified as abnormal in patients with AF in sinus rhythm (diminished function) or dysfunctional (deficient) in the presence of AF.

Perhaps the only other study, as far as we are aware, that assessed afferent regulatory function of the heart in the presence of AF was performed by Gould *et al*. [206] In this study, patients with heart failure with an ejection fraction of <40% were studied either in sinus rhythm or in persistent AF of at least three months duration. The investigators performed a right heart catheterisation study where cardiac noradrenaline spill over (corrected noradrenaline isotope dilution method) was measured in the coronary sinus at baseline, during 20 degrees head-up tilt and during 30 degrees head-up tilt. Baroreceptor unloading using head-up tilt elicits an increase in cardiac sympathetic activity (as measured by cardiac noradrenaline spill over). Patients with concomitant heart failure and AF had a significantly attenuated response to baroreceptor unloading. Low degrees of tilt would be expected to

offload both arterial high-pressure and cardiopulmonary low-pressure baroreceptors. From this study, it was unclear which of these receptors was responsible. The greater the tilt the more arterial (high-pressure) baroreceptors found in the aortic arch and the carotid bodies would play a role in these responses and with lower levels of tilt as seen with this study it is possible that low pressure cardiopulmonary receptors that play a role in regulating cardiac volume are implicated. Aside from the limitation in this study that patients with heart failure and AF were studied (where heart failure can also disturb baroreceptor function),[55] experiments were not performed after restoration of cardiac rhythm and thus it is quite possible that other differences could account for the changes seen. In our study, we have performed repeated autonomic testing in patients with AF and without heart failure, identifying receptor deficits which improve after cardioversion but do not normalise. There is some case evidence of autonomic dysfunction in a patient with persistent AF that manifested in severe orthostatic hypotension, that completely dissipated after cardioversion to restore normal rhythm.[270]

The diminished reflexes in sinus rhythm suggest a background level of remodelling of such autonomic receptors or regulating reflexes of cardiac volume provoke the concept that there may be an effect of burden of AF, which will require further study. Finally, given the presence of similar abnormalities in patients with AF alone; patients in heart failure and concomitant AF;[206] as well as patients with heart failure alone, the presence of such deficits in cardiac volume handling due to deficiencies in cardiopulmonary low-pressure baroreceptor function could provide a mechanistic association of AF with the development of heart failure.[229]

Field et al.[203] (discussed in Chapter 1) assessed arterial high-pressure baroreflex sensitivity in patients with persistent AF, studied immediately after CV (in sinus rhythm) and then at 30 days post-CV. This study showed deficits in baroreceptor sensitivity immediately after CV and extrapolated this finding to be attributable to AF; however, other factors such as dehydration, sedation-related (anaesthetic effects) and the effects of medications and the procedure as well may have accounted for these findings. Baroreflex sensitivity measurement needs to occur during sinus rhythm, and it would not be possible to assess this in the presence of AF. The presence of AF is a major limitation of several cardiovascular autonomic assessments. In our study, we did not assess baroreflex sensitivity and thus subtle abnormalities may be missed. Here we found that there were gross abnormalities of cardiopulmonary low-pressure baroreflexes and that, overall, arterial high-pressure baroreceptor function was preserved and further the assessments that were performed in AF were repeated at least 6 weeks after CV to ensure that any effect of the procedure itself was eliminated.

Limitations

Our interpretations of this study are somewhat limited by low repeat sample sizes. We detected significant improvements in LBNP responses, however we have not been able to identify any improvement in IHR. It is possible that subtle difference in the MAP response (pressor effects in interpretation of IHR are more important than HR responses) may not have been able to be detected due to inadequate statistical power. The HR response is also unusual; where during AF, IHR was associated with a numerically higher (non-significant) increase in HR, however, post-CV, there was a diminished HR response. It is possible that due to severe deficiencies in cardiopulmonary low-pressure baroreceptor function during AF, HR effects are from arterial high-pressure stimulation are heightened in an effort to compensate, however, this is speculative.

Concluding remarks

Clinical Implications

Whilst this study provides evidence of the causal role of AF, it also highlights the potential reversibility of the dysfunctional (absent) reflex response to blood volume-regulating reflexes seen in AF. We have detailed that it is indeed most interesting that these abnormalities could provide a mechanistic link between AF, its progression, the development of heart failure and perhaps also several clinical associations of AF such as falls, dementia, orthostatic hypotension, and syncope. It is reassuring then that this study provides evidence of reversibility and heightens the role of rhythm management in AF; where prior studies that assessed rate vs rhythm management of AF[32, 33] would have entirely missed these aspects of the presence and increasing burden in individuals suffering from AF.

Conclusion

This study demonstrates that the deficiencies of the volume-regulating cardiopulmonary low-pressure baroreceptor reflexes induced by LBNP identified in Chapter 4 are indeed due to the presence of AF and that these reflex abnormalities are reversible after restoration of normal sinus rhythm. Further work (presented in subsequent chapters) is needed to assess the location of the abnormality, assess the effect of catheter-based interventions to treat AF and maintain sinus rhythm as well as assess the role of newer neuromodulating therapies (low-level tragus nerve stimulation) in the treatment of AF.

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Chapter 6: Catheter ablation of Atrial Fibrillation is not associated with destruction of cardiac afferents driving volume-regulating autonomic reflexes: Impact of ablative rhythm intervention

Background

In Chapter 3 and Chapter 4 we have demonstrated that AF patients studied in sinus rhythm (SR) have abnormal (diminished) cardiac volume-sensitive low-pressure baroreflexes elicited by lower body negative pressure (LBNP) and in-AF, these reflexes are dysfunctional. In normal individuals, lower body negative pressure (LBNP) decreases venous return to the heart, unloads cardiopulmonary receptors and results in compensatory systemic vasoconstriction.[97] Atrial stretch receptors, particularly cardiopulmonary receptors found in the pulmonary vein-left atrial junctions are critical mediators of such reflexes.[57, 97] Interestingly, these volume-regulating low-pressure baroreceptors are co-located in veno-atrial tissue,[106, 108] which is where the majority AF triggering atrial ectopic beats originate,[11] and which is also the treatment target for catheter based ablation strategies to manage rhythm in patients with AF.[288] Therefore, on one hand, the management of AF, including using catheter based strategies to isolate the pulmonary veins electrically from the rest of the atria (Pulmonary Vein Isolation, PVI), could ameliorate these reflexes (in a similar way to our finding that the dysfunctional reflexes in-AF improve to on-SR levels after cardioversion, Chapter 5). On the other hand, PVI, by resulting in damage to the cardiac muscle surrounding the pulmonary veins, where these receptors are co-located, could result in unintended nerve disruption.

Catheter-based treatments of AF, with any energy source, typically radiofrequency (heat) thermal energy or cryotherapy (cold) thermal energy result in destruction of cardiac tissue rendering a line of electrical block that effectively isolates any impulses that originate within the pulmonary-vein atrial junction from triggering AF.[11] Prior work demonstrated that circumferential PVI with additional lesions to selectively ablate autonomic areas of the pulmonary vein-atrial junctions which, when stimulated produce vagal reflexes (hypotension, sinus bradycardia or atrioventricular block) results in improved procedural success (freedom of AF at 12 months) and that these are associated with increases in resting heart rates post-PVI.[289] Lesions in addition to PVI to ablate ganglionated (autonomic) plexi, which are a collection of neurons in cardiac tissue, also found surrounding the pulmonary veins in the posterior left atrium[136] can also enhance procedural outcomes.[223] Indeed, as Scherlag *et al*.[290] showed, autonomic ganglion stimulation at the base of the right superior pulmonary vein produced vagally induced heart rate slowing at rest and converted atrial ectopy firing in the pulmonary vein into AF. These areas were associated with complex fractionated electrograms (multi-component, low-frequency, sharp signals) that were found during AF and have also been targeted in clinical trials, with increased procedural success (freedom of AF) over PVI alone.[35] Finally, in a sub analysis of the CIRCA-DOSE study[291] PVI, with any method of thermal energy delivery (cryotherapy or radiofrequency) results in "inadvertent" autonomic alterations exhibited by heart rate variability due to destruction of autonomic ganglia and these correlate with successful outcomes in terms of freedom of AF from a procedure. Thus, whilst there is certainly precedent for autonomic destruction from PVI as well as influences on successful outcome (which may either directly be attributable to procedural success or more likely, represent a marker of procedural success due to durable ablation lesions), there is no data on the effect of PVI on afferent or regulatory autonomic receptors or nerves found in the pulmonary-vein atrial junctions.

Here, we present data from two separate studies; in the first (preliminary study), we evaluated reflex responses to lower body negative pressure (LBNP) in patients post-PVI who were selected after at least three months of their procedure being performed (and prior to 2 years) and where we tested their reflex responses to LBNP, similarly to Chapter 3. There are limitations to this approach, in that the effect of PVI on LBNP is not adequately tested within this study design. Therefore, we had designed and conducted a cohort study before and after PVI, presented below.

In this second, study, we performed an array of clinical autonomic reflex tests in a cohort of patients with AF studied in sinus rhythm (SR) who have been clinically evaluated and have been planned to undergo PVI, prior to PVI as well as post-PVI (in those who consented for repeat evaluation, at least 3 months after PVI). This study was performed in a similar manner to Chapter 4 and Chapter 5.

We hypothesize that whilst AF induces autonomic dysfunction with abnormal reflex responses to decreased venous return, successful pulmonary vein isolation (PVI) could further disrupt volume-regulating reflexes, where other baroreflexes would remain intact.

Methods

The methods and results for this Chapter present separate data from each of the two cohorts; the first is the pilot or preliminary study and the second is a more thorough evaluation of clinical autonomic reflex tests in a cohort of patients undergoing PVI.

The pilot study involved three groups. The first group consisted of twenty patients who had symptomatic paroxysmal AF referred for consideration of pulmonary vein isolation (PVI) and were studied *prior* to PVI. These are presented in Chapter 3.

The second group, presented here, consisted of fourteen patients who were studied following successful treatment with PVI (referred to as the successful PVI group). These patients were only studied (i) after the three-month blanking period post- PVI and (ii) if they remained symptom free up to two years post-PVI. Both groups were compared to fourteen age and sex-matched healthy controls (Control group).

Enrolled patients in either group were free from cardiovascular (coronary artery disease, valvular heart disease or heart failure) and other confounding conditions (autonomic disorders, untreated hypertension, postural hypotension, diabetes mellitus or renal disease). Patients who had received amiodarone during the last six months were also excluded.

The successful PVI group was enrolled from a hospital database of all PVI procedures undertaken for two years prior to enrolment. Patients were excluded if they had multiple PVI procedures, were either within the three-month blanking period at the time of the study or were outside of the two-year window, did not attend regular follow up with the treating

cardiologist, accurate records were not kept in terms of AF recurrence, were not contactable by telephone or did not consent to presenting for the purposes of this study.

Pulmonary Vein Isolation

In the preliminary study, PVI was performed in the fasting state, under general anaesthesia. Three electrode catheters were introduced percutaneously via the right femoral vein. A 7F quadripolar catheter (Biosense-Webster, Diamond Bar, CA, USA) was placed in the coronary sinus and used for pacing and recording. A variable size circumferential decapolar mapping catheter (Lasso, Biosense-Webster, Diamond Bar, CA, USA) and an 8F, 3.5mm irrigated tip ablation catheter (Navistar Thermocool D-F curve, Biosense-Webster, Diamond Bar, CA, USA) were used for mapping and ablation of pulmonary veins (PV). They were introduced to the left atrium via trans-septal puncture. The circumferential mapping catheter was positioned as proximal as possible within each of the PV's and was oversized for stability. Systematic isolation of the four PV's was performed at each ostium, guided by the mapping catheter. Entrance block or the presence of dissociation within the pulmonary veins was the ablation endpoint. All veins were re-checked after 30 minutes observation and further ablation was performed if acute conduction recovery was detected. Ablation success was confirmed in all 14 subjects by symptoms and 3- monthly 24 to 72- hour Holter monitoring. No specific attempts were made to locate or ablate areas associated with cardiac autonomic plexus (ganglionated plexi) as per clinical guidelines.[288]

Experimental protocol

Similar to Chapter 3, the experimental protocol was performed in the fasting state (4 hours) with abstinence from caffeine, alcohol, and strenuous exercise in the preceding 24 hours. All anti-arrhythmic drugs (including ß-blockers) were withheld for five half- lives, symptoms

allowing, prior to the study protocol. All testing was performed in a climate-controlled laboratory with room temperature of 22^{o}C. All patients were in sinus rhythm during the testing protocol. Resting brachial blood pressure was checked in the supine position with a standard mercury sphygmomanometer. Continuous beat-to-beat recordings were obtained using finger photo plethysmography (Nexfin, BMEYE, Amsterdam, The Netherlands)[271] to derive: Mean arterial pressure (MAP, *mmHg*); Heart rate (HR, *bpm*); Stroke volume index (SV, mL/m^2);Cardiac index (CI, $L/min/m^2$); and Systemic vascular resistance index (SVRI, $dynes\ \ s*m^2/cm^5$).

Patients were then subjected to LBNP using a custom-made Perspex chamber placed over the subject's lower limbs with a seal at the iliac crest. A vacuum generator (Model UZ-930; Electrolux; Stockholm, Sweden) was attached to the chamber and connected to a voltage converter, which allowed graduated control of the vacuum intensity (Figure 3A). Thus, negative pressure could be precisely controlled to 0, -20 and -40 mmHg with the aid of an industrial pressure gauge (Ambit Instruments; Wetherill Park, NSW, Australia). All patients underwent a short 2-minute familiarization period with LBNP application followed by a resting period of 10 minutes prior to LBNP protocol commencement. The degree of LBNP intensity was randomly applied for each patient for 10 minutes at each level with a 5-minute break in between. Negative pressure was applied slowly over 30 seconds to minimize patient movement and discomfort.

Clinical autonomic reflex tests in a cohort of patients undergoing PVI

The methods presented below are for the second study we are reporting in this Chapter, specifically evaluating the effect of PVI on clinical autonomic reflex tests in patients with AF, all of whom were studied in sinus rhythm.

Study population

This study comprised consecutively enrolled patients referred to the Centre for Heart Rhythm Disorders with paroxysmal or persistent AF. We consecutively studied patients with paroxysmal/persistent AF who were reviewed by a cardiac electrophysiologist and were selected to undergo PVI. The following were exclusions: amiodarone (preceding 6 months); active malignancy; symptomatic coronary artery disease; significant valvular disease; neurological disorders (Parkinson's disease, autonomic disorders, neuropathy); prior ablations; other arrhythmias; inability to enter a lower body chamber (frail or >120kg); or inability to withhold anti-arrhythmic/anti-hypertensives.

Those who participated in the first visit prior to PVI (pre-PVI) were re-contacted for a post-PVI visit provided they met additional criteria. These prespecified criteria included the following:

1. Absence of any procedural complications (not limited to, but including, significant pericardial effusion requiring drainage, post-procedure pericarditis or stroke).

2. Patients needed to satisfy full clinical follow-up at 1 week, 1, 3 and 6 months following PVI where they had a 12-led electrocardiogram, multi-day (7-day) Holter monitoring.
3. They needed to have been symptom-free and have no documented electrocardiographic evidence of AF (absence of clinical evidence of AF) for >6 months post-PVI.
4. Repeat procedures in the cohort were allowed, provided that, at the time of the post-PVI visit, they had been AF free>6 months.

All participants provided written informed consent and the study was approved by the University of Adelaide human research ethics committee. This study was prospectively registered with the Australian New Zealand Clinical trials registry (**ACTRN12619000186156**).

Patient preparation

All patients withheld alcohol (24 hours) and caffeine (48 hours), refraining from vigorous exercise (48-hours) prior to the study. All anti-arrhythmic (rate and rhythm controlling) and anti-hypertensive medications were withheld for 5 half-lives. Patients fasted for 4 hours allowing water *ad libitum*.

Autonomic testing was performed in a climate-controlled facility (22°C). We collected baseline demographics; risk factors; cardiac chamber measurements (echocardiography); and baseline serum for High-sensitivity CRP (Hs-CRP). Patient preparation was identical to Chapter 4.

Autonomic testing protocol

The autonomic testing protocol in this study were identical to Chapter 4. AF Patients who presented in sinus rhythm (SR) who were selected to undergo PVI as the clinical management strategy for their AF, were studied and then recontacted for a second visit as stipulated above after their PVI had been performed and they had been AF-free>6 months. If they accepted, they had to withhold medications as per the prior protocol and maintain the same preparatory measures and the same autonomic reflex tests were performed in the same laboratory, in the same order as their first visit. Haemodynamic parameters, Systolic blood pressure (SBP); Diastolic pressure (DBP); Mean Arterial Pressure (MAP); Heart rate (HR) were collected continuously for all the three autonomic tests: submaximal isometric handgrip reflex (IHR), Valsalva reflex and Lower Body Negative Pressure (LBNP). Additional LBNP parameters including Forearm blood flow (FBF) and derived Forearm vascular resistance (FVC) were assessed in an identical manner to the experiment performed and described in Chapter 4.

Pulmonary Vein Isolation

PVI was performed in the fasting state, under general anaesthesia. In this study, the method utilised, energy delivery, or any additional lesion sets, aside from PVI, were not stipulated and were performed in accordance with the operator. The predominant procedure was a single ring radiofrequency ablation of all four pulmonary veins (PV) *en bloc*, together with the posterior wall (Si-R-PVI). In one case, wide antral PVI was performed. Three electrode catheters were introduced percutaneously via the right femoral vein under ultrasound guidance. A 7F decapolar steerable catheter (Livewire, Abbott medical, USA) was placed in the coronary sinus and used for pacing and recording. Geometry and voltage maps were created of the left atrium using either a variable size circumferential decapolar mapping

catheter (Lasso, Biosense-Webster, Diamond Bar, CA, USA) or a high-density mapping catheter (HD-Grid Advisor, Abbott medical, USA) during coronary sinus pacing. Maps were created with the EnSite Precision 3-D mapping system (Abbott medical, USA). An 8F, 4mm irrigated tip ablation catheter (Flexability D-F curve, Abbott medical, USA) were used for mapping and ablation in the left atrium. They were introduced to the left atrium via trans-septal puncture (BRK-1 needle, Abbott medical, USA), and an 8.5F SL-0 sheath (Abbott medical, USA) for the mapping catheters and, over-the-wire using a steerable sheath (8.5F Agilis, Abbott medical, USA) for the ablation catheter. Trans-septal puncture was performed with transoesophageal echocardiogram guidance, as well as pressure monitoring and contrast fluoroscopy. Bidirectional block (entrance as well as exit block) and, if present, dissociated firing within the pulmonary veins/posterior box set was the ablation endpoint. All veins were re-checked after an observation period and further ablation was performed if acute conduction recovery was detected to ensure isolation of the PV's.

In one case, cryoablation was performed. Here, we advanced a quadripolar catheter (Supreme, Josephson's, Abbott medical, USA) into the RV from the right femoral vein access site and later positioned in the right subclavian vein for high output pacing to stimulate the right phrenic nerve during right sided PV freezes (recognise injury during energy delivery). Mapping and ablation were performed with a circumferential mapping catheter (Achieve, Medtronic, USA) and the cryo-balloon catheter (Arctic Front, Medtronic USA), delivered in the left atrium via trans-septal puncture as above via a steerable 12F sheath (FlexCath, Medtronic, USA). Each PV was sequentially treated by placing the mapping and ablation cryo-balloon catheter at the ostium of each PV, inflating the cryo-balloon, ensuring occlusion of the PV (using contrast fluoroscopy), and delivering cryotherapy. Ablation was ceased if there was any evidence of phrenic nerve injury (non-capture).

With any of the PVI procedures, no specific attempts were made to locate or ablate areas associated with cardiac autonomic plexus (ganglionated plexi) as per clinical guidelines.[288]

Statistical analysis

Continuous parameters were expressed as mean ± SEM. Categorical variables were expressed as frequencies and percentages and compared using Fishers exact test. Normality was checked and data compared using the students t-test or its non-parametric alternate. Data (pre vs. post) were compared using paired t-tests (normally distributed data) or Wilcoxon signed rank test (non-normally distributed data) as appropriate. Statistical analysis was performed using STATA 16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC) and GraphPad Prism (version 9.02, California, USA). Statistical significance was set at $P < 0.05$.

Results

The first part of this section details the results of the pilot study. Figure 6A presents the CONSORT diagram of the patient groups, both in the original study (AF patients and controls, as well as this group; successful PVI group). The successful PVI group (n=14) was derived from a database of 129 PVI procedures; of whom 48 had a single radiofrequency ablation. 15 were eligible after review of case notes and contact was made. 1 patient withdrew as she was unable to participate in the experimental protocol. The successful PVI group was studied at an average duration of 8 months post ablation (range 3 – 22 months). The baseline characteristics of the included patients are presented in Table 6A.

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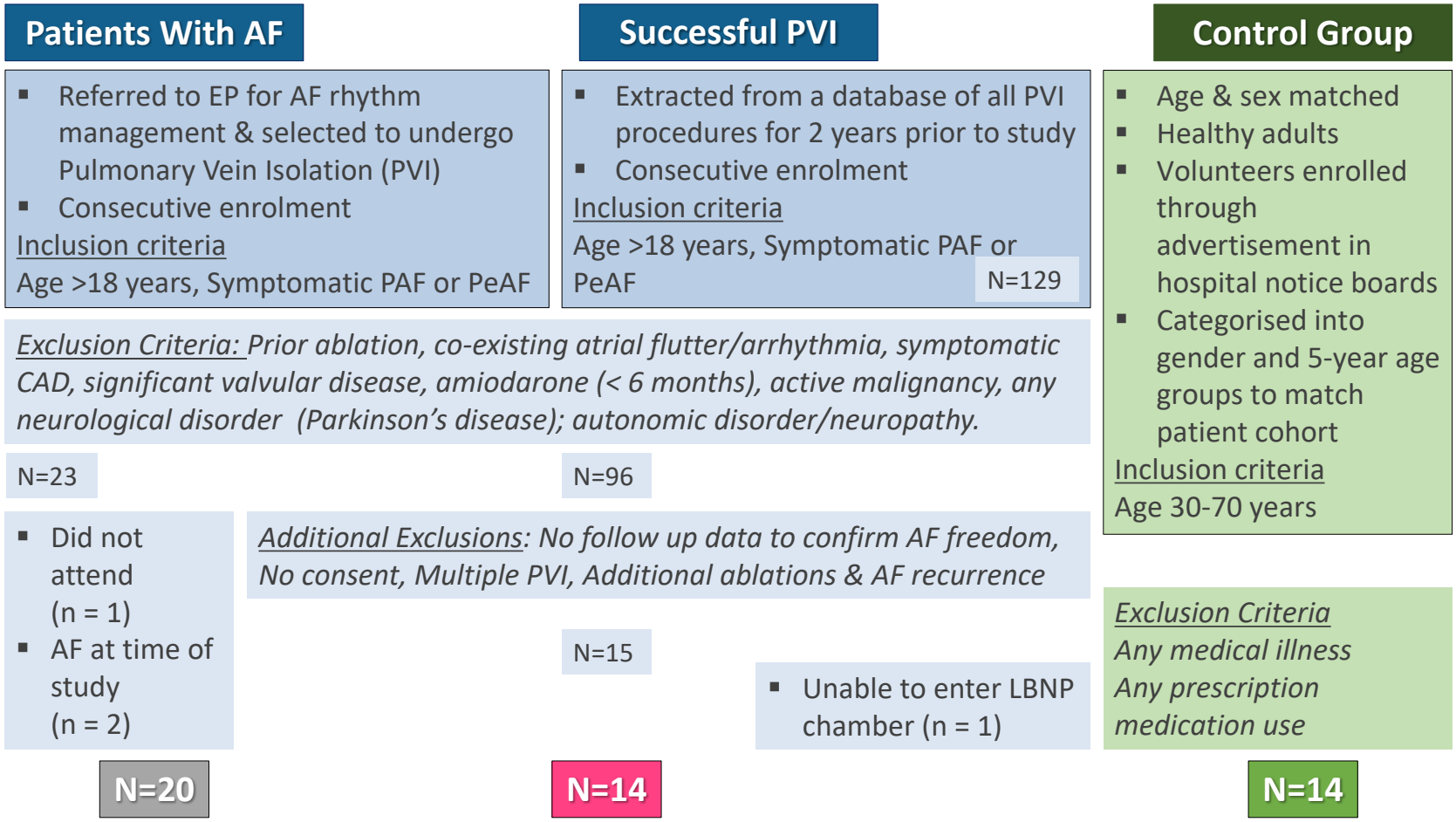
Figure 6A: CONSORT diagram of the pilot study



Enrolment in the pilot study – successful PVI group. Other groups (PAF, studied pre-PVI in SR and control groups as reference).

Table 6A: Baseline characteristics of the successful PVI group in comparison with the pre-PVI (PAF) group and control groups

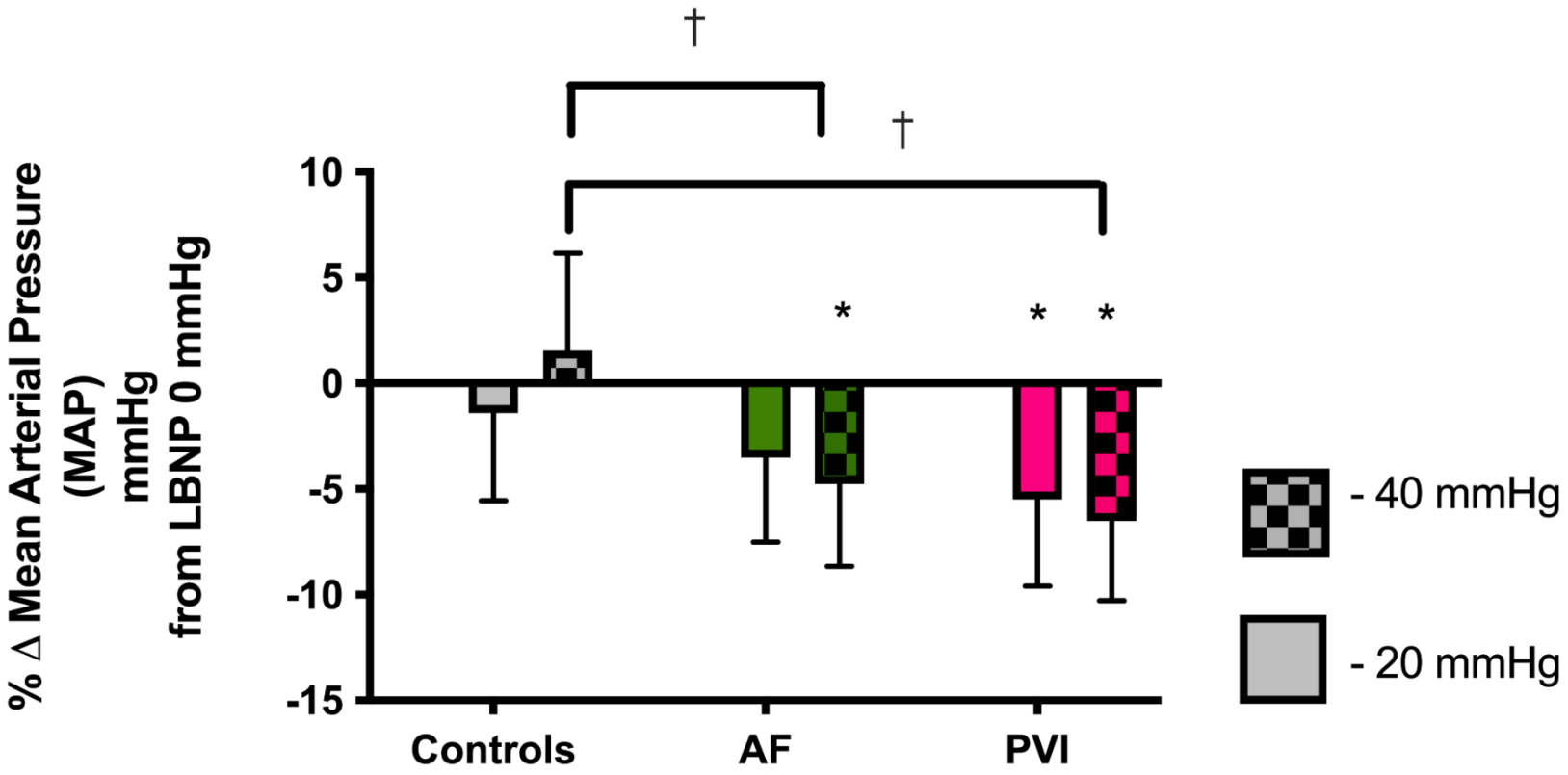
	Controls (n=14)	PAF (n=20)	Successful PVI (n=14)	p-value
Age (years)	55±5	58±10	58±8	0.38
Males, n (%)	9 (64)	13 (65)	12 (86)	0.35
Mean body mass index, kg/m^2	25.6±4	30.3±5	31.0±6	0.02*
Hypertension n (%)	0 (0)	8 (57)	7 (50)	0.99$^\#$
Patients not on any AAD, n (%)	14 (100)	4 (20)	9 (64)	0.01$^{*\#}$
ß-blockers (except Sotalol), n (%)	0 (0)	7 (35)	5 (36)	0.99$^\#$
Sotalol, n (%)	0 (0)	8 (40)	0 (0)	0.01$^{*\#}$
Amiodarone, n (%)	0 (0)	0 (0)	0 (0)	-
Flecainide, n (%)	0 (0)	6 (30)	0 (0)	0.03$^{*\#}$
Digoxin, n (%)	0 (0)	1 (5)	0 (0)	0.99$^\#$
Calcium channel blocker, n (%)	0 (0)	0 (0)	0 (0)	-

AAD, Anti-arrhythmic drugs. Baseline parameters of the PAF and Control groups are presented here for reference (they were presented in Chapter 3).

Haemodynamic responses to LBNP: Successful PVI group MAP response

Baseline MAP was similar across all groups: 97±3 mmHg in the control group, 100±3 mmHg in the PAF group and 98±3 mmHg in the successful PVI group. In the successful PVI group, MAP was significantly lower at -20 mmHg and at -40 mmHg LNBP (Figure 6B, both $p<0.01$). The MAP response to LBNP was different in the successful PVI group ($p=0.03$) in comparison to control subjects. However, this was not different to the PAF group ($p>0.99$).

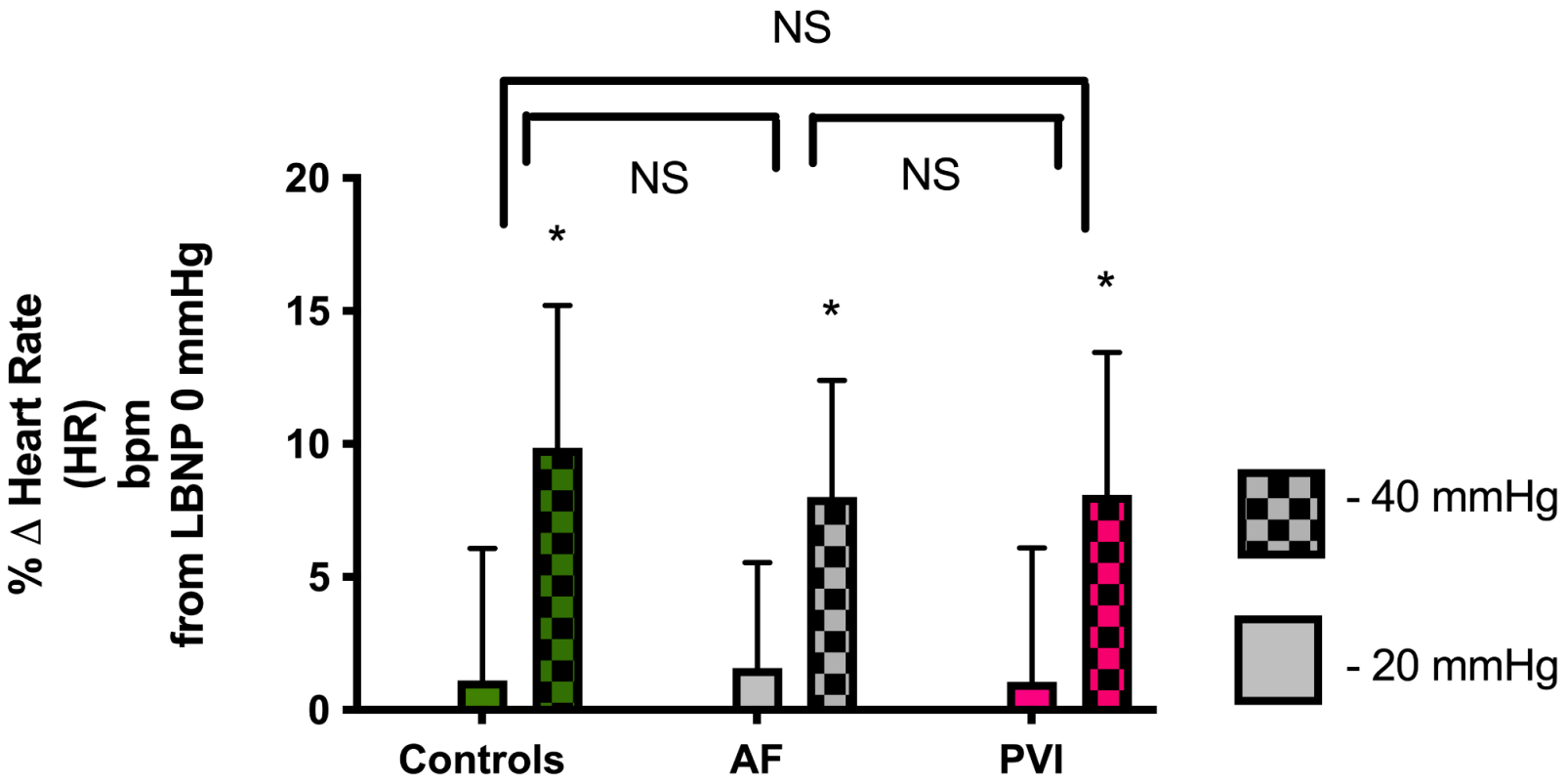
Figure 6B: MAP responses to LBNP in the Successful PVI group



Percentage change in MAP with LBNP (-20 and -40 mmHg) for each group expressed as mean±SEM. *Statistical difference in comparison to baseline at 0 mmHg LBNP ($p<0.05$). †Indicates statistically significant change in response to LBNP between groups ($p<0.05$). NS: non-significant.

Baseline HR was similar between groups (61±2 vs. 57±2 vs. 62±2 bpm in the control vs. PAF vs. post-PVI group respectively, p=0.4). HR did not change at -20 mmHg LNBP, however at - 40 mmHg LBNP there was significant increase in HR consistently in all groups. There was no statistical difference in the HR response to LBNP between the successful PVI and controls or the PAF group. Figure 6C.

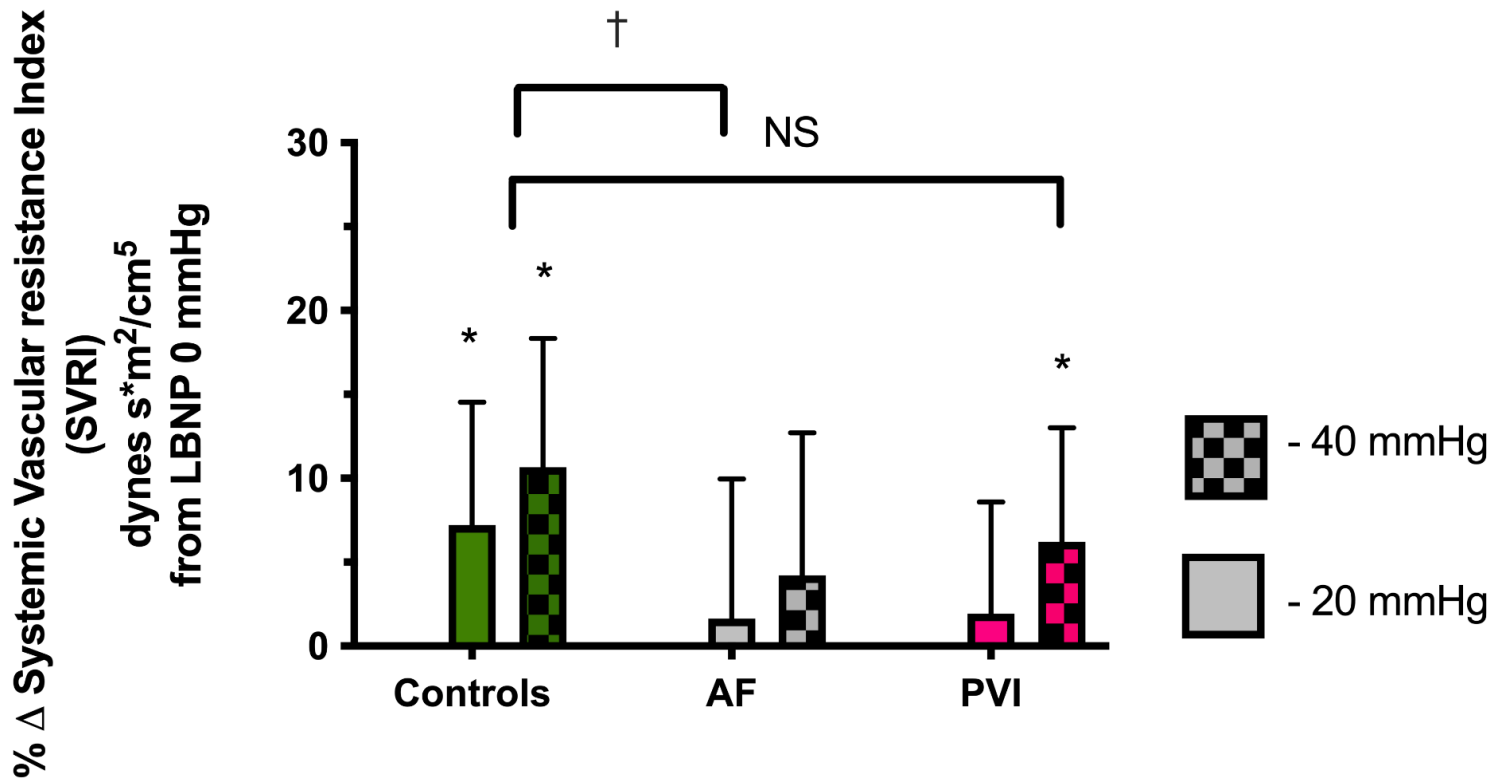
Figure 6C: HR responses to LBNP in the Successful PVI group



Percentage change in HR with LBNP (-20 and -40 mmHg) for each group expressed as mean±SEM. *Statistical difference in comparison to baseline at 0 mmHg LBNP ($p<0.05$). †Indicates statistically significant change in response to LBNP between groups ($p<0.05$). NS: non-significant.

In controls, there was a significant increase in SVRI at both -20 and -40 mmHg LBNP (both $p<0.01$). In the post-PVI group, significant increase in SVRI was only evident at -40 ($p<0.01$) but not -20 mmHg ($p=0.4$) LNBP. Overall SVRI response to LNBP was different between the PAF and control groups ($p=0.04$), where there was an inadequate LBNP response, but not between the post-PVI and control subjects ($p=0.12$). Figure 6D.

Figure 6D: SVRI responses to LBNP in the Successful PVI group



Percentage change in mean SVRI with LBNP (-20 and -40 mmHg) for each group expressed as mean±SEM.*Statistical difference in comparison to baseline at 0 mmH*g* LBNP ($p<0.05$). †Indicates statistically significant change in response to LBNP between groups ($p<0.05$). NS: non-significant.

Table 6B shows the CI and SVI at baseline and after application of -20 and -40 mmHg LBNP as well as the other haemodynamic parameters (MAP, HR and SVRI). CI and SVI were similar at baseline and changed similarly in all 3 groups following application of LBNP at -20 and -40 mmHg. Changes induced by LBNP were statistically significant within each group, but responses were not different between groups (all $p>0.05$).

Table 6B: Raw haemodynamic data including cardiac index & stroke volume index

	LBNP (mmHg)	Controls (n=14)	PAF (n=20)	p-value (vs. Controls)	Successful PVI (n=14)	p-value (vs. Controls)	p-value (vs. PAF)
Mean arterial pressure (*mmHg*)	0	97± 3	100 ± 3	0.04†	98 ± 3	0.03†	NS
	-20	96 ± 3	97 ± 3		93 ± 3**		
	-40	99 ± 3	96 ± 3*		92 ± 2**		
Heart rate (*bpm*)	0	61 ± 2	57 ± 2	NS	62 ± 2	NS	NS
	-20	61 ± 2	58 ± 1		62 ± 4		
	-40	67 ± 2**	61 ± 2***		67 ± 3*		
Systemic Vascular Resistance Index (dynes s* m2/cm5)	0	2720 ± 132	3131 ± 198	0.04†	2740 ± 135	NS	NS
	-20	2916 ± 140	3182 ± 166		2793 ± 120		
	-40	3010 ± 150	3263 ± 167		2910 ± 120		
Cardiac Index ($L/min/m^2$)	0	2.89 ± 0.09	2.70 ± 0.12	NS	2.92 ± 0.10	NS	NS
	-20	2.69 ± 0.08**	2.51 ± 0.09**		2.69 ± 0.09***	NS	
	-40	2.62 ± 0.09**	2.43 ± 0.09***		2.55 ± 0.09***	NS	
Stroke Volume Index ($mL/min/m^2$)	0	45.3 ± 2.1	47.9 ± 1.6	NS	47.5 ± 1.4	NS	NS
	-20	41.8 ± 2.1***	43.8 ± 1.5***		43.5 ± 1.5***	NS	
	-40	37.2 ± 2.5***	39.8 ± 1.5***		38.7 ± 1.6***	NS	

Within group differences: * p< 0.05, ** p< 0.01, *** p< 0.001. Between group differences: †Indicates statistically significant change in response to LBNP between groups (p<0.05). NS: non-significant.

Summary of findings

This study evaluated the cardiac reflex response to LBNP in patients after successful PVI and compared them to healthy control subjects as well as patients pre-PVI (as presented in Chapter 3). Our principal findings are as follows:

Whilst vasoconstrictor responses to LBNP were absent in PAF patients (Chapter 3), no statistical differences were seen between the successful PVI subjects (who were free from the arrhythmia) and the control group. This suggests that whilst there may be some improvement, there is no evidence of further deficit due to PVI.

LBNP lowered both SVI and CI to a similar extent in all the groups, indicating that an equivalent stimulus was applied in each group. Blood pressure was maintained at both levels of LBNP in the control subjects but not in the AF or successful PVI groups in whom MAP fell progressively. This indicates a clear reflex deficit in subjects with AF studied in sinus rhythm, that was not rectified by successful ablative treatment of PAF in the post-PVI group in this study.

The main limitation of this pilot work is that there were different patient cohorts, thus it is not possible to distinguish the effect of PVI from other confounding differences between the two groups. Thus, this is not the best design to test the effect of PVI.

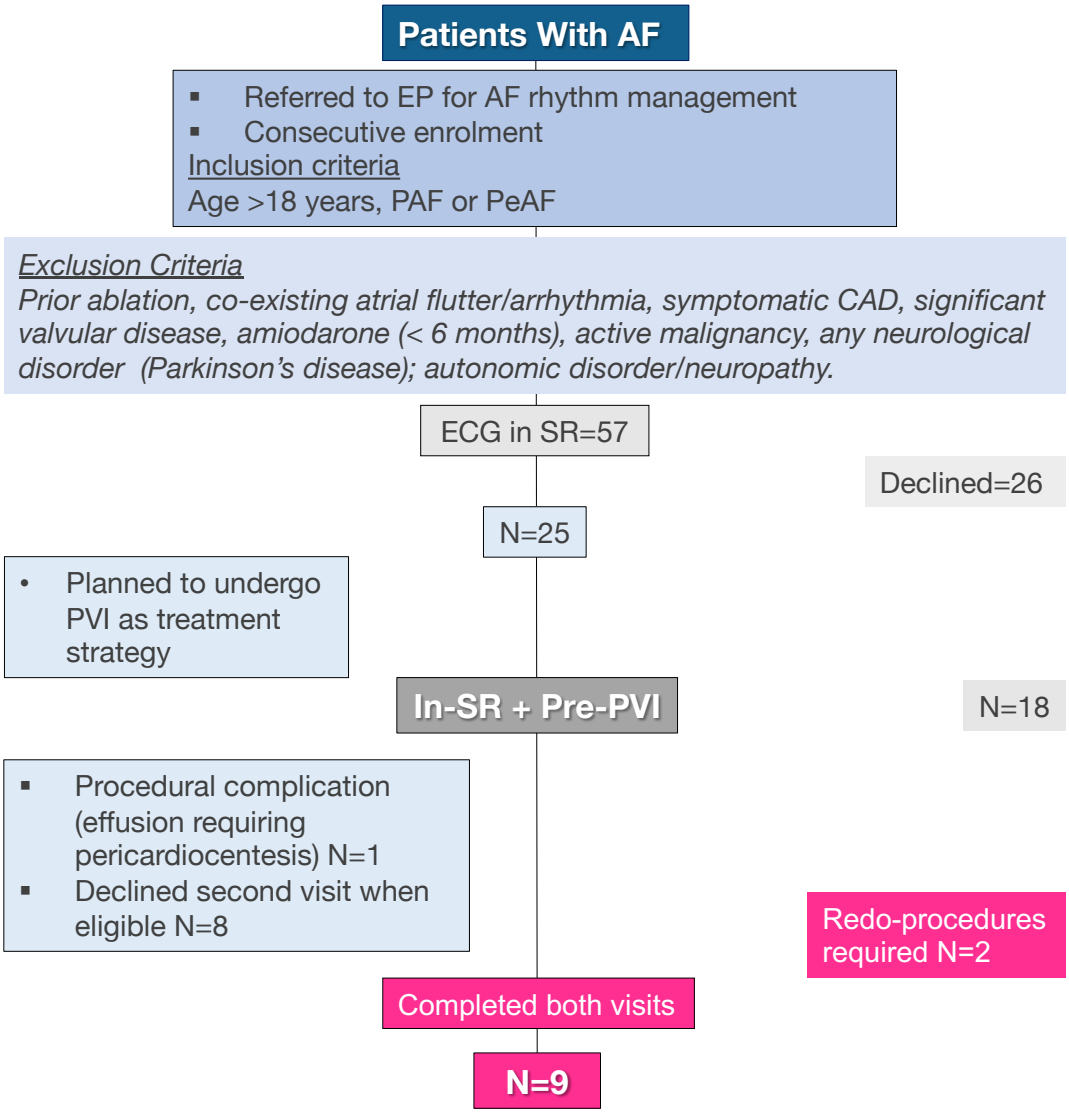
Clinical autonomic reflex tests in a cohort of patients undergoing PVI

18 patients with Paroxysmal or persistent AF who were referred for rhythm management were selected by their treating cardiac electrophysiologist and consented to undergo a catheter-based procedure for their procedure. From these, a cohort of 9 patients were eligible and consented to both study visits. This is presented in Figure 6E: **CONSORT diagram**.

The mean age of the cohort was 64±3 years (78% male); BMI 28±1 kg/m^2; LA size 37±2 ml/m^2; and left ventricular function 65±3%. Baseline (supine, resting) heart rate pre-PVI was 58±5 bpm and post-PVI was 67±3 (P=0.1). Lesion sets that were performed during their AF-ablation procedure were as follows: single ring radiofrequency PVI of all 4 PV and the posterior wall (Si-R-PVI, n=7); cryoablation (n=1) and wide antral PVI (n=1). A redo procedure after initial recurrence was performed in n =2. In these patients, the second study visit was performed if >6 months AF-free after the second procedure. PV±PW entrance/exit block confirmed in all. Additional lesions (which were performed at the operator's discretion) were: further segmental PVI in (n=1); posterior wall lesions in (n=1), and a mitral isthmus line as well as ablation of atrial low-amplitude signals (scar), both, in (n=1).

Symptom and rhythm (12-lead ECG, 4–7-day Holter) follow up (1 week, 1, 3 and 6 months) showed no clinical recurrence in 7of these 9 patients. In the two patients that underwent redo-procedures, mapping demonstrated gaps in the previous lines and reconnection into the single ring in (n=1) and PV reconnection in the patient who originally underwent wide antral PVI (n=1). In both cases, clinical follow up visits and ECG monitoring were repeated after their second ablation procedure at the same time points as above. Baseline characteristics of the cohort are presented in Table 6C.

Figure 6E: CONSORT Diagram



Consort diagram of patient recruitment and establishment of a cohort with completed pre- and post-PVI visits, where PVI resulted in freedom from AF >6months.

Table 6C: Baseline characteristics of the PVI cohort

Baseline Characteristics	**PVI cohort n=9**
Age	63 ± 2
Males; n(%)	7 (78)
Body Mass Index (kg/m2)	28 ± 1
Resting MAP	90 ± 4
Resting HR	67 ± 2
LVEF (%)	65 ± 3
LAVI (ml/m2)	37 ± 2
Ablation lesion sets	
Single ring (PVI and posterior wall)	7(78)
PVI only (cryotherapy)	1(11)
Wide antral PVI (RF)	1(11)
Redo due to recurrence during study	2 (22)
Risk factors	
Hypertension, n(%)	5(56)
Dyslipidaemia	1(11)
Vascular disease	1(11)
OSA	3(33)
Alcohol excess	3(33)
Medications	
ß-blockers (except Sotalol), n(%)	5(56)
Sotalol, n(%)	2(22)
Flecainide, n(%)	3(33)
Central CCB, n(%)	1(11)
Digoxin, n(%)	0(0)
ARB/ACEi	4(44)
Dihydropyridine CCB, n(%)	1(11)
Thiazide diuretic	1(11)

MAP, Mean arterial pressure; HR, Heart rate; LVEF, Left ventricular ejection fraction; LAVI, Left atrial volume indexed to body surface area; CCB, Calcium channel blockers; ARB, Angiotensin receptor blockers; ACEi, Angiotensin-converting enzyme inhibitors.

Submaximal isometric handgrip reflex

Table 6D contains haemodynamic responses to IHR pre- and post-PVI. IHR increased MAP similarly both pre-PVI and post-PVI (+9±2% and +10±3; P=0.99). HR increased slightly pre-PVI in response to IHR (+2±2%) and similarly post-PVI (+2±1%). HR response to IHR was not significant between time points (P=0.99). Figure 6F.

Table 6D: Haemodynamic responses to IHR Pre and Post PVI

	Pre PVI (n=9)		Post PVI (n=9)		P Value
IHR	Pre-IHR	Post-IHR	Pre-IHR	Post-IHR	
MAP	91±6	100±8	94±7	102±6	0.99
SBP	137±11	149±12	138±7	151±6	0.5
DBP	70±5	75±6	73±6	77±5	0.99
HR	58±3	60±4	73±3	75±3	0.99

Data±SEM. Differences between time points (pre- and post-PVI) are represented by P values in the adjacent column. MAP, Mean Arterial Pressure (mmHg); SBP, Systolic Blood Pressure (mmHg); DBP, Diastolic Blood Pressure (mmHg); HR, Heart rate (bpm); $P<0.05$.

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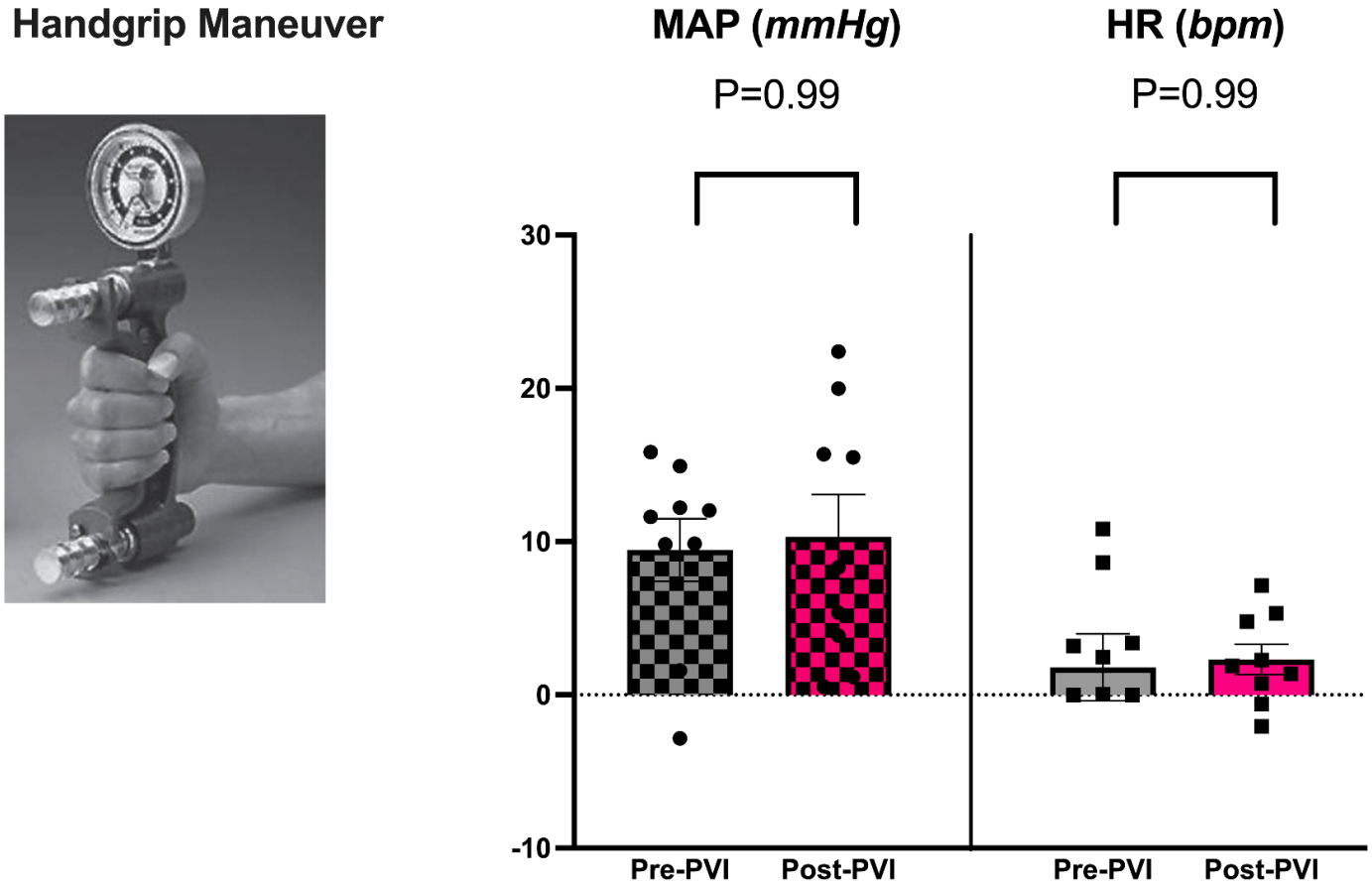
Figure 6F: MAP & HR responses to IHR Pre & Post PVI



Data±SEM. MAP, Mean Arterial Pressure (mmHg) & HR, Heart rate (bpm). IHR haemodynamic responses were compared to corresponding baseline value prior to IHR and were expressed as percentage change. Pre and post comparisons for IHR; $P<0.05$.

Valsalva reflex

Haemodynamic data at each of the two autonomic phases – II and IV are presented in Table 6E. During phase II, pre-PVI MAP increased by +27±7% and post-PVI MAP increased by 22±6%. There was no statistical difference between time-points (P=0.6). HR also increased equivalently between time points (P=0.99). HR increased +21±5% and 11±7% pre- and post-PVI respectively. Figure 6G.

During phase IV, there was an overshoot of both MAP and HR at both time-points (P=0.4 & P=0.7, respectively) as presented in Table 6E.

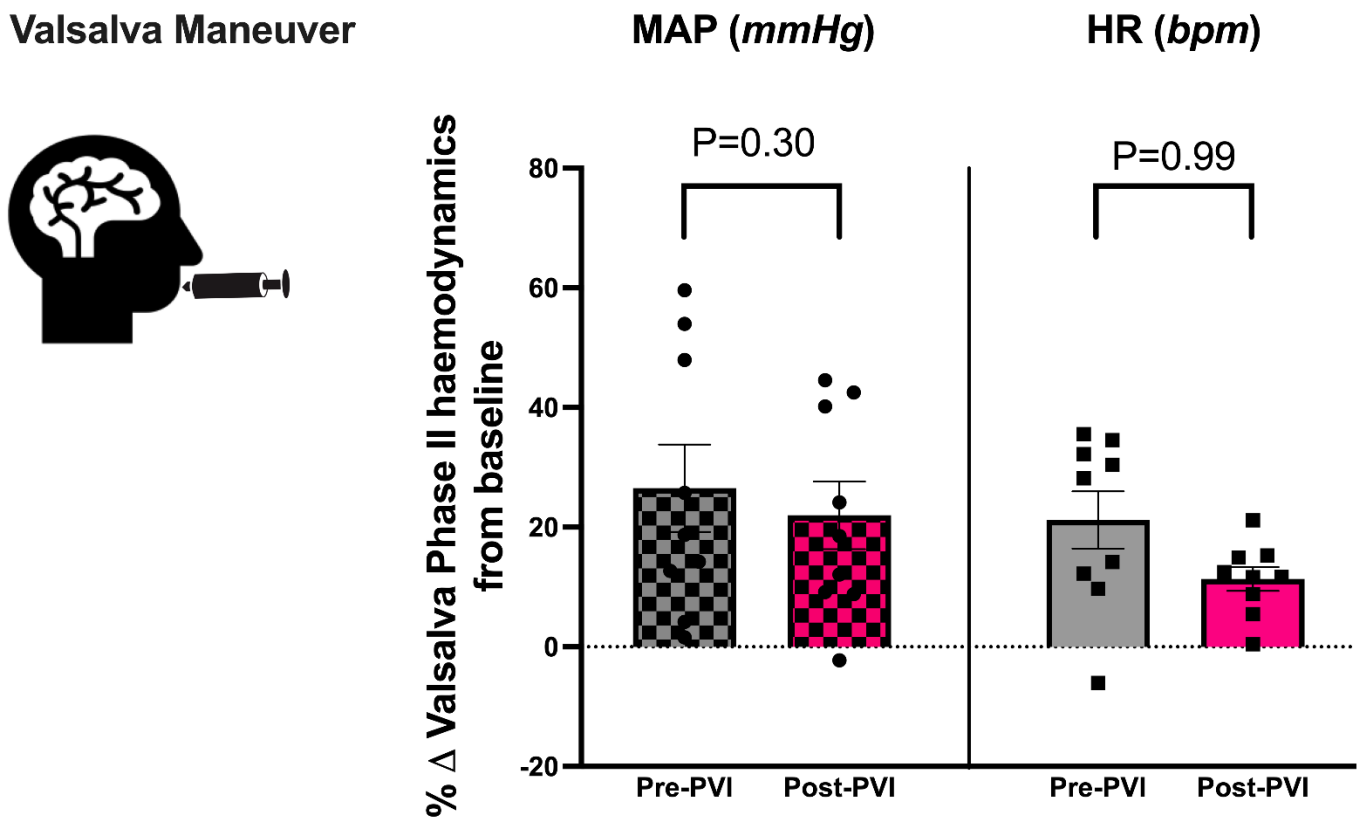
This, similar to our findings in Chapter 4, Valsalva responses were generally preserved in patients with AF, studied in sinus rhythm.

Table 6E: Haemodynamic responses to Valsalva Pre & Post PVI

	Pre PVI (n=9)		Post PVI (n=9)		P Value
Valsalva II	II_{EARLY}	II_{LATE}	II_{EARLY}	II_{LATE}	
MAP	94±8	120±14	94±5	118±10	0.6
SBP	122±11	148±17	124±11	130±9	0.1
DBP	86±8	105±12	86±5	103±10	0.99
HR	73±5	85±5	83±5	91±5	0.2
Valsalva IV	$IV_{BASELINE}$	$IV_{OVERSHOOT}$	$IV_{BASELINE}$	$IV_{OVERSHOOT}$	
MAP	88±6	125±11	92±6	123±7	0.4
SBP	133±10	187±16	136±17	177±9	0.1
DBP	68±5	89±9	72±6	90±5	0.8
HR	58±3	69±5	72±3	82±3	0.7

Data±SEM. Differences between time points (pre- and post-PVI) are represented by P values in the adjacent column. MAP, Mean Arterial Pressure (mmHg); SBP, Systolic Blood Pressure (mmHg); DBP, Diastolic Blood Pressure (mmHg); HR, Heart rate (bpm). Valsalva phases were compared to corresponding baseline value prior to Valsalva and were expressed as percentage change. Between group comparisons for Valsalva for each autonomic phase: $II_{LATE} - II_{EARLY}$ or $IV_{OVERSHOOT} - IV_{BASELINE}$ were compared between time points (Pre vs Post-CV; $P<0.05$).

Figure 6G: MAP & HR responses to Valsalva Pre & Post PVI



Data±SEM. MAP, Mean Arterial Pressure (mmHg) & HR, Heart rate (bpm). Valsalva phases were compared to corresponding baseline value prior to Valsalva and were expressed as percentage change. Pre and post comparisons for Valsalva for Phase II (autonomic phase: $II_{LATE} - II_{EARLY}$; $P<0.05$).

Lower Body Negative Pressure reflex

During LBNP, MAP response was similar pre- (+1.0±4%) and post-PVI (-2.8±2%); P=0.3. HR increased similarly (P=0.3) pre- (16±7%) and post-PVI (7±2%). FBF response, was unchanged (P = 0.9), Figure 6H. FBF decreased pre-PVI (-16±18%) and post-PVI (-21±10%). FVC also decreased pre-PVI (-18±17%) and post-PVI (-19±11%).

At both time-points, this cohort had diminished responses compared to a reference group without AF, presented in Chapter 4.

Table 6F: Haemodynamic responses to LBNP Pre & Post PVI

	Pre PVI (n=9)			Post PVI (n=9)			P Value
LBNP Level	0	-20	-40	0	-20	-40	
MAP	89±5	90±6	90±7	93±5	92±5	91±5	0.3
SBP	130±8	128±9	126±10	139±7	135±7	132±7	0.8
DBP	69±5	72±4	73±5	71±4	72±5	73±5	0.4
HR	58±5	59±4	66±3	67±3	69±3	71±3	0.3
FBF	1.55±0.2	1.09±0.2	1.17±0.2	1.87±0.3	1.56±0.2	1.31±0.1	0.9
FVC	1.77±0.3	1.24±0.2	1.30±0.3	2.08±0.4	1.73±0.2	1.46±0.2	0.99

Data±SEM. Differences between the groups are represented by P values in the adjacent column. MAP, Mean Arterial Pressure (mmHg); SBP, Systolic Blood Pressure (mmHg); DBP, Diastolic Blood Pressure (mmHg); HR, Heart rate (bpm); FBF, Forearm Blood Flow (%/min); FVC, Forearm vascular conductance (100*FBF/MAP, arbitrary units). $P<0.05$.

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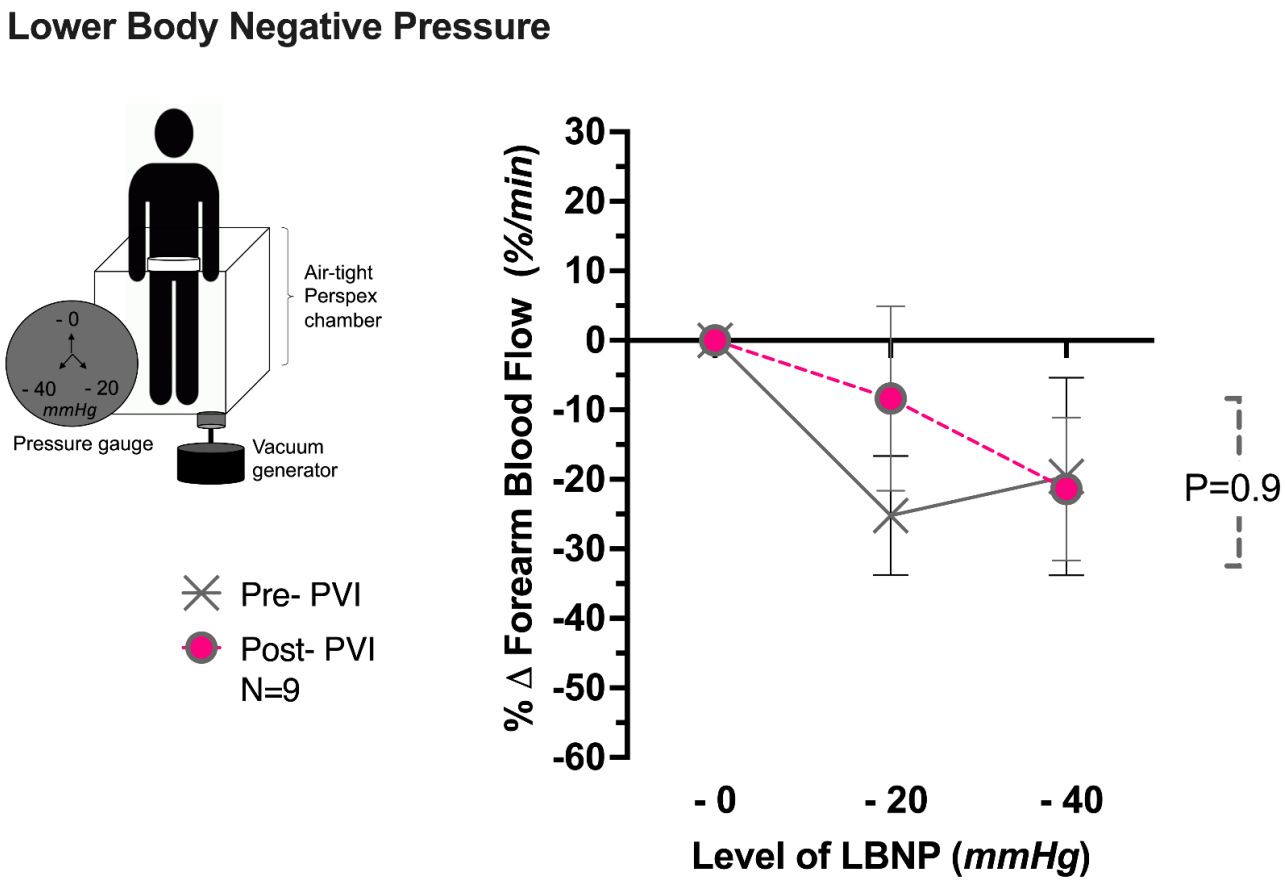
Figure 6H: LBNP vasomotor (FBF) response pre vs post-PVI



Data±SEM. Forearm Blood Flow (FBF) response to LBNP – 20mmHg and – 40 mmHg negative pressure. Expressed as percentage change. Pre and post CV comparisons; P<0.05.

Discussion

A cohort of 9 patients prospectively identified and selected from 18 candidates for PVI underwent clinical autonomic testing before and at least 6 months after a successful PVI procedure. Our principal findings were:

1. There was a background level of autonomic remodelling, exhibited by diminished LBNP reflex response, seen in this cohort similar to the larger cohort of patients with AF studied in SR in Chapter 4 as well as the (separate) cohort, presented in Chapter 3.
2. PVI did not result in further disruption of any of the autonomic reflexes tested and it did not alter this background level of remodelling in terms of LBNP and IHR. Valsalva responses remained intact.
3. Although there was a small increase in baseline HR, measured supine, post-PVI; this was not statistically significant.

We performed three tests of cardiovascular autonomic function- which predominantly assess cardiovascular afferent or regulatory integrity: IHR, Valsalva manoeuvre and LBNP. LBNP (low-level) predominantly tests cardio-pulmonary reflexes from low-pressure (volume-regulating) baroreceptors, Valsalva predominantly tests arterial high-pressure (blood-pressure regulating) baroreceptors and IHR, a muscle somato-sympathethic reflex is modulated by both types of baroreceptors. We have identified (Chapter 4) a severe deficit (dysfunction) of the low-pressure cardiopulmonary baroreflexes that ameliorate with restoration of sinus rhythm (Chapter 5). We have also shown that there is a diminished response to LBNP (cardiopulmonary baroreflexes) in patients with AF studied in sinus rhythm from two

separate cohorts (Chapter 3 and Chapter 4) and, indeed, this background level of autonomic remodelling is seen even within this cohort, however neither of the autonomic reflexes that we have studied are altered by PVI in this study.

The anatomic co-location of the triggering ectopic atrial beats in AF[11] with autonomic afferent (cardiopulmonary, low-pressure, volume-regulating) baroreceptors,[106, 108] which are predominantly responsible for the LBNP reflexes that we have studied;[97] intracardiac neurons[53] and ganglionated plexi[136, 292] are all potentially affected either by the presence of AF (as we have previously shown) as well as by the effect of destruction of this tissue by PVI. Indeed, several studies have identified changes in the autonomic nervous system following ablation (rise in heart rate and accompanying changes in heart rate variability), regardless of the method of ablation performed and that these changes are associated with the success of the procedure.[289, 291]

Specific autonomic ablation targets (such as targeting ganglionated plexi) through the recognition of complex fractionated atrial electrograms,[35] stimulation to produce vagal effects such as sinus bradycardia, atrioventricular block, asystole or hypotension[289] or anatomically choosing sites of ablation,[223] when added to PVI are all associated with increased efficacy of the procedure in maintaining sinus rhythm. Indeed, there is inadvertent neuronal damage of such ganglionated plexi from PVI itself, owing to their close proximity with the pulmonary veins in the posterior left atrium, where it is postulated that perhaps procedural success in AF ablation may be partly attributable to damaging these neuronal collections in the atrium.[136] However, the mechanism surrounding this association is not clear. It is possible that there may be some direct link between such autonomic effects and successful outcomes, or, the alternate explanation is that changes in sinus rate (increases) as well as enhanced sympathetic

balance on sino-atrial control (estimated using spectral heart rate characteristics, or heart-rate variability),[291, 293] are simply a marker of adequate ablation in these areas to produce bidirectional block into and from the pulmonary veins (which is the standard end point of PVI).

The specific autonomic mechanism responsible for the initial "vagal" response - bradycardia, atrio-ventricular block, asystole or hypotension during ablation and ultimate tachycardia from vagal withdrawal (and unbalanced sympathetic activation) could be due to direct stimulation and then damage of the vagal efferent nerves that innervate the sinoatrial node and pass through the epicardial regions surrounding the pulmonary vein-atrial complex and indeed where they may converge in ganglia containing parasympathetic postganglionic neurons to the sino-atrial and atrioventricular nodes.[141, 294]

Aside from the potential efferent mechanisms directly affecting sino-atrial node function, there are several afferent receptors that could cause similar effects. One such afferent receptor could be a sympathetic afferent terminal (forming a network of unmyelinated nerve endings, called C-fibres) that, when stimulated could heighten vagal activity by depressing baroreceptor function, but when destroyed, would result in increased sympathetic balance (and parasympathetic withdrawal) due to its positive feedback nature on sympathetic efferents and its inhibiting effect on parasympathetic efferent control of the heart.[61, 78, 293, 295] Such cardiac sympathetic afferent reflexes are also known as cardio-cardiac reflexes and represent an example of the hierarchical control of the autonomic nervous system of the heart.[52] Alternatively, type B atrial receptors[93] that respond to both decreases as well as increases in blood volume, and are postulated to be low-pressure volume-regulating

cardiopulmonary receptors[93, 97, 108]could be influenced by PVI. This, therefore, was the rationale behind our hypothesis in this study.

LBNP serves to decrease blood volume and these atrial receptors[93, 97, 108] whereas atrial filling (or distension, or perhaps mechanical pressure and then thermal injury from catheters used to ablate the atrial-pulmonary vein complex during PVI) could evoke opposite reflexes and would be expected to cause an impairment in the vasomotor LBNP response. In this study, we have not directly assessed the effect of catheter instrumentation in the heart, or on atrial distension, or mechanical pressure, on these reflex responses. We have sought to answer the question as to whether PVI, which can cause longer term changes in heart rate variability (and heightened sympathetic activity or withdrawal of parasympathetic effects on the sino-atrial node)[289, 291, 293] could also cause deficits in the LBNP response via effects on these volume-regulating low-pressure baroreceptors. As identified, physiologically as Type B Paintal receptors[93, 103] and histologically as myelinated receptors with unmyelinated, arborised endings.[106, 108]

In this small, single-centre cohort of 9 patients, who predominantly had Si-R-PVI, we have not identified any reflex deficit post-PVI with either IHR, Valsalva or LBNP reflexes. This study implies that prior findings of autonomic changes following PVI[289, 291, 293] are not mediated by cardiopulmonary (Paintal Type B) volume-regulating low-pressure baroreceptors. Additionally, there does not appear to be any cardiovascular autonomic reflex deficit (of either baroreceptors, or of regulatory systems) attributable to PVI. In stark contrast, we have demonstrated changes in cardiac afferent reflex function due to the presence of AF, itself, and a background level of remodelling even in sinus rhythm, that PVI does not ameliorate.

Limitations

There are several limitations to this study. First and fore-most, this was a small, single centre study where the predominating lesion set was Si-R-PVI. In choosing patients with a successful clinical outcome, we have mostly eliminated the possibility of an ineffectual ablation, however, this reduced our sample size. We had frequent follow-up, which included multi-day telemetry and clinical consultation. This was a requirement for inclusion in the final cohort. However, we accept that the absence of continuous monitoring with implantable devices is a limitation. We did not acutely measure autonomic function during PVI. This would have been difficult with the equipment required; however, we have not assessed heart rate variability during the procedure in this study, nor did we document any vagal effects during the case produced by ablation. Although this is a limitation, this does not influence our study results and conclusion. Further, whether or not there are acute baroreflex changes during PVI, which may suggest a role of cardiac sympathetic afferent reflexes[61] in the initial vagal stimulation, followed by depressed parasympathetic function and heightened sympathetic tone seen in prior studies following PVI,[289, 291, 293] has not been addressed in our study. Finally, we have focused on potential longer-term effects of PVI, rather than on transitory effects.

Concluding remarks

Clinical implications

These results are encouraging in that wide-antral PVI lesions are not associated with depressed autonomic reflex function. On the other hand, the background level of autonomic dysfunction seen in AF patients studied in sinus rhythm is not ameliorated after PVI alone and therefore, there is need to further understand the mechanisms behind these persisting abnormalities. First, to identify whether these abnormalities are indeed due to AF itself. Although. we have identified clear reflex dysfunction with LBNP during AF and we have observed reflex deficits in AF patients studied in sinus rhythm in two separate studies (cohorts) and so this would seem less likely. Next, we need to understand how these persisting reflex deficits in sinus rhythm contribute to AF pathophysiology in terms of progression of disease, development of clinical sequelae of AF (such as falls, orthostatic intolerance, cognitive impairment, and heart failure) and finally, we need to explore whether neuromodulatory treatments such as low-level tragus nerve stimulation[38, 296] could influence such autonomic reflex abnormalities.

Conclusion

Cardiovascular reflex tests of afferent integrity, in particular, reflex responses to decreased cardiac volume, elicited by cardiopulmonary low-pressure baroreceptors (found in pulmonary vein-atrial junctions) are not altered by PVI. More work to assess specific lesion sets, or targeted ablation of ganglionated plexi, is needed to ensure absence of adverse autonomic remodelling due to catheter ablation for AF.

Chapter 7: Low-level Tragus Nerve Stimulation (LLTS) can reverse abnormalities of the cardiac volume-sensitive reflex due to Atrial Fibrillation: Impact of neuromodulation strategies

Background

The role of neuromodulatory therapies for the management of AF have long been contemplated[52], especially given prior data demonstrating that efferent autonomic activity can trigger AF.[23, 28] Specific potential neuromodulating techniques for the management of AF are undergoing clinical research, such as Low-Level Tragus Nerve Stimulation (LLTS)[38, 277], ganglionated plexus ablation[223], stellate ganglion blockade, renal denervation[149] and epicardial botulism toxin injection.[297-299] There are also some preclinical studies assessing the role of spinal cord stimulation and low level baroreceptor activation therapy.[52] However, neither the precise mechanisms by which they exert their anti-arrhythmic effect, nor their precise effects on the autonomic nervous system are well known.

We have proposed in Chapter 1 that there are multiple avenues, "roads" by which the autonomic nervous system can cause and be mediated in the management of risk factors for AF to alter its progression. Others also indicate that autonomic neuromodulation strategies are "inadvertently" performed pulmonary vein isolation procedures using catheter-based radiofrequency or cryotherapeutic techniques, particularly by damaging ganglionated plexi in the left atrium/pulmonary vein junctions of the heart.[136] Thus, neuromodulation already occurs with multiple therapies that we employ as standard in the management of AF and may contribute to the success of each of these therapies.

LLTS is a relatively new technique with single centre, sham-controlled, double-blinded randomised data to suggest that it decreases AF burden in some individuals.[38] By stimulating the tragus branches of the vagus nerve at low-levels, it is thought to exert its anti-arrhythmic effects by anti-adrenergic and inflammatory mechanisms.[38, 277] What is most interesting is that in this study, TREAT-AF by Stavrakis *et al*.[38] there was an effect that persisted beyond the stimulus and thus it is most likely that there are autonomic remodelling changes, that assist in the reduction of AF burden. The authors performed either sham (n=26) or treatment (n=27) using a transcutaneous nerve stimulator attached to a clip. Treatment (clip placed on the tragus of the ear); which is where nerve endings of the vagus nerve reside, can be stimulated painlessly in this way.[277] Sham procedure stimulated the ear lobe which does not contain any vagal nerve endings.[38] Treatment (tragus, cutaneous nerve activation using this transcutaneous electrical stimulator) was performed for 1 hour a day over a 6-month period. They found that AF burden (2-week electrocardiographic monitoring at baseline, 3 months, and 6 months) decreased by 85% in the active arm compared to placebo from baseline. Heart rate variability alterations as well as reduction in inflammatory markers were observed.[38]

Markers that are associated with the structural substrate involved in AF development (P-wave alternans on electrocardiographic monitoring over 5-minute samples) seemed to predict the utility of LLTS, thereby adding to interplay between anatomic, substrate based remodelling and autonomic remodelling.[296] P-wave alternans is a beat-to-beat variability of the morphology of the atrial “P” wave on electrocardiography.[296] In an animal model of heart failure with preserved ejection fraction, LLTS was associated with attenuation of cardiac inflammation and fibrosis as well as improvements in diastolic function.[300] In humans with heart failure and preserved ejection fraction, acute LLTS appears to improve echocardiographic left ventricular function parameters (global longitudinal strain) and thus,

the structural components of cardiac function appear very much to be intrinsically linked to autonomic function.[301]

We have presented, for the first time, evidence of afferent or regulatory abnormalities in blood volume regulation in the presence of AF (Chapter 4), which are reversible after restoration of sinus rhythm (Chapter 5). This reversibility strongly implicates AF as a cause of this abnormality. Not only is there complete dysfunction during AF, we have also identified in two separate cohorts of patients with AF, persisting abnormalities of the volume-regulating LBNP reflex even in-SR (Chapter 3 and Chapter 4). This suggests a background level of afferent or regulatory abnormality that could potentially underlie the progression of AF.[6] We have furthered this by investigation by examining other reflexes such as the Valsalva manoeuvre and isometric sustained handgrip in order to assist localisation of such abnormalities to dysregulation of cardiopulmonary (low-pressure) baroreflexes initiated by Lower Body Negative Pressure (LBNP). Our work, together with the finding of these reflex abnormalities in cardiac structural diseases such as heart failure and hypertension with left ventricular hypertrophy,[54, 55] suggests that a link between structural abnormalities that are involved with AF, such as heart failure, as well as structural enlargement of the left atrium, which on its own, is associated with the progression of AF.[285]

The autonomic effects of LLTS are incompletely understood[302] In this Chapter, we assess the effect of LLTS in the modulation of such abnormalities in reflex control of blood volume, as assessed by LBNP. In patients with AF, either in-AF or in-SR, we performed LLTS and acutely measured the autonomic effect of this on LBNP responses in these patients. We primarily utilised LLTS to determine whether its effect on vagus nerves upstream of the vasomotor nerves that elicit the motor component of the vasomotor (vasoconstriction effect)

of LBNP at the level of the blood vessels could potentiate the reflex, by passing the afferent volume information-gathering cardiopulmonary low-pressure baroreceptors found in the veno-atrial (pulmonary vein-left atrial junctions) of the heart[97, 106, 108] and therefore provide assistance to us in the further localisation of the abnormalities that we have detected.

Methods

Study population

The study comprised consecutively enrolled patients referred to the Centre for Heart Rhythm Disorders with paroxysmal or persistent AF. The following were exclusions: amiodarone (preceding 6 months); active malignancy; symptomatic coronary artery disease; significant valvular disease; neurological disorders (Parkinson's disease, autonomic disorders, neuropathy); prior ablations; other arrhythmias; inability to enter a lower body chamber (frail or >120kg); or inability to withhold anti-arrhythmic/anti-hypertensives.

We studied AF patients in 2 groups: during sinus rhythm, SR (in-SR) or during AF (in-AF) according to their rhythm at the time of testing. Here, we examined autonomic reflexes before and after LLTS, in those who consented to additional same-day testing of LLTS after the main clinical autonomic reflex protocol.

All participants provided written informed consent and the study was approved by the University of Adelaide human research ethics committee. This study was prospectively registered with the Australian New Zealand Clinical trials registry (ACTRN12619000186156).

Patient preparation, LBNP reflex testing protocol & LLTS

Patient preparation was identical to Chapter 4. In this study, we modified the LBNP testing protocol in Chapter 4, slightly. We attached a vagal nerve stimulator, using an electrode clip, to the right tragus (Parasym Health, London, United Kingdom). Similar to TREAT AF,[38] we determined the pain threshold first by increasing the current (mA) on the vagal stimulator.

Then, we set the current 1mA below this using the following settings: 20Hz at 200μs for 1-hour. LLTS was performed supine within the LBNP chamber at the end of the normal protocol, after which, we repeated LBNP at -40 mmHg only for 5 minutes, with the stimulator active during LBNP.

Haemodynamic parameters, Systolic blood pressure (SBP); Diastolic pressure (DBP); Mean Arterial Pressure (MAP); Heart rate (HR) were measured continuously and collected before (baseline LLTS) and after LBNP at -40 mmHg. Venous occlusion plethysmography parameters such as Forearm blood flow (FBF) and derived Forearm vascular resistance (FVC) responses to LBNP were assessed in an identical manner to that described in Chapter 4.

Statistical analysis

Continuous parameters were expressed as mean ± SEM. Categorical variables were expressed as frequencies and percentages and compared using Fishers exact test. Normality was checked and data compared using the students t-test or its non-parametric alternate. Data (pre vs. post) were compared using paired t-tests (normally distributed data) or Wilcoxon signed rank test (non-normally distributed data) as appropriate. Statistical analysis was performed using STATA 16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC) and GraphPad Prism (version 9.02, California, USA). Statistical significance was set at $P < 0.05$.

Results

10 patients with either paroxysmal or persistent AF, studied in SR and n=5 in-AF participated in this study assessing the effect of LLTS on low-level LBNP (assessing low-pressure, volume-regulating cardiopulmonary baroreceptor reflex function).

LLTS stimulation characteristics

In the in-SR group, mean LLTS pain threshold was 19±1mA and stimulation threshold was 18±1mA. In the in-AF group; mean LLTS pain threshold was 23±2mA and stimulation threshold was 22±2mA.

LLTS stimulation effects on baseline haemodynamic parameters

LLTS did not change resting HR in either in-SR (62±4.7 to 60±5.9bpm; P=0.2) or in-AF (95±7.4 to 94±5.3; P=0.7). Resting MAP after LLTS in-SR was higher (105±4.2mmHg, from 90±4.6; P=0.003). In-AF MAP did not change (110±18.8 to 108±4.7 mmHg; P=0.9). In-SR, the small decrease in MAP following LBNP was not significant (-6±5%; P=0.4) and HR did not reach statistical significance (+11±6%; P=0.1).

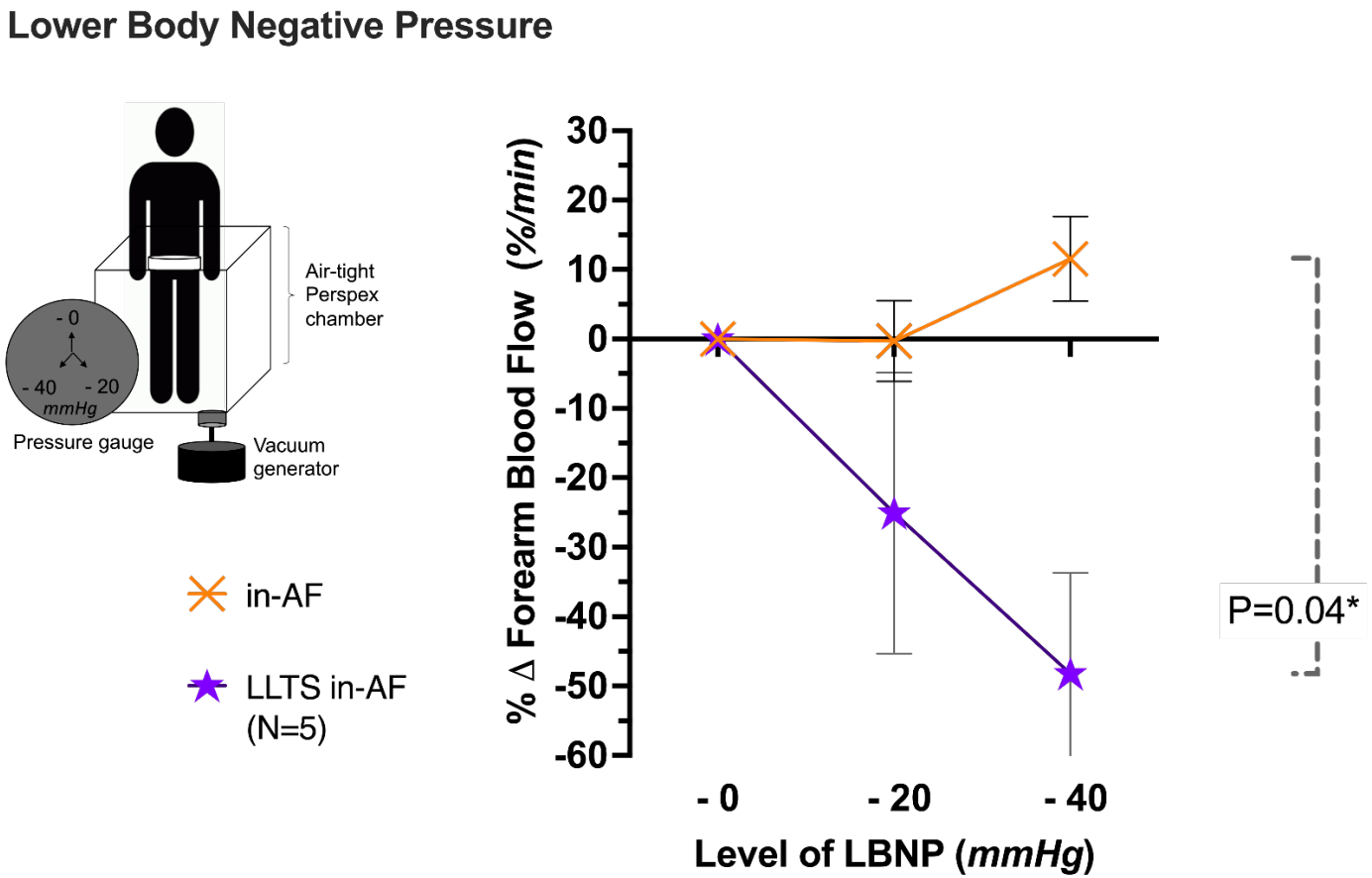
LLTS stimulation effects on LBNP

In-AF neither MAP nor HR following LBNP were statistically significant (-7.6±3.9%; P=0.5 and (-1.6±4.6%; P=0.75).

The in-AF group (n=5) showed that the dysfunctional LBNP response to FBF improved substantially (-48.2±15%; P=0.04). Figure 7A

There was no significant change in LBNP LLTS FBF in-SR (-16.7±16%; P=0.9, n=10) in comparison to LBNP testing in-SR.

Figure 7A: LBNP vasomotor (FBF) response pre vs post-LLTS in-AF



Opt-in (repeated) studies in the in-AF and in-SR group. Forearm Blood Flow, FBF responses to LBNP after Low-level Tragus Nerve Stimulation (LLTS; N=5). LBNP vasoconstriction response to LBNP (negative FBF slope) returned after LLTS on the same-day and during AF. Results expressed as percentage difference (mean ± SEM). *P<0.05.

Discussion

In this study, we examined the effect of active LLTS performed for 1 hour using the same protocol as in the TREAT-AF study[38] on LBNP reflexes in AF patients either in-AF or in-SR. Our principal findings are:

1. LLTS performed during AF restores the dysfunctional LBNP reflex response observed during AF.
2. However, an acute, single LLTS stimulus has no effect on the background level of autonomic remodelling seen in patients with AF studied in-SR.

Tragus nerve stimulation bypasses the cardiac afferent nerves (inputs to central, midbrain, projections of the vagus nerve[38] and sympathetic cervical ganglia[303]), therefore any effect on the abnormal reflex could assist in localizing the site of the abnormality (afferent-receptor or central versus efferent; peripheral vasomotor). The auricular branch of the vagus nerve has afferent endings in the tragus of the ear that can contribute to both vagal and sympathetic centres.[302] In general, the effects of LLTS are likely grossly oversimplified[302] and thus require further work. LLTS can augment cardiac function by modulating autonomic tone.[300, 301] and therefore, it could ameliorate abnormalities in the LBNP (volume-regulating reflexes) we have identified in prior Chapters. Indeed, in this study, we have demonstrated that LLTS has reversed the dysfunctional reflexes that we have observed in during AF. Although LLTS can affect efferent autonomic tone (either central, sympathetic, parasympathetic or both), there is no anatomic possibility that it could directly alter the afferent receptors that mediate the LBNP reflex (cardiopulmonary low-pressure volume-sensitive baroreceptors found in

veno-atrial junctions of the heart and co-located with the anatomic locations of ectopy triggering AF[11]). Thus, in the case of afferent or central abnormality, LLTS may improve reflexes whereas any deficit in the efferent limb of the LBNP reflex (peripheral vasoconstricting sympathetic efferents) would be unaffected. If there were no effect, then, this test would not be of any further assistance.

LLTS effect on LBNP in-SR

In our study, LLTS did not alter the diminished LBNP response in patients with AF studied in-SR. Interpretation of in-SR responses require careful consideration. There may be no effect on this background level of remodelling with LLTS. Alternatively, similar to the findings with P-wave amplitude with LLTS reported by others[296], there may be differing acute vs chronic effects. LLTS is thought to exert its effects through multiple layers of cardiac neural control [52, 286]. Therefore, whilst we have not demonstrated an effect in-SR, the stimulus may have been inadequate, and it is possible that chronic autonomic remodelling changes in patients with AF may require chronic (intermittent) LLTS. This has not been tested in this study and remains an interesting area to pursue in the future.

LLTS effect on LBNP in-AF

In this study, we were able to demonstrate reversal of the dysfunctional LBNP reflex response in patients with AF, during AF. This has several interesting implications. First, it extends our prior work to show that such reflex deficits from AF are reversible. Second, from a mechanistic point of view, reversal with LLTS in-AF modulates peripheral vasomotor function, demonstrating that it is downstream of the defect. As we have stated previously, there is no anatomic direct interaction of the auricular branch of the vagus nerve that is stimulated by LLTS with cardiac-level afferents, and, thus by modulating efferent

sympathetic outflow to peripheral blood vessels (vasoconstriction) during AF, it must mean that the abnormality in-AF must arise either from the afferents of the heart or their inputs into the central regulatory systems in the brain. If no effect of LLTS was seen (as in SR) it would not help us. However, what is clear is that LLTS bypasses cardiac afferents as it inputs directly into the midbrain. However, given that LLTS effects are incompletely understood; with central projections of the tragus nerve identified in not only vagal (midbrain) centers[38] but also in sympathetic ganglia,[303] it cannot assist us in further.

The auricular branch of the vagus nerve, which is what is stimulated by LLTS likely has effects both on sympathetic and parasympathetic nerve fibres en route to the brain, with functional MRI showing activity in vagal centres due to AF as well as the "hijacking" of sympathetic neurons which innervate the superior cervical plexus .[302-304] Indeed, at rest, LLTS could in fact reduce sympathetic tone.[305] This is converse to what we have found, however, there are likely differing effects at different levels of autonomic (and patient) activity. The effect of LLTS on cardiac reflexes has not been evaluated here and was not specifically intended in this study. However, in probing mechanisms- it implicates afferent abnormalities over efferent abnormalities in the abnormal LBNP in-AF response.

Finally, these findings suggest the potential role of neuromodulation treatments, in modifying the autonomic pathophysiology that we have identified in-AF. Thus, although there is a great deal of work that is needed to support this hypothesis, we propose that aside from AF burden reduction in some individuals,[38] LLTS could potentially reverse AF progression and possibly also the progression of the clinical sequelae of AF (heart failure and others identified in prior chapters) and is an important area to direct future research.

Limitations

There are several limitations to this study. First, we did not include a reference group. Secondly, we did not test LLTS with IHR and Valsalva reflexes. Clearly opt-in numbers are lower, and this reflects the difficulty in performing such experiments in patient subjects, especially amidst a pandemic. Thus, despite clear differences in LLTS, interpretations are limited by low repeat sample sizes. Finally, in utilising LLTS to localise the site of the abnormality of LBNP reflexes in AF, it can only infer the likely locations, rather than providing actual evidence as to the neurologic location of the deficit.

Concluding remarks

Clinical Implications

This study provides evidence of afferent >efferent autonomic deficits in the regulatory reflex abnormalities that we have identified to changes in blood volume, elicited by LBNP. Thus, it adds data to the concept of afferent or regulatory autonomic abnormalities in mediating a mechanistic link between anatomic and structural remodelling. These could help us to further define the neuropathophysiology underling the progression of AF, atrial dilatation (structural remodelling) as well as the development of heart failure. The location as well as the reversibility of this with the neuromodulating technique will require further work, however it provides exciting possibilities that we can alter this disease process by altering neurobiology and offers a potential treatment target. Aside from modulating the nervous system, this finding adds to the concept of managing rhythm earlier in patients with AF to prevent its progression and/or the progression to clinically deleterious sequelae such as heart failure.

Conclusion

This study demonstrates that LLTS can reverse the dysfunctional cardiac volume-sensitive reflexes seen during AF. The potential location of the abnormality could lie at either the afferent (cardiac) or regulatory (central input level).

Chapter 8: Abnormal cardiac electrical remodelling in Postural Tachycardia Syndrome (POTS): more evidence of a link between cardiac autonomic & anatomic remodelling

Background

We have proposed an interplay between autonomic and anatomic remodelling in patients with AF (prior chapters). Here, we investigated whether Postural orthostatic tachycardia syndrome (POTS), a known autonomic disorder, conversely, is associated with cardiac structural abnormalities.

Tachycardia with upright posture is a common symptom in individuals with POTS leading to their presentation to cardiologists.[306] POTS is defined as an intolerance to standing characterized by a sustained increase in heart rate; by definition > 30 *beats per minute (bpm)* in adults and > 40 *bpm* in children (12 – 19 years of age) within 10 minutes of standing and without a significant (> 20 *mmHg*) decrease in systolic blood pressure.[307] Symptoms of intolerance, which can often be quite debilitating; include palpitations, lightheadedness, cognitive impairment (or "brain fog"), headache, blurred vision, tremor, generalized weakness, exercise intolerance, decreased gastro-intestinal motility, fatigue and presyncope. Some of these symptoms occur as a consequence of tachycardia itself and others due to a number of proposed pathophysiologic mechanisms, such as generalized sympathetic activation and hypovolemia.[306]

Despite the tachycardia, by which this condition is defined,[307] the heart itself is thought to be structurally and functionally normal. However, others have shown reduced left ventricular mass and blood volume in individuals with POTS as compared to healthy controls, while

systemic autonomic function did not differ.[308] The marked orthostatic tachycardia in POTS could be explained by compensatory physiologic response to a smaller stroke volume due to cardiac atrophy and hypovolemia; also described as a form of cardiac deconditioning.[308-311] Unfortunately, despite the prevailing role of cardiac deconditioning in POTS; treatment aimed at improving body volume either through direct intake or from exercise training regimens are only moderately effective.[312] We hypothesized that the hemodynamic and circulatory challenges the heart is subjected to in POTS could result in abnormal cardiac remodelling.

Methods

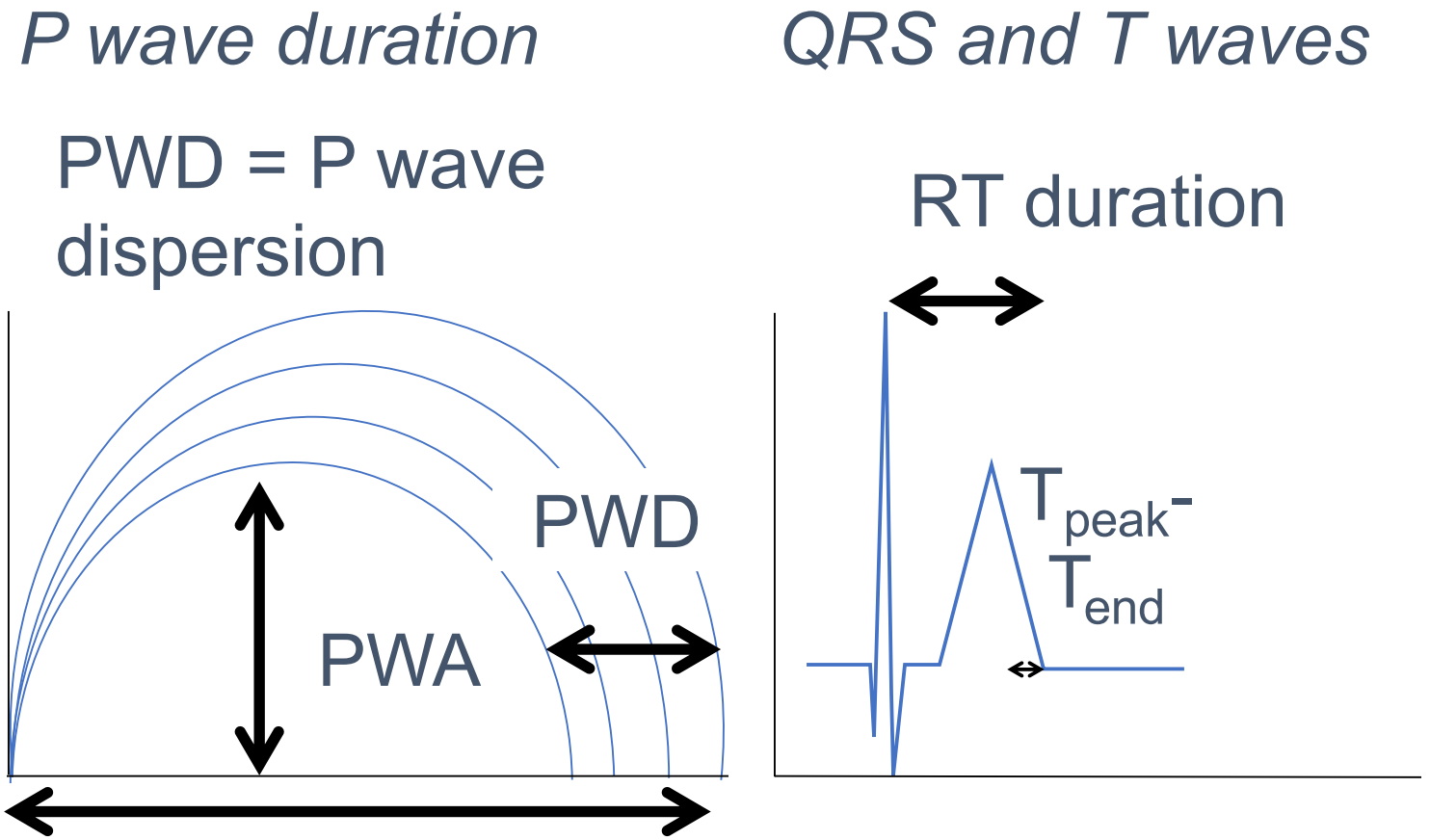
Patient identification

We evaluated prospectively collected, de-identified 12-lead electrocardiogram (ECG) from n=25 patients with newly diagnosed POTS referred to the Centre for Heart Rhythm Disorders (confirmed with a 10-minute standing or tilt-table test) and taken before the initiation of β-blocker or ivabradine; a hyperpolarization-activated cyclic nucleotide-gated channel blocker. We compared these to n=25 age-, sex- and body mass index-matched healthy controls to compare measures of electrical and autonomic remodelling. Controls were selected prospectively from those referred to the Centre for Heart Rhythm Disorders for cardiac evaluation, who did not have a history of POTS or diagnosed cardiac disorder. In order to minimize bias, we applied pre-specified criteria to obtain a random sample of control participants from those referred for evaluation of chest pain or general cardiac check-up, and without symptoms of syncope, structural heart disease on echocardiogram, heart rhythm disorder, conduction abnormalities (including bundle branch block) or cardiac risk factors. In those referred for chest pain evaluation, both atypical symptoms on clinical review as well as negative findings (either anatomic testing to assess coronary artery placque or functional testing through stress testing) were requisite. To produce matching, consecutive controls were matched on a 1:1 basis for age, gender as well as body mass index to a POTS participant. Patients and controls were not on any cardiovascular medications at the time of ECG acquisition. This was to limit the possible effect of medications on cardiac remodelling (particularly β-blockers and ivabradine). This study was approved by the institutional human research ethics committee.

Electrocardiogram analysis

All digitized 12-lead ECGs (25mm/sec paper speed and 10mm/mV) were de-identified and confirmed as normal sinus rhythm tracings. These were analyzed by blinded investigators using an electronic caliper (DigitizeIt v2.2.2; Braunschweig, Germany) to determine heart rate and PR interval in Lead II. P-wave duration (P-wave onset to isoelectric line return) and RT duration (R-wave peak to T-wave end) were measured in each lead. Both P-wave dispersion (PWD) and RT dispersion (RTD), markers of atrial and ventricular conduction delays, were calculated by subtracting the minimum from the maximum of the respective measurements.[313, 314] The peak of the R-wave was used as this is more easily identifiable; and reproducible then the Q-wave (QT interval). P-wave amplitude (PWA) and T_{PEAK}-T_{END} (T-wave peak to iso-electric line return) were determined from lead II, to estimate cardiac autonomic tone.[314, 315] The effect of exercise on sympathetic augmentation and vagal withdrawal is well accepted.[316] Therefore, we also examined cardiac electrical markers which are sensitive to sympathetic stimulation (PWA and T_{PEAK}-T_{END})[315, 317] at peak exercise, where available. ECG analysis is depicted in Figure 8A.

Figure 8A: Electrocardiogram analysis of 12-lead ECG



Analysis parameters taken from 12-lead ECG. P wave and R wave duration calculated from all (available) leads. PWA; P wave amplitude, RT duration and T_{PEAK}-T_{END} taken from lead II.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (version 8.3.0, California, USA). Continuous patient variables were expressed as mean ± SD or median (range) according to data distribution. Categorical variables were expressed as frequencies and percentages and were compared using the χ^2 test or Fisher's exact test as appropriate. Comparisons for continuous data were made using the Students t-test. Statistical significance was set at $P<0.05$.

Results

Baseline patient characteristics are presented in Table 8A. The mean age of the POTS group was 23±11 years with a predominance of females (n = 20; 80%). The mean BMI was 24±6 kg/m^2. The median time from first symptom onset to ECG capture was 12 months. Preceding viral illness and chronic fatigue syndrome were the most frequently associated features. The control group (n=25); were adequately age-, sex- and BMI-matched; (23±10 years, p=0.9; n=20 female, p=0.99 and BMI 23±4 kg/m^2, p=0.7). Compared to controls, individuals with POTS have higher resting heart rate (78±12 vs. 70±10 bpm, p=0.009) and smaller left atrial volume (19±4 vs. 24±1 mL/m^2, p=0.001). Individuals in both groups have normal left ventricular ejection fraction and interventricular septal thickness.

Table 8A: Baseline characteristics of the POTS and control cohorts

	POTS (n=25)	Controls (n=25)	P value
Age (years)	23 ± 11	23 ± 10	0.9
Female gender, n (%)	20 (80%)	20 (80%)	0.99
Body mass index, (kg/m^2)	24 ± 6	23 ± 4	0.7
Resting heart rate (bpm)	78 ± 12	70 ± 10	0.009*
Symptom onset to ECG capture (months)	12 (1-300)	-	-
POTS co-morbidities			
Joint hypermobility/Ehlers Danlos syndrome	2	-	-
Chronic Fatigue Syndrome	5	-	-
Migraines/headaches	3		
Preceding viral illness	5	-	-
Anxiety/depression	4	-	-
Echocardiographic parameters			
Indexed left atrial volume (mL/m^2)	19 ± 4	24 ± 1	0.001*
Left ventricular ejection fraction (%)	66 ± 5	65 ± 5	0.3
Interventricular septal thickness (cm)	0.8 ± 0.1	0.8 ± 0.1	0.6

Values are mean±SD or median (range)s or n (%); p<0.05 for *between group differences.

Individuals with POTS have greater conduction delays in both the atria (PWD: 48±16 vs. 39±12 ms, p=0.03) and ventricles (RTD: 69±24 vs. 53±20 ms, p=0.02) as compared to controls. Baseline T_{PEAK}-T_{END} and PWA did not differ between groups (Table 8B). From the peak exercise ECGs available (n=6 per group) at similar metabolic equivalents (p=0.95), maximum HR (p=0.4) and PR interval shortening (p=0.2) did not differ between groups. PWA increased from baseline in controls but not in patients with POTS (Controls: +0.10±0.06 *mV*, p=0.01; POTS: +0.02±0.06 *mV,* p=0.4), although between group comparison did not reach statistical significance (p=0.08). T_{PEAK}-T_{END} response was seen in both groups but more attenuated in the POTS group at peak exercise (p=0.04).

Table 8B: Electrocardiographic parameters of anatomic (structural) and autonomic remodelling

ECG parameters	POTS (n=25)	Controls (n=25)	P value
Resting parameters			
RR (ms)	785 ± 113	860 ± 128	0.03*
PR (ms)	150 ± 19	146 ± 21	0.5
QRS duration (ms)	82 ± 12	78 ± 15	0.29
QT interval in Lead II (ms)	323 ± 24	323 ± 20	0.98
P wave Dispersion (ms)	48 ± 16	39 ± 12	0.03*
RT Dispersion (ms)	69 ± 24	53 ± 20	0.02*
T_{PEAK}-T_{END} (ms)	76 ± 13	70 ± 10	0.07
PWA (mV)	0.14 ± 0.05	0.14 ± 0.04	0.9
Response to exercise	**(n = 6)**	**(n = 6)**	
Exercise capacity (METs)	10.9 ± 1.4	10.8 ± 2.5	0.95
Δ Heart rate (bpm)	+ 75 ± 25†	+ 89 ± 27†	0.4
Δ PR interval (ms)	- 23 ± 22†	- 41 ± 26†	0.2
Δ T_{PEAK}-T_{END} (ms)	- 20 ± 12†	- 33 ± 6†	0.04*
Δ PWA (mV)	+ 0.02 ± 0.06NS	+ 0.10 ± 0.06†	0.08

Values are mean±SD; p<0.05 for †within group differences and *between group differences. NS = Not statistically significant; METs = Metabolic Equivalents.

Discussion

The principal findings of this study are that individuals with POTS, when compared to age-, sex- and BMI-matched healthy controls, demonstrate: (i) significant atrial and ventricular electrical conduction delay (higher PWD and RTD) despite smaller left atrial size; (ii) an absence of heightened cardiac sympathetic tone (comparable T_{PEAK}-T_{END} and PWA) despite elevated resting heart rate; and (iii) an attenuated cardiac sympathetic response during peak exercise (lower Δ T_{PEAK}-T_{END}). Notably, the PWD seen in individuals with POTS are well above the described normal limits.[313] Taken together, the abnormal cardiac remodelling and intrinsic cardiac autonomic changes point to involvement of the heart in POTS pathophysiology.

Our findings further extend previous observations pertaining to the cardiac origins of POTS with reduced left ventricular mass and hypovolemia underpinning a smaller stroke volume and resultant compensatory orthostatic tachycardia in the absence of autonomic nervous system dysfunction.[308, 318] Hemodynamic assessment using a technique to decrease central venous return (lower body negative pressure) in patients with POTS demonstrated both normal cardiac afferent as well as systemic vasomotor responses.[318] Here, we demonstrate, first, that there is reduction of left atrial volume in POTS individuals; consistent with cardiac MRI findings of lower cardiac volume.[308] Second, we have identified for the first time, evidence of abnormal atrial electrical remodelling reflected by increased PWD (beyond normal limits) in individuals with POTS.[313] Increased PWD signifies both conduction slowing and heterogeneity in the atria[319] which are not expected in the young patient group devoid of co-existing cardiac risk factors, especially given their smaller atrial dimensions. These electrophysiologic changes reflect poor cellular coupling and have been linked to

inflammation and fibrosis due to risk factors such as senescent, obesity and diabetes mellitus and hypertension, as well as cardiovascular conditions such as cardiac failure, myocardial ischemia, and atrial fibrillation.[13, 170, 313, 319, 320]

Third, in addition to features of atrial remodelling in POTS; we have shown an increased RTD, or heterogeneity in ventricular repolarization in the POTS group, which is suggestive of either, or both, abnormal underlying ventricular structural remodelling and cardiac autonomic tone.[314] The spatial dispersion of the QT interval is more complex to interpret than PWD. Whilst studies show that this electrical abnormality of ventricular repolarization can be due to structural remodelling from heart failure as a result of patchy fibrosis and correlated with the degree of left ventricular dysfunction,[321-323] it may also be normal in some patients with heart failure in whom it appears to portend risk of ventricular arrythmia and sudden death.[322, 323] Thus, it may also be a marker of autonomic dysfunction.[324] In our study, T_{PEAK}-T_{END} and PWA were not heightened in individuals with POTS, and were attenuated in response to exercise, indicative of intrinsic cardiac sympathetic abnormalities. Both of these markers have been shown to increase with sympathetic stimulation.[314, 315] In our POTS cohort, there is an incongruence between the elevated resting heart rate; often associated with increased adrenergic state[314] and the absence of changes in resting cardiac autonomic tone. This could be explained by differences in the modulation of heart rate and T_{PEAK}-T_{END}; where the former can be affected by both, increases in circulating catecholamine and efferent cardiac sympathetic nerves, and the latter is modified by efferent input to the heart from the sympathetic (stellate) ganglia alone.[314] Moreover, in our study, the POTS cohort had a normal heart rate response to exercise; whereas there were differences in PWA and T_{PEAK}-T_{END}; indicating abnormalities in intrinsic cardiac sympathetic tone. Therefore, these findings suggest that heart rate increases in POTS may occur due to hypovolemia and cardiac

deconditioning, however co-existing intrinsic cardiac sympathetic abnormalities may be secondary to cardiac remodelling.

Limitations

This work is limited by the non-invasive ECG analysis of electrical and cardiac autonomic remodelling; although there has never been any prior attempt to assess cardiac remodelling in POTS. Given the constraints of the current evidence – it would not be ethically feasible to conduct invasive electrophysiologic mapping or histology based on cardiac biopsy. The limited number of stress ECG available in our study is also a limitation; as the true difference between the groups; particularly with respect to PWA may be underestimated. However, our blinded analysis of ECGs with stringent criteria applied to both patient and ECG selection; together with a control group matched for age, sex and BMI is a strength. Finally, our study cannot distinguish cause from effect with respect to cardiac remodelling in the pathophysiology of POTS. Further work is needed to better characterize these changes and to assess whether treatments that reverse cardiac remodelling offer better outcomes.

Concluding remarks

Clinical implications

This study adds to the concept that there may exist an interplay between disorders of the autonomic nervous system (such as in cardiac dysautonomia) and anatomic disorders (remodelling) found in conditions (arrhythmias) such as AF. This study is preliminary only and serves to substantiate further work. First to invasively assess cardiac structure and function in POTS and secondly, to explore the potential links between autonomic remodelling and structural remodelling in AF and other cardiac arrhythmias and dysautonomia's

Conclusion

This study presents novel evidence implicating the heart in POTS pathophysiology with findings of abnormal cardiac electrical remodelling and intrinsic cardiac autonomic changes. Our findings call for further investigation of cardiac electrical remodelling and sympathetic activity in POTS.

Chapter 9: Conclusions & Future direction

In this thesis we have presented data that examines another facet of the autonomic nervous system and its relationship with AF; that of cardiovascular afferent or regulatory function, where prior work has focussed on the effect of modulating the efferent arm of the autonomic nervous system in alterations of atrial electrophysiology that trigger AF.

The role of the Autonomic nervous system (ANS) historically, has been consigned mostly to the effect of the efferent arms; that of the sympathetic and parasympathetic efferent nerves to the heart.[23, 28] These prior works clearly demonstrate that both arms of the ANS can produce AF. Sympathetic activity results in enhanced automaticity of atrial myocytes, early and delayed after depolarisations due to calcium overload and ryanodine receptor changes, from adrenergic effects on ß-adrenoreceptors and L-type calcium channels. Parasympathetic tone can produce heterogeneity in atrial refractoriness, produced by variable shortening of the atrial effective refractory period by increasing the activity of I_{KACh} (acetyl-choline receptor mediated inward rectifying potassium channel) and thus creating autonomic substrate vulnerable for AF. The combined contribution of both sympathetic and parasympathetic arms appears to be important in the development of AF. Thus, these data further highlight that efferent activity of both “sympathies” can trigger AF, either alone or together, in some combination. However, these mechanisms suggest that the efferent limb of the ANS plays a more prominent role in the triggering of AF[11] early in the disease course and later, there are other factors that occur due to the development of atrial substrate, which, are either induced by AF itself, occur due to modifiable risk factors of AF (such obesity,[8, 9] alcohol,[183] amongst

others[7]), or occur due to non-modifiable causes (such as age, gender or genetic factors).[12] These factors seem to play a more relevant role as AF progresses to more permanent forms. What is not clear, is whether, the ANS plays a role in the progression of AF. Indeed, as we present in Chapter 1, seminal work,[24, 25] which showed the role that AF plays in its self-maintenance, failed to identify efferent autonomic mechanisms (blockade of the sympathetic and parasympathetic nervous input to the heart) as a potential contributor.

Nevertheless, AF itself can provoke changes in the autonomic nervous system. First, through changes in atrial innervation[211, 220, 221, 225] and second, the irregularity of AF itself produces a rise in efferent sympathetic activity at rest.[82] As we summarise in Chapter 1, there are clinical autonomic imbalances that predict the future incidence of AF[31] and, also, appear to result from AF.[31, 226] Thus, there is very likely a bidirectional relationship between AF and the ANS. A strand of evidence to support the role of the ANS in the maintenance or progression of AF come from a curious observation from TREAT-AF.[38] This new neuro-modulation treatment study, whereby Low-level Tragus Nerve Stimulation (LLTS) appears to reduce the burden of AF in some individuals at 6 months follow-up, demonstrated a reduction that persisted well beyond the stimulus (which was only applied for an hour per day). Thus, we propose that there are factors other than the *efferent autonomic innervation* to the heart that are at play in the maintenance and progression of AF.

Herein, we summarise our work, consisting of a review-of-concepts, a systematic review and metanalysis as well as several clinical research studies that explore the function of the afferent or regulatory arm of the ANS in the presence of AF. We also examine the effect of catheter ablation of AF; whereby destruction of pulmonary vein-atrial tissue could potentially

result in destruction of co-located afferent volume-regulating low-pressure baroreceptors.[106, 108]

1. In Chapter 1, we present our review[31] showing that there is considerable evidence to support the concept that a number of risk factors themselves result in shifts in autonomic function. Specifically, a sympathetic shift (and parasympathetic withdrawal) occurs and is quite similar with the shift that occurs due to AF itself. Thus, we propose that risk factors may promote the risk of AF *via* the ANS. Put another way, the ANS may represent the "road" that leads to AF.[31]
2. In Chapter 2, we performed a systematic review and meta-analysis. Here we pooled data to show that AF is an independent risk factor for falls, syncope and AF, in older adults.[226] One study showed that persistent AF was associated with orthostatic intolerance in older adults.[250] Although, there are a number of potential reasons underlying this association and, it is true, also, that association cannot necessarily imply causation, given that orthostatic intolerance can predict onset of future incidence of AF, autonomic dysfunction may indeed play a role in this. Especially, if AF were associated with abnormalities of afferent or regulatory function of blood pressure and volume. Others have shown abnormalities of arterial (high-pressure) baroreflex sensitivity and have attributed this to the presence of AF.[203] What is not known is, whether autonomic afferent dysfunction could compound this risk in older adults.
3. In Chapter 3 we performed a pilot study. Here, we assessed whether patients with highly symptomatic AF, studied in sinus rhythm, SR, have an abnormal reflex response to Lower Body Negative Pressure (LBNP) compared to an age and sex

matched healthy reference group. LBNP decreases atrial volume, and induces reflex vasoconstriction, maintaining blood pressure and volume. At low-levels of negative pressure, this technique predominantly tests volume-regulating low-pressure baroreceptors. We identified, for the first time, that such patients have diminished reflex responses even in SR.[175] On its own, these study findings could occur due to a background variable (such as a risk factor for AF) in these patients, although, we were careful to exclude known causes of autonomic dysfunction, including diabetes mellitus.

4. In Chapter 4, we designed and conducted a study evaluating several cardiovascular autonomic reflexes in patients with AF, either during AF or during SR and compared their responses to an age and sex-matched reference group of participants where, cardiovascular risk factors were permissible. We were also able to match for risk factors. Testing cardiovascular reflexes in the presence of AF is challenging, however, we utilised continuous beat-beat haemodynamic monitoring and venous occlusion plethysmography to evaluate cardiovascular reflexes. We evaluated low-pressure volume regulating using LBNP, high-pressure, blood pressure regulating arterial baroreceptors using Valsalva and both types of baroreflexes using Isometric handgrip reflex, IHR. We identified that the presence of AF did not alter Valsalva reflexes and thus, arterial baroreflex function is grossly normal in AF. IHR was diminished in-AF compared to in-SR and LBNP showed the most significant abnormality. Indeed, in-AF, LBNP reflex was quite dysfunctional (there was no apparent vasomotor response, and perhaps, there was paradoxical vasodilation). In other circumstances, this would occur in late stages of LBNP, or during severe haemorrhagic shock, prior to syncope.[96] This study, with a separate cohort of patients examined, in-SR, exhibited similar findings to the cohort presented in Chapter 3.

There were diminished responses to LBNP in-SR compared to the age-sex and risk factor matched reference group. These findings, as discussed Chapter 4, mirror those found in patients with heart failure, after cardiac transplantation (de-afferentation) and in those with severe left ventricular hypertrophy. The assessment of all these reflex tests, with varied effects on baroreceptor sub-types, assisted us in the localisation of autonomic abnormalities to those of the low-pressure volume-regulating baroreceptors and likely to the afferent, receptor or central (input, or regulatory) limbs of the reflex, given that there were differential effects. We were able to add to this localisation attempt in subsequent chapters.

5. In Chapter 5 we explored the effect of restoring SR (cardioversion). We found that the LBNP response in those patients undergoing cardioversion substantially improved (to in-SR levels). Thus, there is a background level of autonomic remodelling in SR, however, through this study design we were able to demonstrate that a. the dysfunctional LBNP response seen in the in-AF group in Chapter 4 is indeed to the presence of AF and b. there is reversibility of this reflex with rhythm management.
6. In Chapter 6 we explored the hypothesis that catheter ablation for AF (Pulmonary vein isolation, PVI) would further disrupt cardiac afferents responsive to changes in blood volume (low-pressure baroreceptors) found in the veno-atrial junctions of the heart. We found that PVI had no effect on the already diminished response to LBNP and, there was also no effect on the other autonomic reflex tested.
7. In Chapter 7 we utilised a novel neuromodulating technique (LLTS, low-level vagal nerve stimulation) which has been shown to reduce burden of AF in some individuals.[38] We were able to demonstrate that stimulating the tragus for 1 hour and then repeating LBNP, acutely reversed the dysfunctional response to LBNP. This adds great strength to the localisation of the abnormality to the afferent/regulatory

limb (receptors in the heart or in the neuronal circuitry centrally) of the LBNP reflex. Peripheral vasomotor tone increased with LBNP after LLTS and thus would be expected to be normal. Thus, even this experiment has shown a reversibility of this reflex (providing strong evidence against measurement error in AF in Chapter 4) and also suggesting the potential role of neuromodulation treatments, potentially as a reversal of the pathophysiology of AF progression and possibly also the progression of the clinical sequelae of AF and is an important area to direct future research.

8. Thus, we have shown that patients with AF have loss of homeostasis (blood-volume dysregulation) which has implications in terms of a possible mechanism for the progression of AF. Atrial dilatation is associated with AF progression[285] and we propose an intersect between autonomic and anatomic remodelling (atrial dilatation), whereby dysregulation in volume handling (autonomic remodelling), over time, could potentially result in anatomic structural changes that support permanent AF.AF has several sequelae, such as falls, syncope, cognitive impairment, and heart failure, which may also be due to these autonomic abnormalities of reflex control of blood volume and each of these associations warrants further work to elucidate mechanisms and identify treatments to delay or prevent each of these sequelae. Intriguingly, heart failure has been also shown to have abnormalities in the LBNP reflex[55] and thus, supports the concept of a shared mechanism to explain this as a sequela in AF as well.
9. In Chapter 8 we tested this proposed interplay between anatomic and autonomic remodelling in Postural tachycardia syndrome (POTS), a known dysautonomia, where the heart is thought to be structurally normal (not implicated in pathophysiology). We present evidence of abnormal cardiac (structural) remodelling, utilising electrocardiogram markers associated with arrhythmia, including AF). Thus, in a known autonomic cardiac disorder (heart rate increase on changes in posture),

conversely, there is evidence linking autonomic with anatomic remodelling. This study is to be viewed simply as conceptual in nature and was not designed to establish this proposed concept. This study simply adds contextual framework in which to plan further work in this area.

The main message from our study findings is that the presence of AF is associated with severe abnormalities in the blood-volume regulating reflex. These patients exhibit an overall preservation in their ability to acutely regulate blood pressure; however, this study was not designed to assess subtle changes in baroreflex sensitivity. There is convincing evidence of a background level of remodelling in patients with AF (i.e. studied in sinus rhythm); with our observations upheld whether or not the reference group were a completely healthy age and sex-matched cohort (as in Chapter 3) or a risk factor matched reference group (also matched for age and sex, in Chapter 4). Deficiencies in-AF are reversible with restoration of rhythm, to the background level of remodelling. Intriguingly, LLTS can also reverse these changes, and this is an exciting prospect, for neuromodulation as a treatment target in AF.

There are several avenues, from this body of work, that require further exploration. Indeed, as, we have presented in Chapter 1, there is great need to further delineate cardiopulmonary baroreflex control in normal individuals as well as those in AF, from a mechanistic point of view. Investigation into the basic physiology of these receptors requires revival, in the modern era, with newer sophisticated techniques to identify and study the ANS. LBNP is a well-established physiological technique[96] – but more is needed to further refine the anatomy of this reflex and the exact structure and function of the receptors that are stimulated by this technique. Especially interesting is that our findings in AF are similar to those conducted in

heart failure.[108] The pulmonary vein-atrial junction is a complex muscular sleeve[325] that has both anatomic substrate for arrhythmia from muscular discontinuities and acute fibre re-orientation as well as a rich plexus of autonomic nerves. Thus, there is very likely an intersect in the structure (anatomy) of this area as well as the autonomic function in the development of AF. From a mechanical point of view, these sleeves may form a "throttle" regulation of the left atrium as a reservoir.[326] Therefore, our findings, of loss of volume-regulating homeostatic feedback mechanism, in the context of the structure and purpose of the pulmonary vein sleeves in regulating the left atrial reservoir raises the potential link between anatomic and structural remodelling we have discussed in Chapter 4. This link requires careful exploration. First, it may explain the progressive atrial dilatation and progression of AF[285] and second, it could explain the association of AF with heart failure.[229]

To this end, experiments in animal models that explore the direct electrophysiologic activation of cardiopulmonary low-pressure baroreceptors in the context of lower body sequestration of blood, similar the experiments performed during haemorrhage in animals,[93] is required. Similar to prior studies in which AF was induced acutely and cardiopulmonary baroreceptor activity studied from carefully dissected slips of the vagus nerve,[109] further evaluation of both acute AF and chronic AF requires similar study, The effect of a variety of cardiovascular stimuli (intra-atrial or intra-pulmonary vein balloon distension,[99, 327, 328] haemorrhage, both hypotensive and non-hypotensive[93, 329]) require careful evaluation in the presence of AF. In essence, a variety of these historic experiments need to be recapitulated in animal models of AF.

Low-pressure cardiopulmonary receptor stimulation decreases antidiuretic hormone release (resulting in diuresis). We have shown dysfunction during AF (and diminished function in

patients with AF in SR)- this represents a mechanism by which blood volume dysregulation may occur.[58] Conversely, historical studies showed that acutely inducing AF seems to stimulate receptor activity (measured from slips of the vagus nerve,[109] known to be activated by the atrial receptors). This was thought to represent the reflex diuresis seen in AF patients.[109] This finding is not in keeping what we have shown with LBNP; where one would not expect receptor activity, however, there may be differential effects in animal models vs humans.[330] Further, another difference is that in this historical study, acute AF rather than established AF is being evaluated. It is possible that AF may initially potentiate and then, over time, result in receptor function loss. All of this, together with the effect of maintained or chronic AF needs to be re-evaluated given our work.

Indeed, we were only interested in the short term (acute neurodynamic effects of LBNP on vasomotor function) whereas LBNP, is more versatile than this, and several other effects can be investigated. Longer levels of LBNP (in keeping with the presumed effects of deactivation of atrial low-pressure volume-regulating receptors) induces increases in antidiuretic hormone as well as plasma renin.[110] These occur without changes in mean arterial pressure. Thus, LBNP can be capitalised much further in the evaluation of neurohormonal effects of AF. Another advantage of LBNP in clinical AF research, aside from non-invasive assessment in humans rather than AF models, is the ability to measure autonomic reflexes awake, without the interference of anaesthetic agents, nor the requirement for open-chested preparations, which decidedly limit the use of animal models in the assessment of such reflexes.

We have identified that AF is independently associated with the risks of orthostatic intolerance, falls and syncope.[226] Whilst younger patients with chronic AF are less likely affected from low-pressure baroreflex deficits, in older patients, who also have arterial

baroreflex deficiencies,[331] AF could potentiate these clinical problems. There is also compelling evidence to support an independent role of AF in causing cognitive decline and dementia.[228] Further AF, is associated with decreases in cerebral blood flow.[227] LBNP is a useful test in the autonomic modulation (autoregulation) of cerebral blood flow; where it incites decreases in cerebral blood flow and pulsatility at higher levels of LBNP.[96] Therefore, higher levels of LBNP, together with cerebral blood flow measurements could reveal differences in the autonomic regulation of blood volume, which are known to be decreased in AF[227] and thus possibly identify a mechanism by which this occurs in patients with AF. In other words, low-pressure baroreceptor reflexes may also play a role in the development of dementia and cognitive decline, where a clear mechanism has not been thus far identified. If true, this would be of great interest and indeed, would have important clinical ramifications.

In our work, in-AF, we have found an almost paradoxical vasodilatation in AF. This is of great interest and will require further elucidation. At low-levels of LBNP – cardiopulmonary receptors predominate in the vasomotor response to LBNP. Typically, with either progressive haemorrhage[332] or with higher levels of LBNP – there is a period (pre-syncope) where vasodilatation occurs in response to decreased blood volume.[96] This is the first phase of the presyncopal response – but before symptoms occur (MAP decreases but is somewhat maintained >80mmHg and thus cerebral perfusion is somewhat maintained).[333] Whilst the effect described above occurred due to haemorrhage or significant negative pressure-simulating >>1L haemorrhage (where even arterial baroreceptors are at their limits of compensation); the mechanism behind vasomotor tone (or absence of it) is interesting in that it may infact be largely due to the offloading of cardiopulmonary>arterial baroceptors. The specific physiologic mechanism behind the vasodilatation in haemorrhage (with intact receptor function) has not been elucidated and is beyond the scope of our work.

The role of neuromodulation as a treatment strategy is a novel and exciting area of clinical AF research. TREAT-AF[38] is the first promising randomised clinical trial assessing a non-invasive treatment (LLTS) in the management of AF burden. Whilst the response was not universal, there may be autonomic markers that can predict successful outcome.[296] Intriguingly, in our work, we have shown reversibility of dysfunctional cardiopulmonary low-pressure baroreflexes in AF and thus, LLTS may pose additional benefits in AF patients, if this amelioration proves to remain in larger clinical studies specifically examining this. It may confer effects in reduction in the development of heart failure, cognitive decline, falls and syncope, which we have discussed above. All these potential effects warrant further consideration. It is especially important to note that in the presence of AF, ß-blockade is not associated with improved mortality.[34] Thus, whether LLTS, or other neuromodulation strategies could confer such benefits, over ß-blockade, in patients with chronic AF, could represent an exciting further avenue to explore.

Finally, the background remodelling we have identified in AF patients studied in-SR requires further work. Specially to identify what the mechanism might be and what treatments could work to reverse these diminished reflexes. In our efforts, we have not found that PVI to maintain SR has any appreciable impact on ameliorating these reflexes and what is needed is to understand the effect of the burden of AF, which we have not done in this study. Additionally, how risk factors for AF might impact on these reflexes may also be of importance. Any impact on cardiovascular disease that this background level of autonomic afferent remodelling has also needs to be carefully elucidated.

Thus, in our work, we demonstrate the possibility of a new scientific avenue into potentially responsible mechanisms for both AF progression as well as some of the attached clinical sequelae. Future direction of research in this arena needs to include a thorough mechanistic understanding of the implications of these abnormalities in the LBNP reflex in the presence of AF, an understanding of the contribution of AF burden to the underlying autonomic remodelling that we have seen in-SR and finally we need to explore each of the clinical sequelae of AF we have discussed previously; orthostatic intolerance, falls, syncope, heart failure in terms of the potential autonomic contributors to these associations.

Bibliography

1. Gallagher C, Hendriks JM, Giles L, Karnon J, Pham C, Elliott AD, Middeldorp ME, Mahajan R, Lau DH, Sanders P, Wong CX. Increasing trends in hospitalisations due to atrial fibrillation in Australia from 1993 to 2013. Heart Sep 2019;105:1358-1363.

2. Chugh SS, Havmoeller R, Narayanan K, et al. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. Circulation Feb 25 2014;129:837-847.

3. Bassand JP, Accetta G, Camm AJ, et al. Two-year outcomes of patients with newly diagnosed atrial fibrillation: results from GARFIELD-AF. Eur Heart J Oct 7 2016;37:2882-2889.

4. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. Stroke Aug 1991;22:983-988.

5. Roth GA, Mensah GA, Johnson CO, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. J Am Coll Cardiol Dec 22 2020;76:2982-3021.

6. Schotten U, Verheule S, Kirchhof P, Goette A. Pathophysiological mechanisms of atrial fibrillation: a translational appraisal. Physiol Rev Jan 2011;91:265-325.

7. Middeldorp ME, Ariyaratnam J, Lau D, Sanders P. Lifestyle modifications for treatment of atrial fibrillation. Heart Mar 2020;106:325-332.

8. Middeldorp ME, Pathak RK, Meredith M, et al. PREVEntion and regReSsive Effect of weight-loss and risk factor modification on Atrial Fibrillation: the REVERSE-AF study. Europace Dec 1 2018;20:1929-1935.

9. Pathak RK, Middeldorp ME, Meredith M, Mehta AB, Mahajan R, Wong CX, Twomey D, Elliott AD, Kalman JM, Abhayaratna WP, Lau DH, Sanders P. Long-Term Effect of Goal-Directed Weight Management in an Atrial Fibrillation Cohort: A Long-Term Follow-Up Study (LEGACY). J Am Coll Cardiol May 26 2015;65:2159-2169.

10. January CT, Wann LS, Calkins H, et al. 2019 AHA/ACC/HRS Focused Update of the 2014 AHA/ACC/HRS Guideline for the Management of Patients With Atrial Fibrillation: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society in Collaboration With the Society of Thoracic Surgeons. Circulation Jul 9 2019;140:e125-e151.

11. Haïssaguerre M, Jaïs P, Shah DC, Takahashi A, Hocini M, Quiniou G, Garrigue S, Le Mouroux A, Le Métayer P, Clémenty J. Spontaneous Initiation of Atrial Fibrillation by Ectopic Beats Originating in the Pulmonary Veins. New England Journal of Medicine 1998;339:659-666.

12. Andrade JG, Aguilar M, Atzema C, et al. The 2020 Canadian Cardiovascular Society/Canadian Heart Rhythm Society Comprehensive Guidelines for the Management of Atrial Fibrillation. The Canadian journal of cardiology Dec 2020;36:1847-1948.

13. Lau DH, Nattel S, Kalman JM, Sanders P. Modifiable Risk Factors and Atrial Fibrillation. Circulation 2017;136:583-596.

14. Lavie CJ, Pandey A, Lau DH, Alpert MA, Sanders P. Obesity and Atrial Fibrillation Prevalence, Pathogenesis, and Prognosis: Effects of Weight Loss and Exercise. J Am Coll Cardiol Oct 17 2017;70:2022-2035.

15. Abed HS, Samuel CS, Lau DH, et al. Obesity results in progressive atrial structural and electrical remodeling: implications for atrial fibrillation. Heart Rhythm Jan 2013;10:90-100.

16. Mahajan R, Lau DH, Brooks AG, et al. Electrophysiological, Electroanatomical, and Structural Remodeling of the Atria as Consequences of Sustained Obesity. J Am Coll Cardiol Jul 7 2015;66:1-11.

17. Munger TM, Dong YX, Masaki M, et al. Electrophysiological and hemodynamic characteristics associated with obesity in patients with atrial fibrillation. J Am Coll Cardiol Aug 28 2012;60:851-860.

18. Mahajan R, Nelson A, Pathak RK, et al. Electroanatomical Remodeling of the Atria in Obesity: Impact of Adjacent Epicardial Fat. JACC Clin Electrophysiol Dec 2018;4:1529-1540.

19. Hatem SN, Sanders P. Epicardial adipose tissue and atrial fibrillation. Cardiovasc Res May 1 2014;102:205-213.

20. Mishima RS, Verdicchio CV, Noubiap JJ, Ariyaratnam JP, Gallagher C, Jones D, Malik V, Agbaedeng TA, Middeldorp ME, Lau DH, Sanders P, Elliott AD. Self-reported physical activity and atrial fibrillation risk: A systematic review and meta-analysis. Heart Rhythm Dec 19 2020.

21. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol May 19 2004;43:1731-1737.

22. Lau DH, Schotten U, Mahajan R, Antic NA, Hatem SN, Pathak RK, Hendriks JML, Kalman JM, Sanders P. Novel mechanisms in the pathogenesis of atrial fibrillation: Practical applications. European Heart Journal 2016;37:1573-1581.

23. Linz D, Elliott AD, Hohl M, Malik V, Schotten U, Dobrev D, Nattel S, Bohm M, Floras J, Lau DH, Sanders P. Role of autonomic nervous system in atrial fibrillation. Int J Cardiol Jul 15 2019;287:181-188.

24. Wijffels MC, Kirchhof CJ, Dorland R, Allessie MA. Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. Circulation Oct 1 1995;92:1954-1968.

25. Wijffels MC, Kirchhof CJ, Dorland R, Power J, Allessie MA. Electrical remodeling due to atrial fibrillation in chronically instrumented conscious goats: roles of neurohumoral changes, ischemia, atrial stretch, and high rate of electrical activation. Circulation Nov 18 1997;96:3710-3720.

26. Ehrlich JR, Cha TJ, Zhang L, Chartier D, Melnyk P, Hohnloser SH, Nattel S. Cellular electrophysiology of canine pulmonary vein cardiomyocytes: action potential and ionic current properties. J Physiol Sep 15 2003;551:801-813.

27. Smeets JL, Allessie MA, Lammers WJ, Bonke FI, Hollen J. The wavelength of the cardiac impulse and reentrant arrhythmias in isolated rabbit atrium. The role of heart rate, autonomic transmitters, temperature, and potassium. Circulation Research 1986;58:96-108.

28. Chen PS, Chen LS, Fishbein MC, Lin SF, Nattel S. Role of the autonomic nervous system in atrial fibrillation: pathophysiology and therapy. Circ Res Apr 25 2014;114:1500-1515.

29. Iwasaki YK, Nishida K, Kato T, Nattel S. Atrial fibrillation pathophysiology: implications for management. Circulation Nov 15 2011;124:2264-2274.

30. Lau DH, Linz D, Sanders P. New Findings in Atrial Fibrillation Mechanisms. Card Electrophysiol Clin Dec 2019;11:563-571.

31. Malik V, Mishima R, Elliott A, Lau H, Sanders P. The "Road" to Atrial Fibrillation: The Role of the Cardiac Autonomic Nervous System. J Atr Fibrillation Jun-Jul 2020;13:2400.

32. Wyse DG, Waldo AL, DiMarco JP, Domanski MJ, Rosenberg Y, Schron EB, Kellen JC, Greene HL, Mickel MC, Dalquist JE, Corley SD, Atrial Fibrillation Follow-up Investigation of Rhythm Management I. A comparison of rate control and rhythm control in patients with atrial fibrillation. The New England journal of medicine Dec 5 2002;347:1825-1833.

33. Van Gelder IC, Hagens VE, Bosker HA, Kingma JH, Kamp O, Kingma T, Said SA, Darmanata JI, Timmermans AJ, Tijssen JG, Crijns HJ, Rate Control versus Electrical Cardioversion for Persistent Atrial Fibrillation Study G. A comparison of rate control and rhythm control in patients with recurrent persistent atrial fibrillation. The New England journal of medicine Dec 5 2002;347:1834-1840.

34. Kotecha D, Flather MD, Altman DG, et al. Heart Rate and Rhythm and the Benefit of Beta-Blockers in Patients With Heart Failure. J Am Coll Cardiol Jun 20 2017;69:2885-2896.

35. Verma A, Mantovan R, Macle L, De Martino G, Chen J, Morillo CA, Novak P, Calzolari V, Guerra PG, Nair G, Torrecilla EG, Khaykin Y. Substrate and Trigger Ablation for Reduction of Atrial Fibrillation (STAR AF): a randomized, multicentre, international trial. Eur Heart J Jun 2010;31:1344-1356.

36. Kirchhof P, Camm AJ, Goette A, et al. Early Rhythm-Control Therapy in Patients with Atrial Fibrillation. The New England journal of medicine Oct 1 2020;383:1305-1316.

37. Andrade JG, Wells GA, Deyell MW, et al. Cryoablation or Drug Therapy for Initial Treatment of Atrial Fibrillation. The New England journal of medicine Jan 28 2021;384:305-315.

38. Stavrakis S, Stoner JA, Humphrey MB, et al. TREAT AF (Transcutaneous Electrical Vagus Nerve Stimulation to Suppress Atrial Fibrillation): A Randomized Clinical Trial. J Am Coll Cardiol EP Mar 2020;6:282-291.

39. Patterson E, Po SS, Scherlag BJ, Lazzara R. Triggered firing in pulmonary veins initiated by in vitro autonomic nerve stimulation. Heart Rhythm 2005;2:624-631.

40. Bettoni M, Zimmermann M. Autonomic tone variations before the onset of paroxysmal atrial fibrillation. Circulation 2002;105:2753-2759.

41. Viskin S, Golovner M, Malov N, Fish R, Alroy I, Vila Y, Laniado S, Kaplinsky E, Roth A. Circadian variation of symptomatic paroxysmal atrial fibrillation. Data from almost 10 000 episodes. Eur Heart J Oct 1999;20:1429-1434.

42. Lambert EA, Esler MD, Schlaich MP, Dixon J, Eikelis N, Lambert GW. Obesity-Associated Organ Damage and Sympathetic Nervous Activity. Hypertension Jun 2019;73:1150-1159.

43. Gami AS, Hodge DO, Herges RM, Olson EJ, Nykodym J, Kara T, Somers VK. Obstructive Sleep Apnea, Obesity, and the Risk of Incident Atrial Fibrillation. Journal of the American College of Cardiology 2007;49:565-571.

44. Grassi G. Assessment of sympathetic cardiovascular drive in human hypertension: achievements and perspectives. Hypertension Oct 2009;54:690-697.

45. Middlekauff HR, Park J, Moheimani RS. Adverse effects of cigarette and noncigarette smoke exposure on the autonomic nervous system: mechanisms and implications for cardiovascular risk. J Am Coll Cardiol Oct 21 2014;64:1740-1750.

46. Mischel NA, Subramanian M, Dombrowski MD, Llewellyn-Smith IJ, Mueller PJ. (In)activity-related neuroplasticity in brainstem control of sympathetic outflow: unraveling underlying molecular, cellular, and anatomical mechanisms. Am J Physiol Heart Circ Physiol Jul 15 2015;309:H235-243.

47. Voskoboinik A, Prabhu S, Ling LH, Kalman JM, Kistler PM. Alcohol and Atrial Fibrillation: A Sobering Review. J Am Coll Cardiol Dec 13 2016;68:2567-2576.

48. Otake H, Suzuki H, Honda T, Maruyama Y. Influences of autonomic nervous system on atrial arrhythmogenic substrates and the incidence of atrial fibrillation in diabetic heart. International heart journal Sep 2009;50:627-641.

49. Straznicky NE, Grima MT, Sari CI, Eikelis N, Lambert EA, Nestel PJ, Esler MD, Dixon JB, Chopra R, Tilbrook AJ, Schlaich MP, Lambert GW. Neuroadrenergic dysfunction along the diabetes continuum: a comparative study in obese metabolic syndrome subjects. Diabetes Oct 2012;61:2506-2516.

50. Alvarez GE, Beske SD, Ballard TP, Davy KP. Sympathetic neural activation in visceral obesity. Circulation Nov 12 2002;106:2533-2536.

51. Armour JA. Potential clinical relevance of the 'little brain' on the mammalian heart. Exp Physiol Feb 2008;93:165-176.

52. Hanna P, Buch E, Stavrakis S, Meyer C, Tompkins JD, Ardell JL, Shivkumar K. Neuroscientific therapies for atrial fibrillation. Cardiovasc Res Jun 16 2021;117:1732-1745.

53. Ardell JL, Andresen MC, Armour JA, Billman GE, Chen PS, Foreman RD, Herring N, O'Leary DS, Sabbah HN, Schultz HD, Sunagawa K, Zucker IH. Translational neurocardiology: preclinical models and cardioneural integrative aspects. J Physiol Jul 15 2016;594:3877-3909.

54. Mancia G, Grassi G, Giannattasio C. Cardiopulmonary receptor reflex in hypertension. American journal of hypertension Jul 1988;1:249-255.

55. Mohanty PK, Arrowood JA, Ellenbogen KA, Thames MD. Neurohumoral and hemodynamic effects of lower body negative pressure in patients with congestive heart failure. Am Heart J Jul 1989;118:78-85.

56. Mohanty PK, Thames MD, Arrowood JA, Sowers JR, McNamara C, Szentpetery S. Impairment of cardiopulmonary baroreflex after cardiac transplantation in humans. Circulation May 1987;75:914-921.

57. Weisbrod CJ, Arnolda LF, McKitrick DJ, O'Driscoll G, Potter K, Green DJ. Vasomotor responses to decreased venous return: effects of cardiac deafferentation in humans. J Physiol Nov 1 2004;560:919-927.

58. Hainsworth R. Reflexes from the heart. Physiological Reviews 1991;71:617-649.

59. Dawes GS. Studies on veratrum alkaloids; receptor areas in the coronary arteries and elsewhere as revealed by the use of veratridine. J Pharmacol Exp Ther Apr 1947;89:325-342.

60. Bainbridge FA. The influence of venous filling upon the rate of the heart. J Physiol Dec 24 1915;50:65-84.

61. Malliani A, Pagani M, Pizzinelli P, Furlan R, Guzzetti S. Cardiovascular reflexes mediated by sympathetic afferent fibers. Journal of the autonomic nervous system Mar-Apr 1983;7:295-301.

62. Brandle M, Wang W, Zucker IH. Ventricular mechanoreflex and chemoreflex alterations in chronic heart failure. Circ Res Feb 1994;74:262-270.

63. Zucker IH, Wang W, Brandle M, Schultz HD, Patel KP. Neural regulation of sympathetic nerve activity in heart failure. Progress in cardiovascular diseases May-Jun 1995;37:397-414.

64. Zucker IH, Wang W, Schultz HD. Cardiac receptor activity in heart failure: implications for the control of sympathetic nervous outflow. Advances in experimental medicine and biology 1995;381:109-124.

65. Ito M, Zipes DP. Efferent sympathetic and vagal innervation of the canine right ventricle. Circulation Sep 1994;90:1459-1468.

66. Inoue H SBZD. Effects of ischemia on cardiac afferent sympathetic and vagal reflexes in dog. Am J Physiol 1998;255:H26–H35.

67. Zucker IH, Patel KP, Schultz HD. Neurohumoral stimulation. Heart failure clinics Jan 2012;8:87-99.

68. Foreman RD. Mechanisms of cardiac pain. Annu Rev Physiol 1999;61:143-167.

69. Affleck VS, Coote JH, Pyner S. The projection and synaptic organisation of NTS afferent connections with presympathetic neurons, GABA and nNOS neurons in the paraventricular nucleus of the hypothalamus. Neuroscience Sep 6 2012;219:48-61.

70. Reddy MK, Patel KP, Schultz HD. Differential role of the paraventricular nucleus of the hypothalamus in modulating the sympathoexcitatory component of peripheral and central chemoreflexes. American journal of physiology Regulatory, integrative and comparative physiology Sep 2005;289:R789-797.

71. Xu B, Zheng H, Patel KP. Relative contributions of the thalamus and the paraventricular nucleus of the hypothalamus to the cardiac sympathetic afferent reflex. American journal of physiology Regulatory, integrative and comparative physiology Jul 1 2013;305:R50-59.

72. Wang WZ, Gao L, Pan YX, Zucker IH, Wang W. Differential effects of cardiac sympathetic afferent stimulation on neurons in the nucleus tractus solitarius. Neurosci Lett Dec 1 2006;409:146-150.

73. Wang WZ, Gao L, Pan YX, Zucker IH, Wang W. AT1 receptors in the nucleus tractus solitarii mediate the interaction between the baroreflex and the cardiac sympathetic afferent reflex in anesthetized rats. American journal of physiology Regulatory, integrative and comparative physiology Mar 2007;292:R1137-1145.

74. Zahner MR, Li DP, Chen SR, Pan HL. Cardiac vanilloid receptor 1-expressing afferent nerves and their role in the cardiogenic sympathetic reflex in rats. The Journal of Physiology 2003;551:515-523.

75. Wang HJ, Wang W, Cornish KG, Rozanski GJ, Zucker IH. Cardiac sympathetic afferent denervation attenuates cardiac remodeling and improves cardiovascular dysfunction in rats with heart failure. Hypertension Oct 2014;64:745-755.

76. Longhurst JC. Cardiac receptors: their function in health and disease. Progress in cardiovascular diseases Nov-Dec 1984;27:201-222.

77. Longhurst JC, Tjen-A-Looi SC, Fu L-W. Cardiac Sympathetic Afferent Activation Provoked by Myocardial Ischemia and Reperfusion. Annals of the New York Academy of Sciences 2006;940:74-95.

78. Fukuda K, Kanazawa H, Aizawa Y, Ardell JL, Shivkumar K. Cardiac innervation and sudden cardiac death. Circ Res Jun 5 2015;116:2005-2019.

79. Schwartz PJ, Foreman RD, Stone HL, Brown AM. Effect of dorsal root section on the arrhythmias associated with coronary occlusion. Am J Physiol Sep 1976;231:923-928.

80. Vaseghi M, Gima J, Kanaan C, Ajijola OA, Marmureanu A, Mahajan A, Shivkumar K. Cardiac sympathetic denervation in patients with refractory ventricular arrhythmias or electrical storm: intermediate and long-term follow-up. Heart Rhythm Mar 2014;11:360-366.

81. Bourke T, Vaseghi M, Michowitz Y, Sankhla V, Shah M, Swapna N, Boyle NG, Mahajan A, Narasimhan C, Lokhandwala Y, Shivkumar K. Neuraxial modulation for

refractory ventricular arrhythmias: value of thoracic epidural anesthesia and surgical left cardiac sympathetic denervation. Circulation Jun 1 2010;121:2255-2262.

82. Wasmund SL, Li JM, Page RL, Joglar JA, Kowal RC, Smith ML, Hamdan MH. Effect of atrial fibrillation and an irregular ventricular response on sympathetic nerve activity in human subjects. Circulation Apr 22 2003;107:2011-2015.

83. Sleight P. A historical perspective on peripheral reflex cardiovascular control from animals to man. Exp Physiol Aug 2014;99:1017-1026.

84. Chapleau MW, Hajduczok G, Sharma RV, Wachtel RE, Cunningham JT, Sullivan MJ, Abboud FM. Mechanisms of baroreceptor activation. Clin Exp Hypertens Jan-Feb 1995;17:1-13.

85. Seagard JL, van Brederode JF, Dean C, Hopp FA, Gallenberg LA, Kampine JP. Firing characteristics of single-fiber carotid sinus baroreceptors. Circ Res Jun 1990;66:1499-1509.

86. Andresen MC, Hofmann ME, Fawley JA. The unsilent majority-TRPV1 drives "spontaneous" transmission of unmyelinated primary afferents within cardiorespiratory NTS. American journal of physiology Regulatory, integrative and comparative physiology Dec 15 2012;303:R1207-1216.

87. Fan W, Schild JH, Andresen MC. Graded and dynamic reflex summation of myelinated and unmyelinated rat aortic baroreceptors. Am J Physiol Sep 1999;277:R748-756.

88. Donoghue S, Fox RE, Kidd C, Koley BN. The distribution in the cat brain stem of neurones activated by vagal nonmyelinated fibres from the heart and lungs. Quarterly journal of experimental physiology (Cambridge, England) Oct 1981;66:391-404.

89. Mifflin SW. Convergent carotid sinus nerve and superior laryngeal nerve afferent inputs to neurons in the NTS. Am J Physiol Oct 1996;271:R870-880.

90. Goldstein DS, Cheshire WP, Jr. Beat-to-beat blood pressure and heart rate responses to the Valsalva maneuver. Clinical autonomic research : official journal of the Clinical Autonomic Research Society Dec 2017;27:361-367.

91. Bertinieri G, di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G. A new approach to analysis of the arterial baroreflex. Journal of hypertension Supplement : official journal of the International Society of Hypertension Dec 1985;3:S79-81.

92. Hunt BE, Fahy L, Farquhar WB, Taylor JA. Quantification of mechanical and neural components of vagal baroreflex in humans. Hypertension Jun 2001;37:1362-1368.

93. Gupta PD, Henry JP, Sinclair R, Von Baumgarten R. Responses of atrial and aortic baroreceptors to nonhypotensive hemorrhage and to tranfusion. Am J Physiol Dec 1966;211:1429-1437.

94. Kumar P, Prabhakar NR. Peripheral chemoreceptors: function and plasticity of the carotid body. Compr Physiol Jan 2012;2:141-219.

95. Guyenet PG. Regulation of breathing and autonomic outflows by chemoreceptors. Compr Physiol Oct 2014;4:1511-1562.

96. Goswami N, Blaber AP, Hinghofer-Szalkay H, Convertino VA. Lower Body Negative Pressure: Physiological Effects, Applications, and Implementation. Physiol Rev Jan 1 2019;99:807-851.

97. Oren RM, Schobel HP, Weiss RM, Stanford W, Ferguson DW. Importance of left atrial baroreceptors in the cardiopulmonary baroreflex of normal humans. J Appl Physiol (1985) Jun 1993;74:2672-2680.

98. Kappagoda CT, Linden RJ, Mary DA. Reflex increase in heart rate from stimulation of left atrial receptors. J Physiol Jan 1975;244:78p-79p.

99. Kappagoda CT, Linden RJ, Scott EM, Snow HM. Atrial receptors and heart rate: the efferent pathway. J Physiol Aug 1975;249:581-590.

100. Kappagoda CT, Linden RJ, Sivananthan N. The nature of the atrial receptors responsible for a reflex increase in heart rate in the dog. J Physiol Jun 1979;291:393-412.

101. Paintal AS. A study of right and left atrial receptors. J Physiol Jun 29 1953;120:596-610.

102. Paintal AS. The influence of certain chemical substances on the initiation of sensory discharges in pulmonary and gastric stretch receptors and atrial receptors. J Physiol Mar 11 1957;135:486-510.

103. Paintal AS. Natural Stimulation of Type B Atrial Receptors. J Physiol Nov 1963;169:116-136.

104. Hicks MN, Mary DA, Walters GE. Atrial receptor discharge in dogs with chronically induced difference in blood volume. J Physiol Dec 1987;393:491-497.

105. Nonidez JF. Identification of the receptor areas in the venae cavae and pulmonary veins which initiate reflex cardiac acceleration (Bainbridge's reflex). American Journal of Anatomy 1937;61:203-231.

106. Coleridge JC, Hemingway A, Holmes RL, Linden RJ. The location of atrial receptors in the dog: a physiological and histological study. J Physiol Apr 3 1957;136:174-197.

107. Coleridge JCG, Hemingway A, Holmes RL, Linden RJ. The location of atrial receptors in the dog: A physiological and histological study. Journal of Physiology 1957;136:174-197.

108. Zucker IH, Earle AM, Gilmore JP. The Mechanism of Adaptation of Left Atrial Stretch Receptors in Dogs with Chronic Congestive Heart Failure. Journal of Clinical Investigation 1977;60:323-331.

109. Zucker IH, Gilmore JP. Left atrial receptor discharge during atrial arrhythmias in the dog. Circ Res Dec 1973;33:672-677.

110. Norsk P, Ellegaard P, Videbaek R, Stadeager C, Jessen F, Johansen LB, Kristensen MS, Kamegai M, Warberg J, Christensen NJ. Arterial pulse pressure and vasopressin release in humans during lower body negative pressure. Am J Physiol May 1993;264:R1024-1030.

111. Khurana RK, Setty A. The value of the isometric hand-grip test--studies in various autonomic disorders. Clinical autonomic research : official journal of the Clinical Autonomic Research Society Aug 1996;6:211-218.

112. McCloskey DI, Mitchell JH. Reflex cardiovascular and respiratory responses originating in exercising muscle. J Physiol Jul 1972;224:173-186.

113. Coote JH. Physiological significance of somatic afferent pathways from skeletal muscle and joints with reflex effects on the heart and circulation. Brain research Apr 11 1975;87:139-144.

114. McAllen RM. Mediation of the fastigial pressor response and a somatosympathetic reflex by ventral medullary neurones in the cat. J Physiol Nov 1985;368:423-433.

115. Stornetta RL, Morrison SF, Ruggiero DA, Reis DJ. Neurons of rostral ventrolateral medulla mediate somatic pressor reflex. Am J Physiol Feb 1989;256:R448-462.

116. Ruggiero DA, Gomez RE, Cravo SL, Mtui E, Anwar M, Reis DJ. The Rostral Ventrolateral Medulla: Anatomical Substrates of Cardiopulmonary Integration. Cardiorespiratory and Motor Coordination: Springer Berlin Heidelberg; 1991:89-102.

117. Koletsos N, Dipla K, Triantafyllou A, Gkaliagkousi E, Sachpekidis V, Zafeiridis A, Douma S. A brief submaximal isometric exercise test 'unmasks' systolic and diastolic masked hypertension. Journal of hypertension Apr 2019;37:710-719.

118. Booth LC, May CN, Yao ST. The role of the renal afferent and efferent nerve fibers in heart failure. Front Physiol 2015;6:270.

119. Leiria TL, Glavinovic T, Armour JA, Cardinal R, de Lima GG, Kus T. Longterm effects of cardiac mediastinal nerve cryoablation on neural inducibility of atrial fibrillation in canines. Autonomic neuroscience : basic & clinical Apr 26 2011;161:68-74.

120. Randall DC, Brown DR, Li SG, Olmstead ME, Kilgore JM, Sprinkle AG, Randall WC, Ardell JL. Ablation of posterior atrial ganglionated plexus potentiates sympathetic tachycardia to behavioral stress. Am J Physiol Sep 1998;275:R779-787.

121. McGuirt AS, Schmacht DC, Ardell JL. Autonomic interactions for control of atrial rate are maintained after SA nodal parasympathectomy. Am J Physiol Jun 1997;272:H2525-2533.

122. Guyenet PG. The sympathetic control of blood pressure. Nat Rev Neurosci May 2006;7:335-346.

123. Guyenet PG, Stornetta RL, Bochorishvili G, Depuy SD, Burke PG, Abbott SB. C1 neurons: the body's EMTs. American journal of physiology Regulatory, integrative and comparative physiology Aug 1 2013;305:R187-204.

124. Malik V, Holobotovskyy VV, Phillips JK, McKitrick DJ, Arnolda LF. Intrathecal cGMP elicits pressor responses and maintains mean blood pressure during haemorrhage in anaesthetized rats. J Physiol Jun 1 2007;581:543-552.

125. Thornton JM, Aziz T, Schlugman D, Paterson DJ. Electrical stimulation of the midbrain increases heart rate and arterial blood pressure in awake humans. J Physiol Mar 1 2002;539:615-621.

126. Sverrisdottir YB, Green AL, Aziz TZ, Bahuri NF, Hyam J, Basnayake SD, Paterson DJ. Differentiated baroreflex modulation of sympathetic nerve activity during deep brain stimulation in humans. Hypertension May 2014;63:1000-1010.

127. Green A, Wang S, Owen S, Xie K, Liu X, Paterson D, Stein J, Bain P, Aziz T. Deep brain stimulation can regulate arterial blood pressure in awake humans. Neuroreport 2005;16:1741-1745.

128. Vaseghi M, Lux RL, Mahajan A, Shivkumar K. Sympathetic stimulation increases dispersion of repolarization in humans with myocardial infarction. Am J Physiol Heart Circ Physiol May 1 2012;302:H1838-1846.

129. Ajijola OA, Vaseghi M, Zhou W, Yamakawa K, Benharash P, Hadaya J, Lux RL, Mahajan A, Shivkumar K. Functional differences between junctional and extrajunctional adrenergic receptor activation in mammalian ventricle. Am J Physiol Heart Circ Physiol Feb 15 2013;304:H579-588.

130. Butler CK, Smith FM, Cardinal R, Murphy DA, Hopkins DA, Armour JA. Cardiac responses to electrical stimulation of discrete loci in canine atrial and ventricular ganglionated plexi. Am J Physiol Nov 1990;259:H1365-1373.

131. Butler CK, Smith FM, Nicholson J, Armour JA. Cardiac effects induced by chemically activated neurons in canine intrathoracic ganglia. Am J Physiol Oct 1990;259:H1108-1117.

132. Cardinal R, Page P, Vermeulen M, Ardell JL, Armour JA. Spatially divergent cardiac responses to nicotinic stimulation of ganglionated plexus neurons in the canine heart. Autonomic neuroscience : basic & clinical Jan 28 2009;145:55-62.

133. Armour JA. Thoracic Autonomic Nerves Regulating the Canine Heart. Breakdown in Human Adaptation to 'Stress': Springer Netherlands; 1984:931-948.

134. Massari VJ, Johnson TA, Gatti PJ. Cardiotopic organization of the nucleus ambiguus? An anatomical and physiological analysis of neurons regulating atrioventricular conduction. Brain research May 15 1995;679:227-240.

135. Dergacheva O, Weigand LA, Dyavanapalli J, Mares J, Wang X, Mendelowitz D. Function and modulation of premotor brainstem parasympathetic cardiac neurons that control heart rate by hypoxia-, sleep-, and sleep-related diseases including obstructive sleep apnea. Prog Brain Res 2014;212:39-58.

136. Po SS, Nakagawa H, Jackman WM. Localization of left atrial ganglionated plexi in patients with atrial fibrillation: Techniques and technology. Journal of Cardiovascular Electrophysiology 2009;20:1186-1189.

137. Pokushalov E, Romanov A, Artyomenko S, Turov A, Shirokova N, Katritsis DG. Left Atrial Ablation at the Anatomic Areas of Ganglionated Plexi for Paroxysmal Atrial Fibrillation. Pacing and Clinical Electrophysiology 2010;33:1231-1238.

138. Scherlag BJ, Nakagawa H, Jackman WM, Yamanashi WS, Patterson E, Po S, Lazzara R. Electrical stimulation to identify neural elements on the heart: their role in atrial fibrillation. Journal of interventional cardiac electrophysiology : an international journal of arrhythmias and pacing Aug 2005;13 Suppl 1:37-42.

139. Yuan BX, Ardell JL, Hopkins DA, Losier AM, Armour JA. Gross and microscopic anatomy of the canine intrinsic cardiac nervous system. Anat Rec May 1994;239:75-87.

140. Arora RC, Waldmann M, Hopkins DA, Armour JA. Porcine intrinsic cardiac ganglia. Anat Rec A Discov Mol Cell Evol Biol Mar 2003;271:249-258.

141. Ardell JL, Randall WC. Selective vagal innervation of sinoatrial and atrioventricular nodes in canine heart. Am J Physiol Oct 1986;251:H764-773.

142. Yamakawa K, So EL, Rajendran PS, Hoang JD, Makkar N, Mahajan A, Shivkumar K, Vaseghi M. Electrophysiological effects of right and left vagal nerve stimulation on the ventricular myocardium. Am J Physiol Heart Circ Physiol Sep 1 2014;307:H722-731.

143. Kember G, Armour JA, Zamir M. Neural control of heart rate: the role of neuronal networking. J Theor Biol May 21 2011;277:41-47.

144. Ardell JL, Butler CK, Smith FM, Hopkins DA, Armour JA. Activity of in vivo atrial and ventricular neurons in chronically decentralized canine hearts. Am J Physiol Mar 1991;260:H713-721.

145. Murphy DA, Thompson GW, Ardell JL, McCraty R, Stevenson RS, Sangalang VE, Cardinal R, Wilkinson M, Craig S, Smith FM, Kingma JG, Armour JA. The heart reinnervates after transplantation. The Annals of thoracic surgery Jun 2000;69:1769-1781.

146. Vaseghi M, Lellouche N, Ritter H, Fonarow GC, Patel JK, Moriguchi J, Fishbein MC, Kobashigawa JA, Shivkumar K. Mode and mechanisms of death after orthotopic heart transplantation. Heart Rhythm 2009;6:503-509.

147. Linz D, Hohl M, Elliott AD, Lau DH, Mahfoud F, Esler MD, Sanders P, Bohm M. Modulation of renal sympathetic innervation: recent insights beyond blood pressure control. Clinical autonomic research : official journal of the Clinical Autonomic Research Society Aug 2018;28:375-384.

148. Linz D, van Hunnik A, Hohl M, Mahfoud F, Wolf M, Neuberger HR, Casadei B, Reilly SN, Verheule S, Bohm M, Schotten U. Catheter-based renal denervation reduces atrial nerve sprouting and complexity of atrial fibrillation in goats. Circulation Arrhythmia and electrophysiology Apr 2015;8:466-474.

149. Steinberg JS, Shabanov V, Ponomarev D, Losik D, Ivanickiy E, Kropotkin E, Polyakov K, Ptaszynski P, Keweloh B, Yao CJ, Pokushalov EA, Romanov AB. Effect of Renal Denervation and Catheter Ablation vs Catheter Ablation Alone on Atrial Fibrillation Recurrence Among Patients With Paroxysmal Atrial Fibrillation and

Hypertension: The ERADICATE-AF Randomized Clinical Trial. Jama Jan 21 2020;323:248-255.

150. Groh CA, Faulkner M, Getabecha S, Taffe V, Nah G, Sigona K, McCall D, Hills MT, Sciarappa K, Pletcher MJ, Olgin JE, Marcus GM. Patient-reported triggers of paroxysmal atrial fibrillation. Heart Rhythm Jul 2019;16:996-1002.

151. Fedorowski A, Hedblad B, Engstrom G, Gustav Smith J, Melander O. Orthostatic hypotension and long-term incidence of atrial fibrillation: the Malmo Preventive Project. Journal of internal medicine Oct 2010;268:383-389.

152. Agarwal SK, Alonso A, Whelton SP, Soliman EZ, Rose KM, Chamberlain AM, Simpson RJ, Jr., Coresh J, Heiss G. Orthostatic change in blood pressure and incidence of atrial fibrillation: results from a bi-ethnic population based study. PloS one 2013;8:e79030.

153. Lampert R, Burg MM, Jamner LD, Dziura J, Brandt C, Li F, Donovan T, Soufer R. Effect of beta-blockers on triggering of symptomatic atrial fibrillation by anger or stress. Heart Rhythm Aug 2019;16:1167-1173.

154. Sgoifo A, de Boer SF, Westenbroek C, Maes FW, Beldhuis H, Suzuki T, Koolhaas JM. Incidence of arrhythmias and heart rate variability in wild-type rats exposed to social stress. Am J Physiol Oct 1997;273:H1754-1760.

155. Eaker ED, Sullivan LM, Kelly-Hayes M, D'Agostino RB, Sr., Benjamin EJ. Anger and hostility predict the development of atrial fibrillation in men in the Framingham Offspring Study. Circulation Mar 16 2004;109:1267-1271.

156. Agarwal SK, Norby FL, Whitsel EA, Soliman EZ, Chen LY, Loehr LR, Fuster V, Heiss G, Coresh J, Alonso A. Cardiac Autonomic Dysfunction and Incidence of Atrial Fibrillation: Results From 20 Years Follow-Up. J Am Coll Cardiol Jan 24 2017;69:291-299.

157. Middeldorp ME, Pathak RK, Meredith M, et al. PREVEntion and regReSsive Effect of weight-loss and risk factor modification on Atrial Fibrillation: the REVERSE-AF study. EP Europace 2018;20:1929-1935.

158. Grassi G, Seravalle G, Cattaneo BM, Bolla GB, Lanfranchi A, Colombo M, Giannattasio C, Brunani A, Cavagnini F, Mancia G. Sympathetic Activation in Obese Normotensive Subjects. Hypertension 1995;25:560-563.

159. Grassi G, Seravalle G, Colombo M, Bolla G, Cattaneo BM, Cavagnini F, Mancia G. Body Weight Reduction, Sympathetic Nerve Traffic, and Arterial Baroreflex in Obese Normotensive Humans. Circulation 1998;97:2037-2042.

160. Grassi G, Seravalle G, Dell'Oro R, Turri C, Pasqualinotto L, Colombo M, Mancia G. Participation of the Hypothalamus-Hypophysis Axis in the Sympathetic Activation of Human Obesity. Hypertension 2001;38:1316-1320.

161. Scherrer U, Randin D, Tappy L, Vollenweider P, Jequier E, Nicod P. Body fat and sympathetic nerve activity in healthy subjects. Circulation Jun 1994;89:2634-2640.

162. Spraul M, Ravussin E, Fontvieille AM, Rising R, Larson DE, Anderson EA. Reduced sympathetic nervous activity. A potential mechanism predisposing to body weight gain. Journal of Clinical Investigation 1993;92:1730-1735.

163. Lambert EA, Rice T, Eikelis N, Straznicky NE, Lambert GW, Head GA, Hensman C, Schlaich MP, Dixon JB. Sympathetic activity and markers of cardiovascular risk in nondiabetic severely obese patients: the effect of the initial 10% weight loss. American journal of hypertension Oct 2014;27:1308-1315.

164. Dimitri H, Ng M, Brooks AG, et al. Atrial remodeling in obstructive sleep apnea: implications for atrial fibrillation. Heart Rhythm Mar 2012;9:321-327.

165. Huang B, Liu H, Scherlag BJ, Sun L, Xing S, Xu J, Luo M, Guo Y, Cao G, Jiang H. Atrial fibrillation in obstructive sleep apnea: Neural mechanisms and emerging therapies. Trends Cardiovasc Med Jan 22 2020.

166. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort: The framingham heart study. Jama 1994;271:840-844.

167. Huxley RR, Lopez FL, Folsom AR, Agarwal SK, Loehr LR, Soliman EZ, Maclehose R, Konety S, Alonso A. Absolute and attributable risks of atrial fibrillation in relation to optimal and borderline risk factors: the Atherosclerosis Risk in Communities (ARIC) study. Circulation Apr 12 2011;123:1501-1508.

168. Lau DH, Mackenzie L, Kelly DJ, et al. Short-term hypertension is associated with the development of atrial fibrillation substrate: a study in an ovine hypertensive model. Heart Rhythm Mar 2010;7:396-404.

169. Lau DH, Mackenzie L, Kelly DJ, et al. Hypertension and atrial fibrillation: evidence of progressive atrial remodeling with electrostructural correlate in a conscious chronically instrumented ovine model. Heart Rhythm Sep 2010;7:1282-1290.

170. Lau DH, Shipp NJ, Kelly DJ, et al. Atrial arrhythmia in ageing spontaneously hypertensive rats: unraveling the substrate in hypertension and ageing. PLoS One 2013;8:e72416.

171. Kistler PM, Sanders P, Dodic M, Spence SJ, Samuel CS, Zhao C, Charles JA, Edwards GA, Kalman JM. Atrial electrical and structural abnormalities in an ovine model of chronic blood pressure elevation after prenatal corticosteroid exposure: implications for development of atrial fibrillation. Eur Heart J Dec 2006;27:3045-3056.

172. Grassi G, Cattaneo BM, Seravalle G, Lanfranchi A, Mancia G. Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. Hypertension Jan 1998;31:68-72.

173. Hering D, Kara T, Kucharska W, Somers VK, Narkiewicz K. Longitudinal tracking of muscle sympathetic nerve activity and its relationship with blood pressure in subjects with prehypertension. Blood Press Jun 2016;25:184-192.

174. Vranish JR, Holwerda SW, Young BE, Credeur DP, Patik JC, Barbosa TC, Keller DM, Fadel PJ. Exaggerated Vasoconstriction to Spontaneous Bursts of Muscle Sympathetic Nerve Activity in Healthy Young Black Men. Hypertension Jan 2018;71:192-198.

175. Malik V, McKitrick DJ, Lau DH, Sanders P, Arnolda LF. Clinical evidence of autonomic dysfunction due to atrial fibrillation: implications for rhythm control strategy. Journal of interventional cardiac electrophysiology : an international journal of arrhythmias and pacing Apr 2019;54:299-307.

176. Huxley RR, Filion KB, Konety S, Alonso A. Meta-analysis of cohort and case-control studies of type 2 diabetes mellitus and risk of atrial fibrillation. The American journal of cardiology 2011;108:56-62.

177. Xiong Z, Liu T, Tse G, Gong M, Gladding PA, Smaill BH, Stiles MK, Gillis AM, Zhao J. A Machine Learning Aided Systematic Review and Meta-Analysis of the Relative Risk of Atrial Fibrillation in Patients With Diabetes Mellitus. Frontiers in Physiology 2018;9:835.

178. Donnellan E, Aagaard P, Kanj M, Jaber W, Elshazly M, Hoosien M, Baranowski B, Hussein A, Saliba W, Wazni O. Association Between Pre-Ablation Glycemic Control and Outcomes Among Patients With Diabetes Undergoing Atrial Fibrillation Ablation. JACC Clin Electrophysiol Aug 2019;5:897-903.

179. Sanders P, Malik V, Lau DH. Glycemic Control in Atrial Fibrillation: A Sweet Spot of Risk Factor Modification. JACC Clin Electrophysiol Aug 2019;5:904-906.

180. Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic Autonomic Neuropathy. Diabetes Care 2003;26:1553-1579.

181. Rizzo MR, Sasso FC, Marfella R, et al. Autonomic dysfunction is associated with brief episodes of atrial fibrillation in type 2 diabetes. J Diabetes Complications Jan-Feb 2015;29:88-92.

182. Gallagher C, Hendriks JML, Elliott AD, Wong CX, Rangnekar G, Middeldorp ME, Mahajan R, Lau DH, Sanders P. Alcohol and incident atrial fibrillation - A systematic review and meta-analysis. Int J Cardiol Nov 1 2017;246:46-52.

183. Voskoboinik A, Kalman JM, De Silva A, et al. Alcohol Abstinence in Drinkers with Atrial Fibrillation. The New England journal of medicine Jan 2 2020;382:20-28.

184. Harper C. The neurotoxicity of alcohol. Hum Exp Toxicol Mar 2007;26:251-257.

185. Koskinen P, Virolainen J, Kupari M. Acute alcohol intake decreases short-term heart rate variability in healthy subjects. Clinical science (London, England : 1979) Aug 1994;87:225-230.

186. Rossinen J, Viitasalo M, Partanen J, Koskinen P, Kupari M, Nieminen MS. Effects of acute alcohol ingestion on heart rate variability in patients with documented coronary artery disease and stable angina pectoris. The American journal of cardiology Feb 15 1997;79:487-491.

187. Maki T, Toivonen L, Koskinen P, Naveri H, Harkonen M, Leinonen H. Effect of ethanol drinking, hangover, and exercise on adrenergic activity and heart rate variability in patients with a history of alcohol-induced atrial fibrillation. The American journal of cardiology Aug 1 1998;82:317-322.

188. Carter JR, Stream SF, Durocher JJ, Larson RA. Influence of acute alcohol ingestion on sympathetic neural responses to orthostatic stress in humans. Am J Physiol Endocrinol Metab May 2011;300:E771-778.

189. Narkiewicz K, Cooley RL, Somers VK. Alcohol potentiates orthostatic hypotension : implications for alcohol-related syncope. Circulation Feb 1 2000;101:398-402.

190. Abdel-Rahman AR, Merrill RH, Wooles WR. Effect of acute ethanol administration on the baroreceptor reflex control of heart rate in normotensive human volunteers. Clinical science (London, England : 1979) Jan 1987;72:113-122.

191. Chamberlain AM, Agarwal SK, Folsom AR, Duval S, Soliman EZ, Ambrose M, Eberly LE, Alonso A. Smoking and incidence of atrial fibrillation: results from the Atherosclerosis Risk in Communities (ARIC) study. Heart Rhythm Aug 2011;8:1160-1166.

192. Cryer PE, Haymond MW, Santiago JV, Shah SD. Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. The New England journal of medicine Sep 9 1976;295:573-577.

193. Haass M, Kubler W. Nicotine and sympathetic neurotransmission. Cardiovascular drugs and therapy Jan 1997;10:657-665.

194. Middlekauff HR, Park J, Agrawal H, Gornbein JA. Abnormal sympathetic nerve activity in women exposed to cigarette smoke: a potential mechanism to explain increased cardiac risk. Am J Physiol Heart Circ Physiol Nov 15 2013;305:H1560-1567.

195. Elliott AD, Maatman B, Emery MS, Sanders P. The role of exercise in atrial fibrillation prevention and promotion: Finding optimal ranges for health. Heart Rhythm Nov 2017;14:1713-1720.

196. Elliott AD, Linz D, Mishima R, Kadhim K, Gallagher C, Middeldorp ME, Verdicchio CV, Hendriks JML, Lau DH, La Gerche A, Sanders P. Association between physical activity and risk of incident arrhythmias in 402 406 individuals: evidence from the UK Biobank cohort. Eur Heart J Apr 14 2020;41:1479-1486.

197. Elliott AD, Linz D, Verdicchio CV, Sanders P. Exercise and Atrial Fibrillation: Prevention or Causation? Heart, lung & circulation Sep 2018;27:1078-1085.

198. Estes NAM, 3rd, Madias C. Atrial Fibrillation in Athletes: A Lesson in the Virtue of Moderation. JACC Clin Electrophysiol Sep 2017;3:921-928.

199. Guasch E, Benito B, Qi X, et al. Atrial fibrillation promotion by endurance exercise: demonstration and mechanistic exploration in an animal model. J Am Coll Cardiol Jul 2 2013;62:68-77.

200. Stambler B, Turner D, Guo C, GUO G, Ellenbogen K. CHARACTERIZATION OF THE IN-VIVO CARDIAC ELECTROPHYSIOLOGIC EFFECTS OF ATRIAL-NATRIURETIC-FACTOR (ANF). Paper presented at: Circulation1994.

201. Goette A, Honeycutt C, Langberg JJ. Electrical remodeling in atrial fibrillation. Time course and mechanisms. Circulation Dec 1 1996;94:2968-2974.

202. Segerson NM, Sharma N, Smith ML, et al. The effects of rate and irregularity on sympathetic nerve activity in human subjects. Heart Rhythm Jan 2007;4:20-26.

203. Field ME, Wasmund SL, Page RL, Hamdan MH. Restoring Sinus Rhythm Improves Baroreflex Function in Patients With Persistent Atrial Fibrillation. Journal of the American Heart Association 2016;5:e002997.

204. Ikeda T, Murai H, Kaneko S, Usui S, Kobayashi D, Nakano M, Ikeda K, Takashima S-I, Kato T, Okajima M, Furusho H, Takamura M. Augmented single-unit muscle sympathetic nerve activity in heart failure with chronic atrial fibrillation. 2012;590:509-518.

205. Marrouche NF, Brachmann J, Andresen D, et al. Catheter Ablation for Atrial Fibrillation with Heart Failure. The New England journal of medicine Feb 1 2018;378:417-427.

206. Gould PA, Yii M, Esler MD, Power JM, Kaye DM. Atrial fibrillation impairs cardiac sympathetic response to baroreceptor unloading in congestive heart failure. European Heart Journal 2005;26:2562-2567.

207. van den Berg MP, Hassink RJ, Tuinenburg AE, van Sonderen EF, Lefrandt JD, de Kam PJ, van Gelder IC, Smit AJ, Sanderman R, Crijns HJ. Quality of life in patients with paroxysmal atrial fibrillation and its predictors: importance of the autonomic nervous system. Eur Heart J Feb 2001;22:247-253.

208. Fassoulaki A, Theodoraki K, Melemeni A. Pharmacology of sedation agents and reversal agents. Digestion 2010;82:80-83.

209. Lu F, Lin J, Benditt DG. Conscious sedation and anesthesia in the cardiac electrophysiology laboratory. J Cardiovasc Electrophysiol Feb 2013;24:237-245.

210. Liu X, Rabin PL, Yuan Y, Kumar A, Vasallo P, 3rd, Wong J, Mitscher GA, Everett THt, Chen PS. Effects of anesthetic and sedative agents on sympathetic nerve activity. Heart Rhythm Dec 2019;16:1875-1882.

211. Jayachandran JV, Sih HJ, Winkle W, Zipes DP, Hutchins GD, Olgin JE. Atrial fibrillation produced by prolonged rapid atrial pacing is associated with heterogeneous changes in atrial sympathetic innervation. Circulation Mar 14 2000;101:1185-1191.

212. Olgin JE, Sih HJ, Hanish S, Jayachandran JV, Wu J, Zheng QH, Winkle W, Mulholland GK, Zipes DP, Hutchins G. Heterogeneous atrial denervation creates substrate for sustained atrial fibrillation. Circulation Dec 8 1998;98:2608-2614.

213. Morillo CA, Klein GJ, Jones DL, Guiraudon CM. Chronic rapid atrial pacing. Structural, functional, and electrophysiological characteristics of a new model of sustained atrial fibrillation. Circulation Mar 1 1995;91:1588-1595.

214. Gaspo R, Bosch RF, Talajic M, Nattel S. Functional mechanisms underlying tachycardia-induced sustained atrial fibrillation in a chronic dog model. Circulation Dec 2 1997;96:4027-4035.

215. Fareh S, Villemaire C, Nattel S. Importance of refractoriness heterogeneity in the enhanced vulnerability to atrial fibrillation induction caused by tachycardia-induced atrial electrical remodeling. Circulation Nov 17 1998;98:2202-2209.

216. Satoh T, Zipes DP. Unequal atrial stretch in dogs increases dispersion of refractoriness conducive to developing atrial fibrillation. J Cardiovasc Electrophysiol Sep 1996;7:833-842.

217. Misier AR, Opthof T, van Hemel NM, Defauw JJ, de Bakker JM, Janse MJ, van Capelle FJ. Increased dispersion of "refractoriness" in patients with idiopathic paroxysmal atrial fibrillation. J Am Coll Cardiol Jun 1992;19:1531-1535.

218. Calkins H, Allman K, Bolling S, Kirsch M, Wieland D, Morady F, Schwaiger M. Correlation between scintigraphic evidence of regional sympathetic neuronal dysfunction and ventricular refractoriness in the human heart. Circulation Jul 1993;88:172-179.

219. Inoue H, Zipes DP. Results of sympathetic denervation in the canine heart: supersensitivity that may be arrhythmogenic. Circulation Apr 1987;75:877-887.

220. Chang CM, Wu TJ, Zhou S, Doshi RN, Lee MH, Ohara T, Fishbein MC, Karagueuzian HS, Chen PS, Chen LS. Nerve sprouting and sympathetic hyperinnervation in a canine model of atrial fibrillation produced by prolonged right atrial pacing. Circulation Jan 2 2001;103:22-25.

221. Yu Y, Wei C, Liu L, Lian AL, Qu XF, Yu G. Atrial fibrillation increases sympathetic and parasympathetic neurons in the intrinsic cardiac nervous system. Pacing and clinical electrophysiology : PACE Nov 2014;37:1462-1469.

222. Armour JA, Murphy DA, Yuan BX, Macdonald S, Hopkins DA. Gross and microscopic anatomy of the human intrinsic cardiac nervous system. Anat Rec Feb 1997;247:289-298.

223. Katritsis DG, Pokushalov E, Romanov A, Giazitzoglou E, Siontis GC, Po SS, Camm AJ, Ioannidis JP. Autonomic denervation added to pulmonary vein isolation for paroxysmal atrial fibrillation: a randomized clinical trial. J Am Coll Cardiol Dec 17 2013;62:2318-2325.

224. Deneke T, Chaar H, de Groot JR, Wilde AA, Lawo T, Mundig J, Bosche L, Mugge A, Grewe PH. Shift in the pattern of autonomic atrial innervation in subjects with persistent atrial fibrillation. Heart Rhythm Sep 2011;8:1357-1363.

225. Gussak G, Pfenniger A, Wren L, et al. Region-specific parasympathetic nerve remodeling in the left atrium contributes to creation of a vulnerable substrate for atrial fibrillation. JCI Insight Oct 17 2019;4.

226. Malik V, Gallagher C, Linz D, Elliott AD, Emami M, Kadhim K, Mishima R, Hendriks JML, Mahajan R, Arnolda L, Sanders P, Lau DH. Atrial Fibrillation Is Associated With Syncope and Falls in Older Adults: A Systematic Review and Meta-analysis. Mayo Clinic proceedings Apr 2020;95:676-687.

227. Gardarsdottir M, Sigurdsson S, Aspelund T, Gardarsdottir VA, Forsberg L, Gudnason V, Arnar DO. Improved brain perfusion after electrical cardioversion of atrial fibrillation. Europace Apr 1 2020;22:530-537.

228. Diener H-C, Hart RG, Koudstaal PJ, Lane DA, Lip GYH. Atrial Fibrillation and Cognitive Function. Journal of the American College of Cardiology 2019;73:612-619.

229. Anter E, Jessup M, Callans DJ. Atrial fibrillation and heart failure: Treatment considerations for a dual epidemic. Circulation 2009;119:2516-2525.

230. Lakkireddy D, Atkins D, Pillarisetti J, Ryschon K, Bommana S, Drisko J, Vanga S, Dawn B. Effect of yoga on arrhythmia burden, anxiety, depression, and quality of life in paroxysmal atrial fibrillation: the YOGA My Heart Study. J Am Coll Cardiol Mar 19 2013;61:1177-1182.

231. Feinberg WM, Blackshear JL, Laupacis A, Kronmal R, Hart RG. Prevalence, age distribution, and gender of patients with atrial fibrillation. Analysis and implications. Archives of internal medicine Mar 13 1995;155:469-473.

232. Wong CX, Brooks AG, Leong DP, Roberts-Thomson KC, Sanders P. The Increasing Burden of Atrial Fibrillation Compared With Heart Failure and Myocardial Infarction: A 15-Year Study of All Hospitalizations in Australia. Archives of internal medicine May 14 2012;172:739-740.

233. Gallagher C, Hendriks JM, Giles L, Karnon J, Pham C, Elliott AD, Middeldorp ME, Mahajan R, Lau DH, Sanders P, Wong CX. Increasing trends in hospitalisations due to atrial fibrillation in Australia from 1993 to 2013. Heart (British Cardiac Society) Apr 1 2019.

234. Bunch TJ, Weiss JP, Crandall BG, May HT, Bair TL, Osborn JS, Anderson JL, Muhlestein JB, Horne BD, Lappe DL, Day JD. Atrial fibrillation is independently associated with senile, vascular, and Alzheimer's dementia. Heart rhythm Apr 2010;7:433-437.

235. Kalantarian S, Stern TA, Mansour M, Ruskin JN. Cognitive impairment associated with atrial fibrillation: a meta-analysis. Annals of internal medicine Mar 5 2013;158:338-346.

236. Wong CX, Gan SW, Lee SW, Gallagher C, Kinnear NJ, Lau DH, Mahajan R, Roberts-Thomson KC, Sanders P. Atrial fibrillation and risk of hip fracture: A population-based analysis of 113,600 individuals. International journal of cardiology Sep 15 2017;243:229-232.

237. Wallace ER, Siscovick DS, Sitlani CM, Dublin S, Mitchell P, Robbins JA, Fink HA, Cauley JA, Buzkova P, Carbone L, Chen Z, Heckbert SR. Incident atrial fibrillation and the risk of fracture in the cardiovascular health study. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA Feb 2017;28:719-725.

238. Jansen S, Frewen J, Finucane C, de Rooij SE, van der Velde N, Kenny RA. AF is associated with self-reported syncope and falls in a general population cohort. Age and ageing Jul 2015;44:598-603.

239. Sanders NA, Ganguly JA, Jetter TL, Daccarett M, Wasmund SL, Brignole M, Hamdan MH. Atrial fibrillation: an independent risk factor for nonaccidental falls in older patients. Pacing and clinical electrophysiology : PACE Aug 2012;35:973-979.

240. O'Neal WT, Qureshi WT, Judd SE, Bowling CB, Howard VJ, Howard G, Soliman EZ. Effect of Falls on Frequency of Atrial Fibrillation and Mortality Risk (from the REasons for Geographic And Racial Differences in Stroke Study). The American journal of cardiology Oct 15 2015;116:1213-1218.

241. Scott ME, Patterson GC. Cardiac output after direct current conversion of atrial fibrillation. British heart journal Jan 1969;31:87-90.

242. Kannus P, Parkkari J, Koskinen S, Niemi S, Palvanen M, Jarvinen M, Vuori I. Fall-induced injuries and deaths among older adults. Jama May 26 1999;281:1895-1899.

243. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. Jama Apr 19 2000;283:2008-2012.

244. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. [Web page]. 2012; http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 1 August 2019.

245. Chen X, Van Nguyen H, Shen Q, Chan DK. Characteristics associated with recurrent falls among the elderly within aged-care wards in a tertiary hospital: the effect of cognitive impairment. Archives of gerontology and geriatrics Sep-Oct 2011;53:e183-186.

246. Hung CY, Wu TJ, Wang KY, Huang JL, Loh el W, Chen YM, Lin CS, Lin CH, Chen DY, Tang YJ. Falls and Atrial Fibrillation in Elderly Patients. Acta Cardiologica Sinica Sep 2013;29:436-443.

247. Arita T, Suzuki S, Yagi N, et al. Impact of Atrial Fibrillation on Falls in Older Patients: Which is a Problem, Existence or Persistence? Journal of the American Medical Directors Association 2018.

248. Sule S, Palaniswamy C, Aronow WS, Ahn C, Peterson SJ, Adapa S, Mudambi L. Etiology of syncope in patients hospitalized with syncope and predictors of mortality and rehospitalization for syncope at 27-month follow-up. Clinical cardiology Jan 2011;34:35-38.

249. Chen L, Chen MH, Larson MG, Evans J, Benjamin EJ, Levy D. Risk factors for syncope in a community-based sample (the Framingham Heart Study). The American journal of cardiology May 15 2000;85:1189-1193.

250. McNicholas T, Tobin K, O'Callaghan S, Kenny RA. Is orthostatic hypotension more common in individuals with atrial fibrillation?-Findings from The Irish Longitudinal Study on Ageing (TILDA). Age and ageing Nov 1 2017;46:1006-1010.

251. Bhangu J, McMahon CG, Hall P, Bennett K, Rice C, Crean P, Sutton R, Kenny RA. Long-term cardiac monitoring in older adults with unexplained falls and syncope. Heart (British Cardiac Society) May 2016;102:681-686.

252. Vanegas DI, Jiménez NJ, Rincón CA, Hernández MA, Valderrama ZL. Experiencia clínica con el uso del monitor cardiaco implantable. Revista Colombiana de Cardiología 2017;24:223-229.

253. Brunckhorst, Lemola, Holzmeister, Scharf, Altwegg, Binggeli, Hellermann, Enseleit, Duru. Synkopenabklärung mittels implantierbarem Loop Recorder – Langzeiterfahrung eines Zentrumspitals. Praxis 2005/01/01 2005;94:105-112.

254. Arita T, Suzuki S, Yagi N, et al. Impact of Atrial Fibrillation on Falls in Older Patients: Which is a Problem, Existence or Persistence? Journal of the American Medical Directors Association Jun 2019;20:765-769.

255. Rawles JM. What is meant by a "controlled" ventricular rate in atrial fibrillation? Heart 1990;63:157-161.

256. Anter E, Jessup M, Callans DJ. Atrial fibrillation and heart failure: treatment considerations for a dual epidemic. Circulation May 12 2009;119:2516-2525.

257. Ricci F, De Caterina R, Fedorowski A. Orthostatic hypotension: Epidemiology, prognosis, and treatment. Journal of the American College of Cardiology 2015;66:848-860.

258. Oren RM, Schobel HP, Weiss RM, Stanford W, Ferguson DW. Importance of left atrial baroreceptors in the cardiopulmonary baroreflex of normal humans. Journal of applied physiology (Bethesda, Md : 1985) 1993;74:2672-2680.

259. Field ME, Wasmund SL, Page RL, Hamdan MH. Restoring Sinus Rhythm Improves Baroreflex Function in Patients With Persistent Atrial Fibrillation. Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease 02/23 11/25/received
01/13/accepted 2016;5:e002997.

260. Malik V, McKitrick DJ, Lau DH, Sanders P, Arnolda LF. Clinical evidence of autonomic dysfunction due to atrial fibrillation: implications for rhythm control strategy. Journal of Interventional Cardiac Electrophysiology 2019.

261. Brignole M, Gianfranchi L, Menozzi C, Raviele A, Oddone D, Lolli G, Bottoni N. Role of autonomic reflexes in syncope associated with paroxysmal atrial fibrillation. Journal of the American College of Cardiology Oct 1993;22:1123-1129.

262. Donoghue OA, Jansen S, Dooley C, De Rooij S, Van Der Velde N, Kenny RA. Atrial fibrillation is associated with impaired mobility in community-dwelling older adults. Journal of the American Medical Directors Association Dec 2014;15:929-933.

263. Fumagalli S, Potpara TS, Bjerregaard Larsen T, Haugaa KH, Dobreanu D, Proclemer A, Dagres N. Frailty syndrome: an emerging clinical problem in the everyday management of clinical arrhythmias. The results of the European Heart Rhythm Association survey. Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology Nov 1 2017;19:1896-1902.

264. Polidoro A, Stefanelli F, Ciacciarelli M, Pacelli A, Di Sanzo D, Alessandri C. Frailty in patients affected by atrial fibrillation. Archives of gerontology and geriatrics Nov-Dec 2013;57:325-327.

265. Thiyagarajah A, Lau DH, Sanders P. Atrial fibrillation and conduction system disease: the roles of catheter ablation and permanent pacing. Journal of interventional cardiac electrophysiology : an international journal of arrhythmias and pacing Aug 2018;52:395-402.

266. Liu L, Nattel S. Differing sympathetic and vagal effects on atrial fibrillation in dogs: role of refractoriness heterogeneity. American Journal of Physiology - Heart and Circulatory Physiology 1997;273:H805-816.

267. Lemola K, Chartier D, Yeh YH, Dubuc M, Cartier R, Armour A, Ting M, Sakabe M, Shiroshita-Takeshita A, Comtois P, Nattel S. Pulmonary vein region ablation in experimental vagal atrial fibrillation : Role of pulmonary veins versus autonomic ganglia. Circulation 2008;117:470-477.

268. Po SS, Scherlag BJ, Yamanashi WS, Edwards J, Zhou J, Wu R, Geng N, Lazzara R, Jackman WM. Experimental model for paroxysmal atrial fibrillation arising at the pulmonary vein-atrial junctions. Heart Rhythm 2006;3:201-208.

269. Brignole M, Gianfranchi L, Menozzi C, Raviele A, Oddone D, Lolli G, Bottoni N. Role of autonomic reflexes in syncope associated with paroxysmal atrial fibrillation. Journal of the American College of Cardiology 1993;22:1123-1129.

270. Reato S, Baratella MC, D'Este D. Persistent atrial fibrillation associated with syncope due to orthostatic hypotension: A case report. Journal of Cardiovascular Medicine 2009;10:866-868.

271. Nicia SB, Veelen TA, Stens J, Koopman MMW, Boer C. Detection of volume loss using the Nexfin device in blood donors. Anaesthesia 2016;71:163-170.

272. Cooke WH, Ryan KL, Convertino VA. Lower body negative pressure as a model to study progression to acute hemorrhagic shock in humans. J Appl Physiol (1985) Apr 2004;96:1249-1261.

273. Nixon JV, Murray RG, Leonard PD, Mitchell JH, Blomqvist CG. Effect of large variations in preload on left ventricular performance characteristics in normal subjects. Circulation 1982;65:698-703.

274. Modesti PA, Polidori G, Bertolozzi I, Vanni S, Cecioni I. Impairment of cardiopulmonary receptor sensitivity in the early phase of heart failure. Heart Jan 2004;90:30-36.

275. Lok NS, Lau CP. Abnormal vasovagal reaction, autonomic function, and heart rate variability in patients with paroxysmal atrial fibrillation. Pacing and clinical electrophysiology : PACE Feb 1998;21:386-395.

276. Heusser K, Schobel HP, Adamidis A, Fischer T, Frank H. Cardiovascular Effects of Beta-Blockers with and without Intrinsic Sympathomimetic Activity. Kidney and Blood Pressure Research 2002;25:34-41.

277. Stavrakis S, Humphrey MB, Scherlag BJ, Hu Y, Jackman WM, Nakagawa H, Lockwood D, Lazzara R, Po SS. Low-level transcutaneous electrical vagus nerve stimulation suppresses atrial fibrillation. J Am Coll Cardiol Mar 10 2015;65:867-875.

278. Chapleau MW, Hajduczok G, Abboud FM. Peripheral and central mechanisms of baroreflex resetting. Clin Exp Pharmacol Physiol Suppl 1989;15:31-43.

279. Smith G, Boyle MJ. The 10 mL syringe is useful in generating the recommended standard of 40 mmHg intrathoracic pressure for the Valsalva manoeuvre. Emerg Med Australas Dec 2009;21:449-454.

280. Shoemaker JK, Hogeman CS, Sinoway LI. Sympathetic responses to Valsalva's manoeuvre following bed rest. Can J Appl Physiol Jun 2003;28:342-355.

281. Campbell MR, Charles JB. Historical Review of Lower Body Negative Pressure Research in Space Medicine. Aerosp Med Hum Perform Jul 2015;86:633-640.

282. Wolthuis RA, Bergman SA, Nicogossian AE. Physiological effects of locally applied reduced pressure in man. Physiol Rev Jul 1974;54:566-595.

283. Trimarco B, Lembo G, De Luca N, Volpe M, Ricciardelli B, Condorelli G, Rosiello G, Condorelli M. Blunted sympathetic response to cardiopulmonary receptor unloading in hypertensive patients with left ventricular hypertrophy. A possible compensatory role of atrial natriuretic factor. Circulation Oct 1989;80:883-892.

284. Wythe S, Davies T, Martin D, Feelisch M, Gilbert-Kawai E. Getting the most from venous occlusion plethysmography: proposed methods for the analysis of data with a rest/exercise protocol. Extrem Physiol Med 2015;4:8.

285. De With RR, Marcos EG, Dudink E, Spronk HM, Crijns H, Rienstra M, Van Gelder IC. Atrial fibrillation progression risk factors and associated cardiovascular outcome in well-phenotyped patients: data from the AF-RISK study. Europace Mar 1 2020;22:352-360.

286. Salavatian S, Beaumont E, Longpre JP, Armour JA, Vinet A, Jacquemet V, Shivkumar K, Ardell JL. Vagal stimulation targets select populations of intrinsic cardiac neurons to control neurally induced atrial fibrillation. Am J Physiol Heart Circ Physiol Nov 1 2016;311:H1311-H1320.

287. Uradu A, Wan J, Doytchinova A, Wright KC, Lin AYT, Chen LS, Shen C, Lin SF, Everett THt, Chen PS. Skin sympathetic nerve activity precedes the onset and termination of paroxysmal atrial tachycardia and fibrillation. Heart Rhythm Jul 2017;14:964-971.

288. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Europace Nov 2016;18:1609-1678.

289. Pappone C, Santinelli V, Manguso F, et al. Pulmonary Vein Denervation Enhances Long-Term Benefit after Circumferential Ablation for Paroxysmal Atrial Fibrillation. Circulation 2004;109:327-334.

290. Scherlag BJ, Yamanashi W, Patel U, Lazzara R, Jackman WM. Autonomically induced conversion of pulmonary vein focal firing into atrial fibrillation. J Am Coll Cardiol Jun 7 2005;45:1878-1886.

291. Tang LYW, Hawkins NM, Ho K, Tam R, Deyell MW, Macle L, Verma A, Khairy P, Sheldon R, Andrade JG, Investigators C-DS. Autonomic Alterations After Pulmonary Vein Isolation in the CIRCA-DOSE (Cryoballoon vs Irrigated Radiofrequency Catheter Ablation) Study. J Am Heart Assoc Feb 2021;10:e018610.

292. Pauza DH, Skripka V, Pauziene N, Stropus R. Morphology, distribution, and variability of the epicardiac neural ganglionated subplexuses in the human heart. Anat Rec Aug 1 2000;259:353-382.

293. Hsieh MH, Chiou CW, Wen ZC, Wu CH, Tai CT, Tsai CF, Ding Ya, Chang MS, Chen Sa. Alterations of heart rate variability after radiofrequency catheter ablation of focal atrial fibrillation originating from pulmonary veins. Circulation 1999;100:2237-2243.

294. Lazzara R, Scherlag BJ, Robinson MJ, Samet P. Selective in situ parasympathetic control of the canine sinoatrial and atrioventricular nodes. Circ Res Mar 1973;32:393-401.

295. Marron K, Wharton J, Sheppard MN, Fagan D, Royston D, Kuhn DM, de Leval MR, Whitehead BF, Anderson RH, Polak JM. Distribution, morphology, and

neurochemistry of endocardial and epicardial nerve terminal arborizations in the human heart. Circulation Oct 15 1995;92:2343-2351.

296. Kulkarni K, Singh JP, Parks KA, Katritsis DG, Stavrakis S, Armoundas AA. Low-Level Tragus Stimulation Modulates Atrial Alternans and Fibrillation Burden in Patients With Paroxysmal Atrial Fibrillation. J Am Heart Assoc Jun 15 2021;10:e020865.

297. de Groot JR. Botulinum toxin injection in the autonomic ganglion plexi to prevent postoperative atrial fibrillation: More than a cosmetic treatment. Heart Rhythm Feb 2019;16:185-186.

298. Romanov A, Pokushalov E, Ponomarev D, et al. Long-term suppression of atrial fibrillation by botulinum toxin injection into epicardial fat pads in patients undergoing cardiac surgery: Three-year follow-up of a randomized study. Heart Rhythm Feb 2019;16:172-177.

299. Waldron NH, Cooter M, Haney JC, et al. Temporary autonomic modulation with botulinum toxin type A to reduce atrial fibrillation after cardiac surgery. Heart Rhythm Feb 2019;16:178-184.

300. Zhou L, Filiberti A, Humphrey MB, Fleming CD, Scherlag BJ, Po SS, Stavrakis S. Low-level transcutaneous vagus nerve stimulation attenuates cardiac remodelling in a rat model of heart failure with preserved ejection fraction. Exp Physiol Jan 2019;104:28-38.

301. Tran N, Asad Z, Elkholey K, Scherlag BJ, Po SS, Stavrakis S. Autonomic Neuromodulation Acutely Ameliorates Left Ventricular Strain in Humans. J Cardiovasc Transl Res Jun 2019;12:221-230.

302. Cakmak YO. Concerning Auricular Vagal Nerve Stimulation: Occult Neural Networks. Front Hum Neurosci 2019;13:421.

303. Matthews B, Robinson PP. The course of postganglionic sympathetic fibres distributed with the facial nerve in the cat. Brain research Sep 10 1986;382:55-60.

304. Cakmak YO, Cotofana S, Jager C, Morawski M, Sora MC, Werner M, Hammer N. Peri-arterial Autonomic Innervation of the Human Ear. Sci Rep Jul 31 2018;8:11469.

305. Clancy JA, Mary DA, Witte KK, Greenwood JP, Deuchars SA, Deuchars J. Non-invasive vagus nerve stimulation in healthy humans reduces sympathetic nerve activity. Brain Stimul Nov-Dec 2014;7:871-877.

306. Bryarly M, Phillips LT, Fu Q, Vernino S, Levine BD. Postural Orthostatic Tachycardia Syndrome: JACC Focus Seminar. J Am Coll Cardiol Mar 19 2019;73:1207-1228.

307. Sheldon RS, Grubb BP, 2nd, Olshansky B, et al. 2015 heart rhythm society expert consensus statement on the diagnosis and treatment of postural tachycardia syndrome, inappropriate sinus tachycardia, and vasovagal syncope. Heart Rhythm Jun 2015;12:e41-63.

308. Fu Q, Vangundy TB, Galbreath MM, Shibata S, Jain M, Hastings JL, Bhella PS, Levine BD. Cardiac origins of the postural orthostatic tachycardia syndrome. J Am Coll Cardiol Jun 22 2010;55:2858-2868.

309. Masuki S, Eisenach JH, Schrage WG, Johnson CP, Dietz NM, Wilkins BW, Sandroni P, Low PA, Joyner MJ. Reduced stroke volume during exercise in postural tachycardia syndrome. J Appl Physiol (1985) Oct 2007;103:1128-1135.

310. Joyner MJ, Masuki S. POTS versus deconditioning: the same or different? Clin Auton Res Dec 2008;18:300-307.

311. Gaffney FA, Nixon JV, Karlsson ES, Campbell W, Dowdey AB, Blomqvist CG. Cardiovascular deconditioning produced by 20 hours of bedrest with head-down tilt (-5 degrees) in middle-aged healthy men. Am J Cardiol Oct 1 1985;56:634-638.

312. Wells R, Elliott AD, Mahajan R, Page A, Iodice V, Sanders P, Lau DH. Efficacy of Therapies for Postural Tachycardia Syndrome: A Systematic Review and Meta-analysis. Mayo Clin Proc Aug 2018;93:1043-1053.

313. Perez-Riera AR, de Abreu LC, Barbosa-Barros R, Grindler J, Fernandes-Cardoso A, Baranchuk A. P-wave dispersion: an update. Indian Pacing Electrophysiol J Jul - Aug 2016;16:126-133.

314. Yagishita D, Chui RW, Yamakawa K, Rajendran PS, Ajijola OA, Nakamura K, So EL, Mahajan A, Shivkumar K, Vaseghi M. Sympathetic nerve stimulation, not circulating norepinephrine, modulates T-peak to T-end interval by increasing global dispersion of repolarization. Circ Arrhythm Electrophysiol Feb 2015;8:174-185.

315. Field ME, Donateo P, Bottoni N, et al. P-Wave Amplitude and PR Changes in Patients With Inappropriate Sinus Tachycardia: Findings Supportive of a Central Mechanism. J Am Heart Assoc Apr 19 2018;7.

316. Christensen NJ, Galbo H. Sympathetic nervous activity during exercise. Annu Rev Physiol 1983;45:139-153.

317. Korantzopoulos P, Letsas KP, Christogiannis Z, Kalantzi K, Massis I, Milionis HJ, Pappas C, Goudevenos JA. Exercise-induced repolarization changes in patients with stable coronary artery disease. Am J Cardiol Jan 2011;107:37-40.

318. Fu Q, Arbab-Zadeh A, Perhonen MA, Zhang R, Zuckerman JH, Levine BD. Hemodynamics of orthostatic intolerance: implications for gender differences. Am J Physiol Heart Circ Physiol Jan 2004;286:H449-457.

319. Magnani JW, Williamson MA, Ellinor PT, Monahan KM, Benjamin EJ. P wave indices: current status and future directions in epidemiology, clinical, and research applications. Circ Arrhythm Electrophysiol Feb 2009;2:72-79.

320. Lau DH, Psaltis PJ, Mackenzie L, et al. Atrial remodeling in an ovine model of anthracycline-induced nonischemic cardiomyopathy: remodeling of the same sort. J Cardiovasc Electrophysiol Feb 2011;22:175-182.

321. Berger RD, Kasper EK, Baughman KL, Marban E, Calkins H, Tomaselli GF. Beat-to-beat QT interval variability: novel evidence for repolarization lability in ischemic and nonischemic dilated cardiomyopathy. Circulation Sep 2 1997;96:1557-1565.

322. Pye M, Quinn AC, Cobbe SM. QT interval dispersion: a non-invasive marker of susceptibility to arrhythmia in patients with sustained ventricular arrhythmias? 1994;71:511-514.

323. Barr CS, Naas A, Freeman M, Lang CC, Struthers AD. QT dispersion and sudden unexpected death in chronic heart failure. Lancet Feb 5 1994;343:327-329.

324. Wei K, Dorian P, Newman D, Langer A. Association between QT dispersion and autonomic dysfunction in patients with diabetes mellitus. Journal of the American College of Cardiology 1995;26:859-863.

325. Tan AY, Li H, Wachsmann-Hogiu S, Chen LS, Chen P-S, Fishbein MC. Autonomic Innervation and Segmental Muscular Disconnections at the Human Pulmonary Vein-Atrial Junction. Journal of the American College of Cardiology 2006;48:132-143.

326. Burch GE, Romney RB. Functional anatomy and throttle valve action on the pulmonary veins. American heart journal 1954;47:58-66.

327. Ledsome JR, Linden RJ. The role of left atrial receptors in th diuretic response to left atrial distension. J Physiol Sep 1968;198:487-503.

328. Kidd C, Ledsome JR, Linden RJ. The effect of distension of the pulmonary vein-atrial junction on activity of left atrial receptors. J Physiol Dec 1978;285:445-453.

329. Gilmore JP, Zucker IH. Discharge of type B atrial receptors during changes in vascular volume and depression of atrial contractility. J Physiol Jun 1974;239:207-223.

330. Schuttler D, Bapat A, Kaab S, Lee K, Tomsits P, Clauss S, Hucker WJ. Animal Models of Atrial Fibrillation. Circ Res Jun 19 2020;127:91-110.

331. Cleroux J, Giannattasio C, Bolla G, Cuspidi C, Grassi G, Mazzola C, Sampieri L, Seravalle G, Valsecchi M, Mancia G. Decreased cardiopulmonary reflexes with aging in normotensive humans. Am J Physiol Sep 1989;257:H961-968.

332. Barcroft H, Edholm OG, McMichael J, Sharpey-Schafer EP. PosthÆmorrhagic Fainting. The Lancet 1944/04/15/ 1944;243:489-491.

333. Schiller AM, Howard JT, Convertino VA. The physiology of blood loss and shock: New insights from a human laboratory model of hemorrhage. Exp Biol Med (Maywood) Apr 2017;242:874-883.

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Appendix

Editorial commentary of our work

Editorial commentary on our published work (already in print or planned at the time of submission of this thesis):

1. Hu T, Noheria A, Asirvatham SJ. Atrial Fibrillation and Falls: A Mechanistic or Age-Confounded Relationship? Mayo Clinic proceedings Apr 2020;95:632-635.
2. Planned commentary for accepted article in J Am Coll Cardiol EP, as communicated by EIC J Am Coll Cardiol EP; of – work presented in Chapter 4, Chapter 5 and Chapter 7.