

The effects of adiposity and obesity on cardiac electrophysiology

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Statement of Originality

In accordance with Imperial College London regulations, I declare that all the work within this thesis is my own, unless specifically stated or referenced otherwise in the text.

Dr Kiran Haresh Kumar Patel

September 2023

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Shri Ganeshaya namah

Shri Saraswataye namah

Shri Gurubhyo namah

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Abstract

Obesity is an independent risk factor for cardiac arrhythmias. Although this is an active field of enquiry, there are several unknowns that have informed the studies contained in this thesis. First, the effect of increasing adiposity on ventricular repolarisation abnormalities has not been studied amongst the UK population. Second, it is unknown if surgical weight reduction reverses any subclinical proarrhythmic electrophysiological remodelling in arrhythmia-free obese patients. Third, the mechanisms by which epicardial fat confers higher risks of arrhythmias has not been studied.

Amongst the UK Biobank population, increasing adiposity and cardiometabolic ill-health were associated with QTc prolongation and their effect on QTc interval was additive when they co-existed. The relationships between QTc interval and increasing adiposity and cardiometabolic ill-health, respectively, were not modulated by individuals' genetics. Ventricular arrhythmias were more prevalent in individuals with cardiometabolic ill-health, with or without concurrent obesity, compared to obesity alone.

Non-invasive electrocardiographic imaging in morbidly obese and non-obese individuals without a history of cardiac arrhythmias showed that total atrial activation times, dispersion of total atrial activation and atrial activation gradients were greater in obese patients and persisted after bariatric surgery. By contrast, spatial ventricular repolarisation gradients reversed after bariatric surgery in obese patients, to the extent that there was no statistical difference between non-obese and post-operative

obese individuals. Bariatric surgery was also associated with partial improvements in glycaemic control, autonomic function and regression of left ventricular mass as well as epicardial fat volume.

Paired human epicardial adipose tissue (EAT) and subcutaneous adipose tissue (SAT) biopsies were harvested from patients undergoing cardiothoracic surgery and cultured to generate adipose-conditioned media. EAT-conditioned media was associated with a higher concentration of profibrotic adipokine Activin A compared to SAT. Neonatal ventricular myocytes incubated with EAT showed greater conduction slowing in response to pacing under identical experimental conditions compared to SAT.

These results show that increasing adiposity and obesity are associated with abnormal ventricular repolarisation that reverses with weight reduction, although atrial activation abnormalities may persist even after weight reduction. These findings may reflect differential mechanisms of remodelling and that are partly attributable to EAT.

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List of Abbreviations

AF	Atrial fibrillation
ANOVA	Analysis of variance
AP	Action potential
APD	Action potential duration
ARlc	Corrected activation-recovery interval
AT	Activation time
ATG	Activation time gradient
BF	Body fat
BMI	Body mass index
BSP	Body surface potentials
BSPM	Body surface potential mapping
CRP	C-reactive protein
CT	Computed tomography
Cx	Connexin
DAD	Delayed after-depolarisation
EAD	Early after-depolarisation
EAT	Epicardial adipose tissue
ECG	Electrocardiogram
ECGi	Electrocardiographic imaging

EF	Ejection fraction
ELISA	Enzyme-linked immunosorbent assay
GWAS	Genome-wide association study
HbA1c	Glycosylated haemoglobin
HG	Hip girth
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A
IL	Interleukin
LV	Left ventricle
LVEDP	Left ventricular end diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end systolic volume
MEA	Multielectrode array
MRI	Magnetic resonance imaging
n	Number (sample size)
ns	not significant
NRVM	Neonatal rat ventricular myocytes
PRS	Polygenic risk score
PVC	Premature ventricular complex
QTc	Corrected QT (interval)
RT	Repolarisation time

RTG	Repolarisation time gradient
SAT	Subcutaneous adipose tissue
SGLT2i	Sodium-glucose co-transporter 2 inhibitor
SNP	Single nucleotide polymorphism
TAT	Total activation time
TDI	Townsend deprivation index
TGF- β	Tissue growth factor-beta
TNF- α	Tumour necrosis factor alpha
TRT	Total repolarisation time.
VF	Ventricular fibrillation
VT	Ventricular tachycardia
WG	Waist girth
WHR	Waist:hip ratio

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ventricular repolarisation heterogeneity – mechanistic insights into fat-related arrhythmic risk. 6-8 June. Manchester, UK.

Prizes and Awards

Royal Society of Medicine Cardiology President's prize 2023 - finalist

Mechanisms underpinning reversibility of arrhythmic risk in obesity with weight loss.

British Cardiovascular Society Annual Conference “Best of the Best” clinical abstracts 2022 – runner-up

Bariatric surgery reverses ventricular repolarisation heterogeneity – mechanistic insights into fat-related arrhythmic risk.

National Heart and Lung Institute Research Away Day 2022 - best flash presentation

Weight loss following bariatric surgery reduces repolarisation heterogeneity to ameliorate arrhythmic risk in obese patients.

Heart Rhythm Society Travel Scholarship recipient 2022

Repolarisation gradients decrease after bariatric surgery in obese patients.

Cardiac Electrophysiology Society Young Investigator Award - finalist

Repolarisation gradients decrease after bariatric surgery in obese patients.

The National Heart and Lung Foundation and the Michael Alberman Travel

Award 2020

The award was given to support a presentation at the European Society of Cardiology European Heart Rhythm Association Congress, Vienna, Austria. Owing to the coronavirus pandemic, the award was subsequently used to support a presentation at Heart Rhythm Society, San Francisco, USA.

Chapter 1: Introduction

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1.1. Overview

The work presented in this thesis investigated the effects of increasing adiposity and obesity on electrophysiological remodelling. The aim of this chapter is to provide the reader with sufficient background to contextualise the work presented subsequently in this thesis. As such, this chapter includes an introduction to basic cardiac electrophysiology including the mechanisms underpinning atrial and ventricular arrhythmias. It then discusses obesity as an arrhythmic risk factor, the extent to which this risk is conferred directly by adiposity and indirectly by obesity-related cardiometabolic ill-health, as well as the mechanisms underpinning obesity-related arrhythmogenesis focussing on epicardial fat. It then outlines the management options for obesity, and finally explains how proarrhythmic substrates may be studied non-invasively. Where appropriate, subsequent chapters will reflect and expand upon the principles and evidence outlined in this chapter to provide more detailed rationale and context for the results contained therein.

1.2. Basic cardiac electrophysiology

In sinus rhythm, the electrical impulse is generated in the sinoatrial node and propagates across the atria to reach the atrioventricular node. Following a physiological delay, the impulse is conducted down the bundle of His and Purkinje

fibres and delivered across the ventricles. The Bundle of His, which splits into the right and left bundle branches, is a subendocardial structure and therefore the activation wavefront propagates from endo- to epi-cardium via the Purkinje system. These anatomical structures constitute the intrinsic conduction system of the heart.

Myocardial tissue is also an electrical syncytium; each cardiomyocyte is electrically coupled to its neighbouring cells via specialised proteins called gap junctions. Gap junctions comprise of two connexon hemichannels, each of which are made of six connexins. Connexins (Cx) are transmembrane proteins arranged to form a central pore through which ions and small molecules (<1 kiloDalton) can pass. They are numbered according to their molecular weight in kiloDaltons and the most common connexins in the human heart are Cx40, Cx43 and Cx45. The alignment of two connexons between adjacent cardiomyocytes forms a channel through which ions may pass, thus enabling one cell to activate its neighbour to facilitate a rapid activation wavefront to spread across the myocardium. Similarly, gap junctions also ensure that the myocardium recovers in a relatively homogenous fashion and within a narrow timeframe following activation by enabling passive flow of charge from one cell to the next. Gap junctions are so-called because, although alignment of protein complexes facilitates molecular and ionic communication, adjacent cells are not in intimate contact and are separated by a small “gap”.

The electrical activation (depolarisation) and recovery (repolarisation) of cardiomyocytes is described by the action potential and is determined by a complex balance of ionic currents across the cell membrane. Although the currents and ion

channels determining the action potential differ across the atrial, ventricular and specialised conducting cardiomyocytes, the cardiac action potential may be considered as comprising five phases and can be summarised as follows. First, phase 0 is an upstroke that is generated by a rapid intracellular influx of sodium ions via voltage-gated sodium ($\text{Na}_v1.5$) channels, encoded by *SCN5A* and co-assembled with $\beta1-4$ proteins, resulting in the resting membrane potential to change from negative to +30-40mV. This process constitutes cellular depolarisation. Next, phase 1 is defined by a small downstroke denoting rapid repolarisation by a transient efflux of potassium chloride ions (I_{to}). The transient outward potassium current in humans is facilitated by $\text{K}_v4.3$ and $\text{K}_v4.2$ α -subunits co-assembled with KChIP2 and DPP proteins (3), and to a lesser extent by $\text{K}_v1.4$ α channel subunits (4). This is followed by a longer phase 2 comprising of slower repolarisation. The plateau is characteristic of phase 2 and is produced by the efflux of intracellular potassium ions via the rapid delayed rectifier outward current (I_{Kr}) conducted via the $\text{K}_v11.1$ pore-forming unit, also known as human ether-à-go-go related gene (hERG), and the slow rectifier current (I_{Ks}) via the $\text{K}_v7.1$ unit and other subunits encoded by *KCNQ1*, *KCNE1-5* genes (3). This is partly offset by an intracellular influx of calcium ions via L-type channels ($\text{Ca}_v1.2$) ($I_{Ca,L}$), and to lesser extent sodium ions (3). $I_{Ca,L}$ is also responsible for calcium-induced calcium release from the sarcoplasmic reticulum that facilitates excitation-contraction coupling (5), and importantly, for depolarisation (phase 0) of the sinoatrial and atrioventricular nodal cells rather than the sodium current observed in other cardiomyocytes (6). In phase 3, efflux of potassium occurs via the inward rectifier potassium current (I_{K1}) passing through $\text{K}_{ir}2.1$, $\text{K}_{ir}2.2$, $\text{K}_{ir}2.3$, and $\text{K}_{ir}2.4$ pore-forming proteins encoded by *KCNJ2*, *KCNJ12*, *KCNJ4*, and *KCNJ14* genes, respectively (7), which is represented by the downward

deflection and return to resting membrane potential signifying completion of repolarisation. Phase 4 is the resting membrane potential and is partly maintained by the $\text{Na}^+\text{-K}^+$ pump, which exchanges 3 Na^+ for 2 K^+ and thereby carries a net outward current, and other pumps such as the $\text{Na}^+\text{-Ca}^{2+}$ exchanger. In pacemaker atrial cells, the resting membrane potential becomes increasingly positive over time, until a threshold (-60mV) is reached to trigger phase 0. The action potentials of sinoatrial, atrial and atrioventricular nodal cells do not exhibit phase 2 or 3. By contrast, ventricular and Purkinje cardiomyocytes have a more stable negative resting membrane potential remains stable (-90mV) and their action potentials are characterised by phases 0-4. The action potential of different cardiomyocytes in relation to body surface electrical recordings of the heart on the electrocardiogram (ECG) is shown in Figure 1.1.

1.3. Atrial arrhythmias

Atrial arrhythmias encompass a range of arrhythmias arising superior to the atrio-ventricular septum. This includes atrial fibrillation (AF), atrial tachycardia and atrial ectopy. Amongst all cardiac arrhythmias, AF is the most common sustained tachyarrhythmia (8, 9) and is characterised by chaotic and ineffective atrial contraction that reduces ventricular filling and consequently cardiac output. More importantly, AF renders the left atrium vulnerable to thrombus formation that may embolise to cause stroke and other systemic ischaemic events. The mechanisms underpinning AF are incompletely understood and is the subject of intense academic endeavour. Although it is accepted that triggers in the pulmonary veins are responsible for the initiation of AF in a vulnerable substrate (10), various hypotheses have been proposed to explain its maintenance. These include rotors and spiral

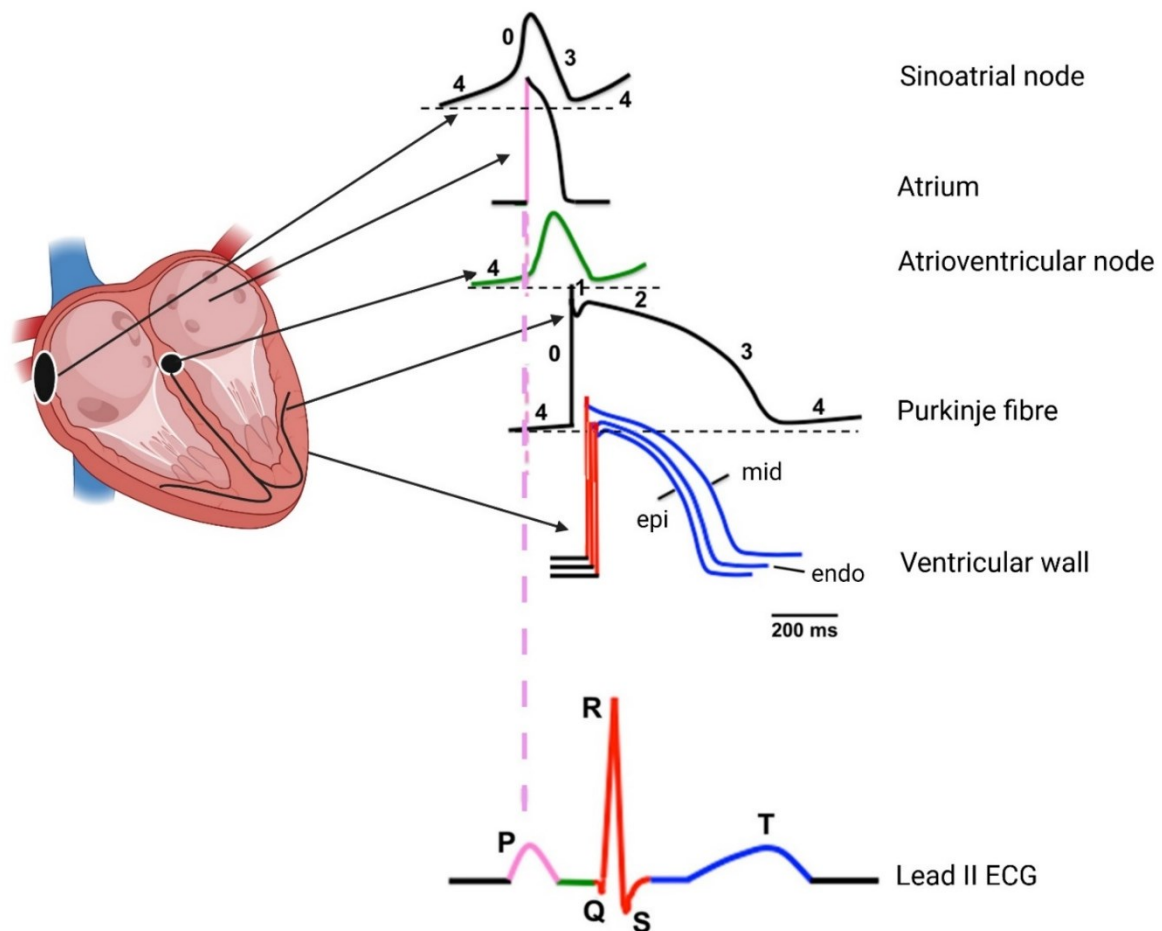


Figure 1.1: Action potentials in the heart. Action potentials in the heart vary morphologically as determined by the ion channels and electrophysiological properties that determine their individual characteristics. The corresponding deflections on the 12-lead ECG (lead II) is shown (colour coded). Numbers represent the phases ascribed to each part of action potential. Epi, epicardium; mid, mid-myocardium; endo, endocardium. Adapted from Varro et al, 2021 (14).

waves (11), which may anchor at anatomical structures or drift through the myocardium, and multiple wavelets which randomly propagate through the atria (12, 13).

1.4. Ventricular arrhythmias

The term ventricular arrhythmias usually encompass ventricular tachycardia (VT) and ventricular fibrillation (VF). However, it can also include ventricular extrasystoles, which are premature or ectopic contractions that arise from the ventricles.

VT is defined as three or more consecutive beats arising from ventricular tissue rather than specialised conducting cells at a rate of more than 100 beats per minute.

VT may be classed as monomorphic or polymorphic, depending on the QRS morphology, self-terminating, non-sustained or sustained based on duration, and haemodynamically stable or unstable based on clinical manifestation. Non-sustained VT self-terminates within 30 seconds of onset, whereas sustained VT persists beyond this threshold. Individuals with haemodynamically stable VT typically have a recordable and clinically acceptable blood pressure; unstable VT usually present with signs of circulatory compromise and may degenerate into VF.

VF is an irregular ventricular tachycardia with a heart rate usually exceeding 300 beats per minute and is characterised by chaotic contractions, manifesting as a highly variable cycle length and QRS morphology. VF is synonymous with cardiac arrest and in some instances may be preceded by VT. Up to a quarter of cardiovascular deaths occur suddenly and may be attributed to idiopathic ventricular fibrillation in structurally normal hearts and no history of cardiovascular disease (15, 16). It follows that subclinical conduction and/or repolarisation abnormalities that may not be detected using standard 12-lead ECG contribute to these statistics.

1.5. Mechanisms of cardiac arrhythmias

The three mechanisms underpinning tachyarrhythmias are re-entry, triggered activity (extra-systoles) and automaticity. These mechanisms, particularly re-entry, may be understood in the historical context in which they were described.

1.5.1. Historical context

In 1849 Ludwig and Hoffa were amongst the first to observe chaotic ventricular contractions. In 1874, Vulpian described this as “mouvement fibrillaire” which later became known as “ventricular fibrillation” and in 1889 was proposed to be mechanism underlying sudden cardiac death (17). In 1898, W T Porter suggested that this rhythm was the consequence of abnormal conduction (18). Wiggers described VF as an uncoordinated type of contraction that produces no functional cardiac output that quickly results in loss of consciousness and death without restoration of co-ordinated contractions (19).

The mechanisms underlying ventricular arrhythmias began to be investigated in the early 20th century. In 1908, Mayer described the phenomenon of re-entry in a ring of tissue from a jellyfish (*Medusa Cassiopeia*) (20). He observed that it was possible to generate a circular activation wavefront by stimulating the ring on one side and introducing a region of local conduction block on another. Provided that the local block was removed before the wavefront reached it and the tissue had recovered its excitability, the activation wavefront would continue to propagate indefinitely. In 1914, Garrey showed that unidirectional block was a pre-requisite to sustain the propagating activation wavefront (21). He also noted that hearts of larger mammals

such as dogs rarely recovered from fibrillation, unlike hearts of smaller animals like mice that rarely failed to recover (21). Also in 1914, Mines applied these concepts in closed loops of myocardial tissue (22). His experiments showed how in the wake of conduction in healthy tissue, the refractory tail prevents re-activation of segments that have just been activated. However, in diseased myocardium, delayed conduction allows refractory tissue to recover excitability and can be re-activated when the activation wavefront returns to its origin. In this way, slow conduction and a short refractory period can sustain tachycardia that can degenerate into fibrillation (Figure 1.2) (22). Furthermore, by applying single electrical shocks to Langendorff-perfused rabbit hearts at different points during the cardiac cycle, Mines showed that only a critically-timed stimulus in the “vulnerable phase” of systole initiated ventricular fibrillation, and not at other times of the cardiac cycle in which it resulted in non-conducted ectopy (22). This was also confirmed by De Boer (23). Indeed, later in 1940, Shannon and Wiggers also described experimental findings of how turtle ventricles were not completely refractory throughout systole but were responsive to stimuli in the latter third of this phase of the cardiac cycle (24). Given that a stimulus was of sufficient strength, it was able to induce an extra contraction before the ventricle had completely recovered (24). These early experiments demonstrated how conduction slowing, unidirectional conduction block and ectopic stimuli render the myocardium vulnerable to life-threatening arrhythmias. Although these findings were reported in 1914, synchronised cardioversion for arrhythmias only became available in an integrated system approximately fifty years after Mines’ discoveries (25).

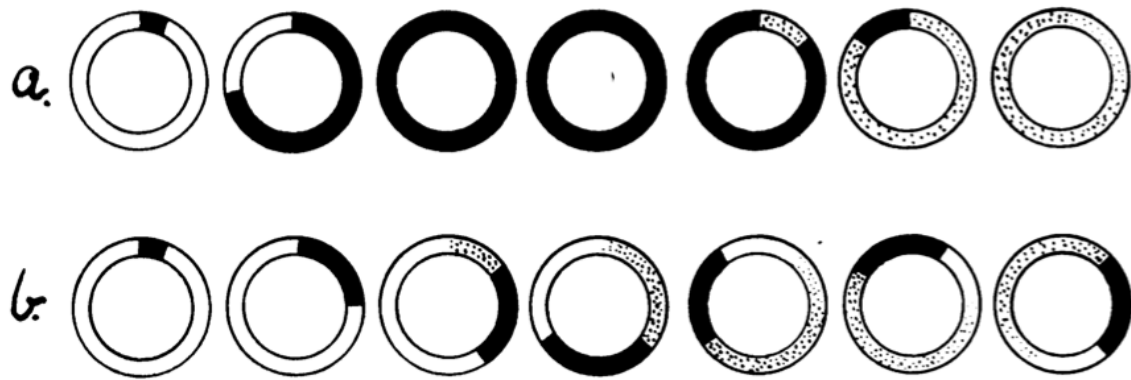


Figure 1.2: Mechanism of re-entry. Original figure reproduced from Mines' description of re-entry (26). In the top panel (**a**), an impulse leaves a trail of absolute refractory tissue shown in black. As the excitation wavefront propagates faster than the rate of recovery of the refractory tissue in its wake, the tissue is rendered in a state of total and absolute refractoriness (black ring) resulting in termination of the impulse. As the tissue recovers, it becomes relatively refractory (stippled) and eventually recovers to enable a new impulse to be propagated. In the bottom panel (**b**), if conduction is slowed or absolute refractory period is shortened, the tissue in the wake of the activation wavefront recovers from its absolute and relative refractoriness enabling the wavefront to propagate indefinitely in a circus movement.

In addition to conduction slowing and the critically-timed extra-stimuli, in 1963 Han and Moe hypothesised that non-uniform recovery following excitation, i.e. temporal heterogeneity or increased dispersion of repolarisation, would facilitate re-entry in the context of repetitive extra-stimuli (27). In open-chested dogs, they showed that dispersion of repolarisation increased significantly immediately after a premature stimulus in a train of regular paced beats. Moreover, they suggested that

heterogeneity of repolarisation would continue to increase with each successive premature beat and a train of premature beats, eventually leading to fibrillation. In experiments reminiscent of those performed by Mines, Allessie et al also showed that tachycardia could be initiated and sustained in isolated rabbit left atrial tissue by a single extra electrical stimulus delivered after the refractory period in a train of paced beats (28). Importantly, the tachycardia was maintained by a circus movement akin to the mechanism described by Mines(22). Subsequently, they showed that local conduction block occurred in regions with delayed recovery of excitability, so that heterogeneity of repolarisation resulted in conduction delay in the context of a critically timed premature impulse (29, 30). Combined with experimental work investigating the effects of conduction slowing and extra-stimuli in the early 20th century, these results on the non-uniform recovery of excitation describe the fundamental principles of re-entrant arrhythmias in normal and diseased hearts.

1.5.2. Re-entry

Re-entry is the most common mechanism of cardiac arrhythmias. As described in the previous section, it usually occurs in a substrate with an underlying predilection for non-uniform propagation of an activation wavefront in a closed-loop or spiral-wave fashion that overwhelms the intrinsic conduction pathways (31). The result is either a dyssynchronous contraction or abnormally fast heart rate that renders the myocardial contraction ineffective to maintain cardiac output (32). Functional re-entry, as first described by Garrey (21) occurs when an excitation wavefront propagate around refractory tissue in the absence of any structural abnormalities, such as scar tissue, to cause conduction block. Indeed, the “leading circle” re-entry described by Allessie et al comprised a core of the permanently refractory tissue

around which an excitation wavefront could propagate indefinitely (30). Functional re-entry may also occur around tissue that is excitable but not excited, resulting in spiral waves or rotors around these regions. In either case, steep gradients between areas of early and late repolarisation create unidirectional block of the subsequent wave of depolarisation thus promoting re-entry. In these instances, a critically timed premature beat occurring within a critical region can be particularly arrhythmogenic. This has been shown in individuals with a history of ventricular arrhythmias and in silico models (Cluitmans et al 2021). Spatial gradients of repolarisation are discussed in the following section.

1.5.3. Spatial gradients of repolarisation

Dispersion of repolarisation refers to the spatial distribution of repolarisation in the substrate of interest. An increased global dispersion of repolarisation denotes a relatively large difference between the earliest and latest repolarising regions. Following activation, an increased global dispersion of repolarisation may not be proarrhythmic if early and late repolarising regions are sufficiently anatomically distant, such that the latter is able to recover fully before the arrival of a subsequent activation wavefront. On the other hand, an increased dispersion of repolarisation between anatomically neighbouring sites, i.e. increased *local* dispersion, may result in functional conduction block if an activation wavefront encounters refractory tissue adjacent to a tissue that has recovered excitability and thereby facilitate re-entry by mechanisms described in the previous sections (see sections on historical context and re-entry). For this reason, repolarisation dispersion can be expressed as a function of the anatomical distance between early and late repolarising regions and have been referred to as spatial gradients of repolarisation (33). This concept also

explains why increased *local* spatial heterogeneity of repolarisation is a more important determinant of arrhythmic risk than prolongation of *global* repolarisation per se (34). Indeed, increasing the difference in repolarisation times between adjacent sites resulting in a steep spatial repolarisation time gradient (RTG) has been shown to be proarrhythmic in atrial and ventricular tissue. For example, in open-chest dogs regional warming and cooling of the ventricles augmented the difference in monophasic action potential duration between neighbouring regions and, upon stimulation with a premature stimulus from a region of early repolarisation, resulted in conduction delay that facilitated and sustained re-entry (35). Similarly, carbamylcholine-induced increased dispersion of repolarisation in rabbit atrial tissue also resulted in conduction block in the context of a critically-timed premature impulse arising from a region of early repolarisation (28, 29). Subsequent to these experimental observations, animal and computational studies have shown that re-entry along the border of early and late repolarising areas, i.e. the RTG, is responsible for the induction and maintenance of polymorphic VT (33). Clinically, the proarrhythmic myocardial substrate has also been shown to be characterised by steep RTG in several at-risk groups, including individuals with long QT syndrome (36) and Brugada syndrome (37) as well as survivors of idiopathic VF (38).

1.5.4. Triggered arrhythmias

Premature beats may occur spontaneously during phases 2 or 3 of an action potential and are known as early afterdepolarisations (EADs) (39). EADs may render the myocardium vulnerable to arrhythmias (32). For instance, APD prolongation which may be caused by myocardial inflammation for example, promotes EADs by facilitating the time-dependent recovery of L-type voltage-gated calcium channels

that had become inactivated after depolarisation. Intracellular calcium influx during repolarisation results in EADs which in turn cause non-uniform recovery and thus unidirectional conduction block and re-entry. The myocardium is particularly vulnerable to re-entry by this mechanism when a single critically timed EAD or series of EADs result in conducted premature ventricular contractions (PVCs) to sustain focal arrhythmias.

Premature beats may occur as delayed afterdepolarisations (DADs) during phase 4 of an action potential, i.e. after repolarisation and before the next action potential. Efflux of calcium ions from the sarcoplasmic reticulum, for example with increased sympathetic tone or autonomic dysfunction, causes cytosolic calcium concentration to increase and myocardial cells to reach depolarisation threshold sooner resulting in a premature contraction.

1.5.5. Enhanced automaticity

Enhanced automaticity describes the phenomenon in which myocardial cells depolarise with greater frequency. This can occur in the context of a normal physiological response, for example, during exercise and increased sympathetic drive. In this context, enhanced automaticity of cardiomyocytes in the conducting system may not necessarily result in arrhythmias. However, enhanced automaticity of other non-specialised cells may do, particularly if their rate of electrical discharge exceeds the intrinsic rate of pacemaker cells located in the sinus node, or an extra-stimulus coincides at a critical time with normally conducted impulses.

1.6. Substrate versus trigger

In normal healthy myocardium, the atria and ventricles are unlikely to either spontaneously initiate or maintain arrhythmias due to electrophysiological homeostatic mechanisms that tightly regulate depolarisation and repolarisation. On the other hand, an electrically or structurally remodelled substrate in itself may not initiate an arrhythmia in the absence of a trigger, such as an ectopic beat. Similarly, a trigger is unlikely to result in the maintenance of an arrhythmia in the absence of the remodelled substrate. Extra-cardiac factors, notably the autonomic nervous system, are also important modulators of arrhythmias. Thus, it can be concluded that any cardiac arrhythmia requires a vulnerable substrate and a trigger to exist under conditions that can sustain it. These requirements are known as Coumel's triangle of arrhythmogenesis (40). A vulnerable substrate may be characterised by electrical remodelling, for example by alterations in ion channel and gap junction function and expression, as well as microscopic and macroscopic structural remodelling which may, for example, be caused by fatty infiltration or dilatation, respectively. The substrate and/or triggers may evolve with time, thereby increasing vulnerability and as well burden and severity of arrhythmias. For instance, paroxysmal AF is believed to be initiated by triggers arising from the pulmonary veins in a functionally vulnerable substrate, for example due to inflammation or ischaemia (10). Over time, paroxysms of AF induce structural remodelling characterised by fibrosis and atrial dilatation which underlies its progression from paroxysmal to persistent, and eventually permanent, AF (41).

1.7. Ventricular arrhythmias in the general and obese population

Consistent with Coumel's triangle of arrhythmogenesis (42), ventricular arrhythmias occur infrequently in the general population in the context of a normal QTc interval and absence of other risk factors, triggers or external factors. Even in instances when QTc is sufficiently prolonged in a non-obese population to trigger ectopy as a consequence of increased spatial heterogeneity of repolarisation, ventricular arrhythmias may not be sustained in the absence of an abnormal substrate to sustain them (38). Nevertheless, although VT and PVCs are more likely to occur in structurally abnormal hearts, ventricular arrhythmias (VA) may occur in individuals amongst the general population with no apparent structural heart disease and account for up to 10% of all VT (43). Furthermore, in that population-based study age- and sex-adjusted incidence of idiopathic VA was estimated to be 51.86/100,000 (95% CI 47.72–56.01) and increased with age. Although the incidence of VT was similar between males and females, age-adjusted incidence of symptomatic PVCs was higher in females than males (46.2/100,000, 95% CI 40.9–51.6 vs 20.5/100,000, 95% CI 16.8–24.3). Idiopathic VA-induced cardiomyopathy and PVC leading to more sinister VA occurred rarely suggesting that in the general population VAs are usually either transient without significant sequelae or associated with a terminal event (43). By contrast, in a separate study obese individuals have been shown to experience a greater burden of PVCs (44) and a higher rate of appropriate shock therapy from implantable cardiac defibrillators (45) compared to their non-obese counterparts amongst the general population. These data suggest that obese hearts represent a remodelled substrate in which PVC are triggered more readily leading to a greater prevalence of sustained sinister VA than in leaner hearts.

1.8. Inflammation and arrhythmic risk

One of the many mechanisms by which the myocardium may be rendered a proarrhythmic substrate is by local and systemic inflammation. For instance, amongst 5806 individuals followed up for almost 7 years, Aviles et al. showed that those in the highest C-reactive protein (CRP) quartile ($>3.41\text{mg/L}$) had almost two-fold higher AF burden (7.4% vs 3.7%), as well as 31% greater risk of developing AF in the future, than those in the lowest quartile ($<0.97\text{mg/L}$) (46). Moreover, one standard deviation increase in CRP levels was associated with a 24% greater risk of developing AF (46). Similarly, in the prospective Copenhagen City Heart study, there was a two-fold increase in the risk of AF in individuals of the highest CRP quintile, suggesting that inflammation was a predictor of incident AF (47). Consistent with the association between baseline CRP and arrhythmic risk, both local (e.g. myocarditis and pericarditis) and systemic (e.g. sepsis and post cardiothoracic surgery) inflammatory responses have been implicated in arrhythmogenesis (48, 49, 50, 51). For example, patients with severe sepsis have a six-fold greater risk of new-onset AF compared to non-septic patients, which increased further with transition to septic shock (52, 53). These findings were corroborated in a meta-analysis of 11 studies by Kuipers et al. who demonstrated that compared to an absence of sepsis, AF risk incremented by 8%, 10% and 23% in sepsis, severe sepsis and septic shock, respectively (54). This association extended to other types of arrhythmias including atrial flutter, paroxysmal supraventricular tachycardia, VT and VF (50). Similarly, acute phase reactions post-cardiothoracic surgery, quantified by rise in CRP and C3- and C4-CRP complexes has shown to correlate with the occurrence of arrhythmia, while other inflammatory biomarkers (IL-6, IL-8 and IL-10) have demonstrated at

greater concentrations in individuals with post-operative AF than those in sinus rhythm (51, 55).

The association between inflammation and arrhythmogenesis is also supported by immunohistochemical analysis of atrial appendageal tissue (56, 57), demonstrating infiltration of immunologically active macrophages that is consistent with an increased neutrophil:leucocyte ratio observed in AF patients (58). Furthermore, AF is associated with increased expression of monocyte chemoattractant-1 (MCP-1), intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in the endocardium which promotes recruitment of inflammatory cells (56, 57, 58, 59).

These lines of enquiry supporting the role of inflammation in arrhythmogenesis is strengthened by the reversibility observed with anti-inflammatory agents. A meta-analysis of 50 randomised controlled trials demonstrated that low (<1000mg) and medium (1000-10,000mg) dose of corticosteroid reduced CRP and IL-6 concentrations, and risk of AF by 26% in the context of post-cardiac surgery (60). Similarly, 0.5mg colchicine twice daily vs control halved the incidence of post-operative AF in patients undergoing cardiac surgery (12.0% vs 22.0%) and pulmonary vein isolation (16.0% vs 33.5%), respectively (61, 62). In patients with elevated CRP, the JUPITER trial demonstrated 27% decrease in the incidence of AF in those taking rosuvastatin 20mg daily compared to control, an effect that may be explained by the suppression of the inflammatory mediators (63, 64).

1.9. Obesity

Obesity is characterised by increased body mass as a consequence of excess adipose volume. It is a complex multifactorial disease caused by biological, psychosocial, economic and environmental factors (65). Importantly, obesity represents a chronic low-grade systemically pro-inflammatory state. Adipocytes hypertrophy with increasing adiposity creating a hypoxic environment and inducing genetic mutations which results in up- and down-regulation of pro- and anti-inflammatory mediators, respectively (66, 67). Before discussing the evidence supporting obesity as proarrhythmic risk factor, the following sections will provide a synopsis on the classification of obesity, its epidemiology and related cardiovascular adaptations.

1.9.1. Definition

Body mass index (BMI), measured by dividing weight (kilograms) by height² (metres), is the most commonly used index to measure body weight, and forms the basis for the majority of healthcare recommendations and guidelines. This is because it is quick and easy to implement and can be used to track changes in whole body adiposity over time. A healthy weight by BMI is considered 18.5-24.9kg/m² and overweight 25-29.9 kg/m². Thus, the widely accepted definition of obesity is a BMI equal to or greater than 30kg/m². Subclassifications of obesity include grade 1 obesity, which is defined as 30-34.9kg/m², grade 2 as BMI 35-39.9kg/m² and grade III (morbidly obese) as BMI 40kg/m² or greater. These definitions of obesity by BMI are inherently flawed as it is an imprecise measure of adiposity. For this reason, according to the UK obesity management guidelines (68), waist girth (WG) which is an indicator of abdominal visceral adiposity may be a

useful adjunct measure to assess health risks in overweight and obese individuals. WG greater than 94cm is considered high and greater than 102cm very high in males. In females, the same thresholds are 80cm and 88cm, respectively. One of the limitations of WG, however, is that there is no consensus on the anatomical landmarks to guide measurement which results in intra- and inter-individual variability. Other anthropometric indices may also be used to quantify adiposity, such as waist:hip ratio (WHR) and waist:height ratio, although these do not yield significantly more information on adiposity than WG. Neck circumference is neither commonly used nor widely referenced in studies. Abdominal diameter is a surrogate for WG but more difficult to measure clinically (69). For these reasons, BMI and WG are the mainstay metrics for quantifying weight and adiposity in guidelines (68, 70, 71).

1.9.2. Epidemiology of obesity

The incidence of obesity has at least doubled since 1980 (Figure 1.3) and affects over 600 million adults worldwide (72). According to the Global Burden of Disease group, more than a third of the world's population is either overweight or obese (73). The prevalence of obesity is increasing even in low- and middle-income countries which has been attributed to urbanisation, accessibility to energy-dense food over traditional diets and a cultural shift towards Western lifestyles (74). In the UK, two thirds of the population is overweight or obese (75). Over the past decade, the economic costs of obesity have been brought into sharp focus by several studies. In 2011, a simulation model using data from the USA and UK suggested that by 2030 there would be 65 million and 11 million more obese adults in the USA and UK,

respectively, which would increase the number of patients with diabetes by up to 8 million, heart disease and stroke by 7 million, and cancer by 0.5 million at the expense of 55 million quality-adjusted life years (76). These were expected to add up to £2 billion to healthcare costs in the UK each year (76). Another meta-analysis estimated that, compared to individuals with healthy weight, excess weight or obesity increased UK healthcare costs by as much as 12% and 36%, respectively (77). These excess costs were borne from a higher demand for medications, hospitalisation and ambulatory care, and were greater in females than males (77). Indeed, prescription costs incurred by a morbidly obese patient over a three-year period are ten times greater than that of a normal weight patient (78). Similarly, the cost of delivering general practice services to obese patients is almost three times greater than that for normal weight patients, and over a three-year period the average total healthcare resource utilisation costs by a morbidly obese patient is approximately £10,500 (78). In 2019, obesity was responsible for over 1 million hospital admissions in the UK, accounting for £6.5 billion of the NHS' annual spend (79). Most recently, analysis on 310,913 UK Biobank participants showed that a single unit increase in body mass index (BMI) decreased quality adjusted life years by 0.65% (95% CI: 0.49-0.81%) per year and increased annual total healthcare costs by £42.23 (95% CI: £32.95-£51.51) per person (80). Given that arrhythmic risk naturally increases with age, and with many Western hemisphere countries reporting aging populations (81), the obesity epidemic is likely to add to the burden of arrhythmias in the coming years. In short, obesity is a burdensome healthcare and economic problem that shows no sign of abating.

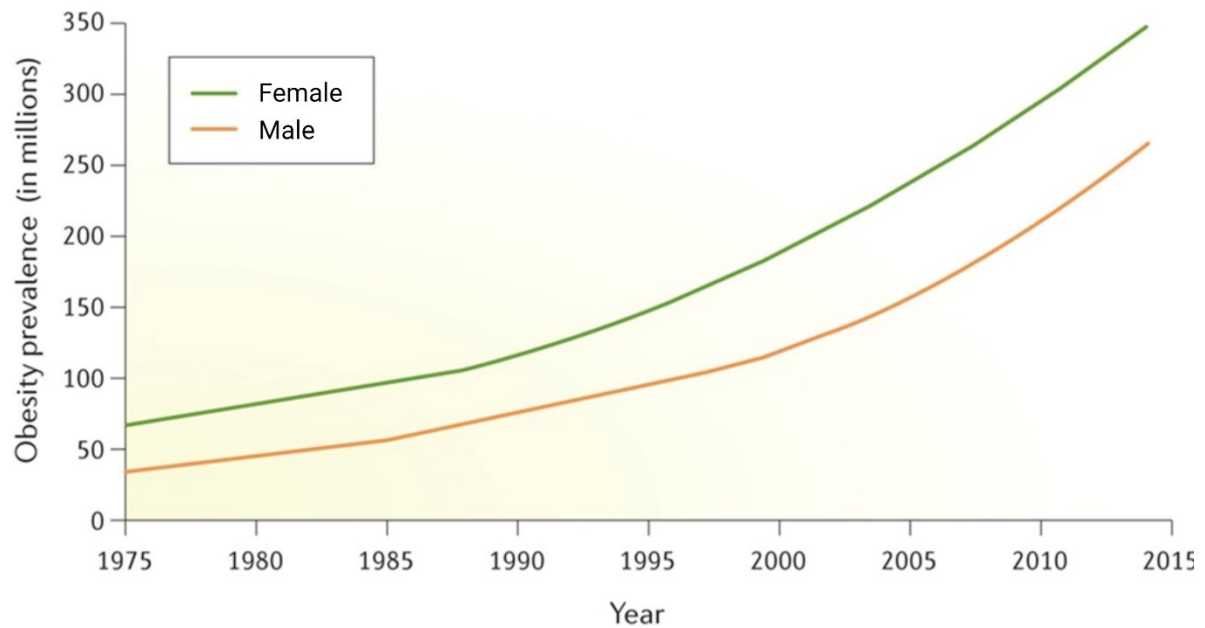


Figure 1.3: Global prevalence of obesity from 1975 to 2015. Obesity has more than doubled since 1975 and is consistently more prevalent in females than males. Original figure reproduced and adapted from Gonzalez-Muniesa et al (82).

1.9.3. Cardiovascular adaptations in obesity

Obesity is associated with several haemodynamic adaptations. Increasing adiposity results in increased circulating blood volume and a concurrent reduction in systemic vascular resistance, resulting in atrial stretch, increased stroke volume and cardiac output (83). In chronic obesity, these adaptations may cause further atrial dilatation as well as concentric or eccentric left ventricular hypertrophy, resulting in raised left ventricular filling pressures and wall stress that predispose to diastolic dysfunction(83). Systolic dysfunction occurs when left ventricular hypertrophy is inadequate to match the demands of increased wall stress, and the left ventricle begins to fail (84). This exacerbates left atrial dilatation (85) and causes pulmonary venous hypertension (84). When combined with hypoxia and acidosis from OSA due to mechanical compression of the respiratory tract in obesity (86), these culminate in

pulmonary arterial hypertension, right ventricular hypertrophy and right ventricular failure, and eventually, congestive cardiac failure (84). These physiological adaptations are influenced by severity of obesity, degree of abnormal pre- and after-loading conditions, and extent of neurohormonal and metabolic abnormalities, such as increased sympathetic tone, renin-angiotensin-aldosterone system activation, insulin resistance and lipotoxicity from adipose tissue (84). These structural, neuroendocrine, metabolic and lipotoxic effects of increasing adiposity directly and indirectly cause structural and electrical remodelling of the atria and ventricles. The following sections will discuss the evidence for obesity as a risk factor for atrial and ventricular arrhythmias, and is also represented in Figure 1.4.

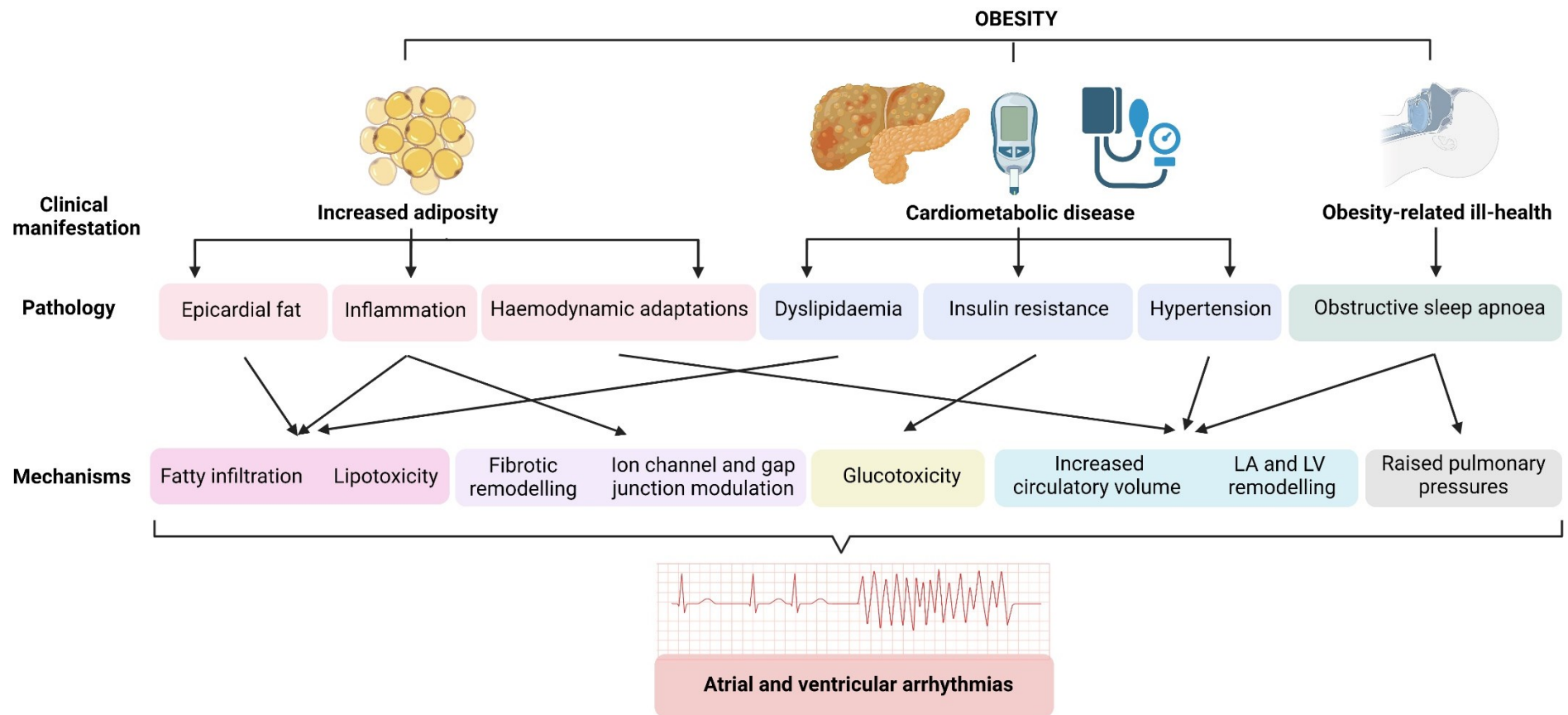


Figure 1.4: Mechanisms underpinning obesity as a risk factor for atrial and ventricular arrhythmias. The higher risk of cardiac arrhythmias in obesity is conferred by increasing adiposity, concurrent cardiometabolic disease and related ill-health. These result in adverse electrophysiological remodelling of the atria and ventricles to create a proarrhythmic substrate.

1.9.4. Obesity and atrial arrhythmic risk

In age and sex-adjusted multivariable analyses of 13,382 echocardiograms, obese patients had increased odds (OR 2.53, 95% CI 2.30 to 2.75) of left atrial enlargement, in addition to other parameters of diastolic dysfunction (85). A prospective study of 1212 adults followed-up over 10 years showed that obesity and hypertension were independent predictors of left atrial volume after adjusting for age, sex, cardiovascular disease and other cardiovascular risk factors (87). Furthermore, linear regression analyses demonstrated that the effect of obesity was almost twice that of hypertension, suggesting obesity is the more important risk factor for left atrial enlargement. The Framingham Heart Study also showed that left atrial dilatation was a mediator of atrial arrhythmias associated with increasing BMI (88).

Electroanatomic adaptations of the atria in obesity manifest as changes P wave indices (PWI) on 12-lead ECG, which are the features characterising right and left atrial electrical depolarisation (89). P-wave prolongation, increased dispersion of P-wave (difference between the longest and shortest P-wave) and P-wave axis deviation have been associated with atrial remodelling in echocardiographic studies (90, 91), as well as with increased risk of AF (92, 93, 94), in the Atherosclerosis Risk in Communities study (ARIC) and the Framingham Heart Study. Furthermore, increasing BMI was associated with abnormal PWIs even after adjusting for age, sex, ethnicity and pericardial fat volume among 996 participants in a computed tomography (CT) sub-study of the Multi-Ethnic Study of Atherosclerosis (MESA) (95). Subsequently, a larger investigation of 14,433 participants enrolled in the prospective ARIC study showed progressive abnormalities in PWIs with increasing weight after adjusting for traditional cardiovascular risk factors (96).

Atrial dilatation and the consequent distortion of its electroanatomic architecture causes atrial conduction slowing and increases conduction heterogeneity which are hallmarks of a proarrhythmic substrate (97, 98). In ovine models, increasing weight is associated with increasing left atrial volume, fibrosis, inflammation and lipidosis, which increases conduction heterogeneity and spontaneous as well as inducible AF (97) (Figure 1.5). These findings have been corroborated in atrial tissue of obese patients that are similarly characterised by larger areas of low voltage, slowed conduction and fractionated electrograms compared to individuals with normal BMI (99) (Figure 1.6).

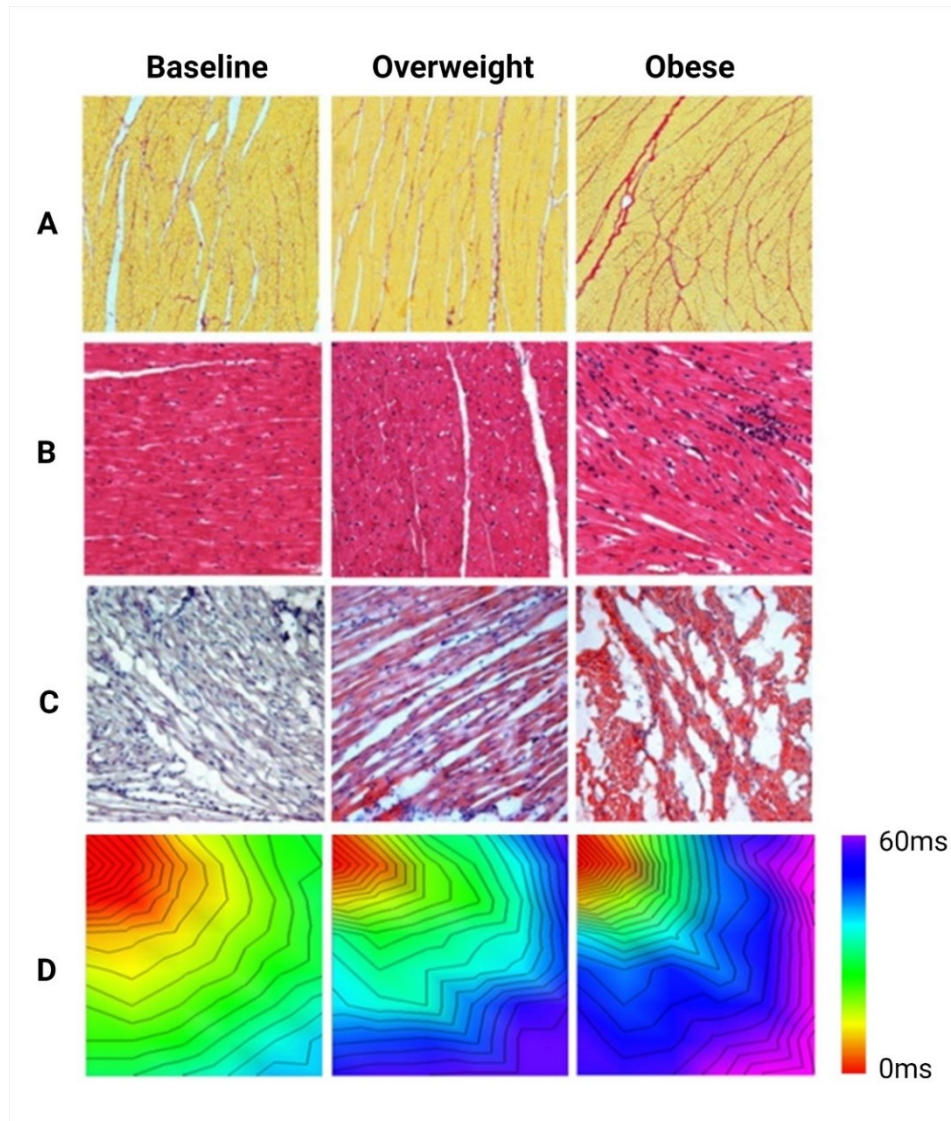


Figure 1.5: Obesity causes adverse structural and electrophysiological remodelling in ovine atria. (A-C): Histological analysis of atrial tissue from sheep at baseline and after a high-fat diet resulting in overweight and obese animals. Picrosirius **(A)**, haematoxylin and eosin **(B)** and Oil Red O **(C)** staining show greatest degree of fibrosis, inflammatory infiltrates and lipidosis, respectively, in the obese group. **(D):** Activation isochronal maps of left atrial appendage paced at 400ms shows slower conduction in the tissues from overweight and obese sheep (blue and purple, respectively). Figure adapted from Abed et al (97)

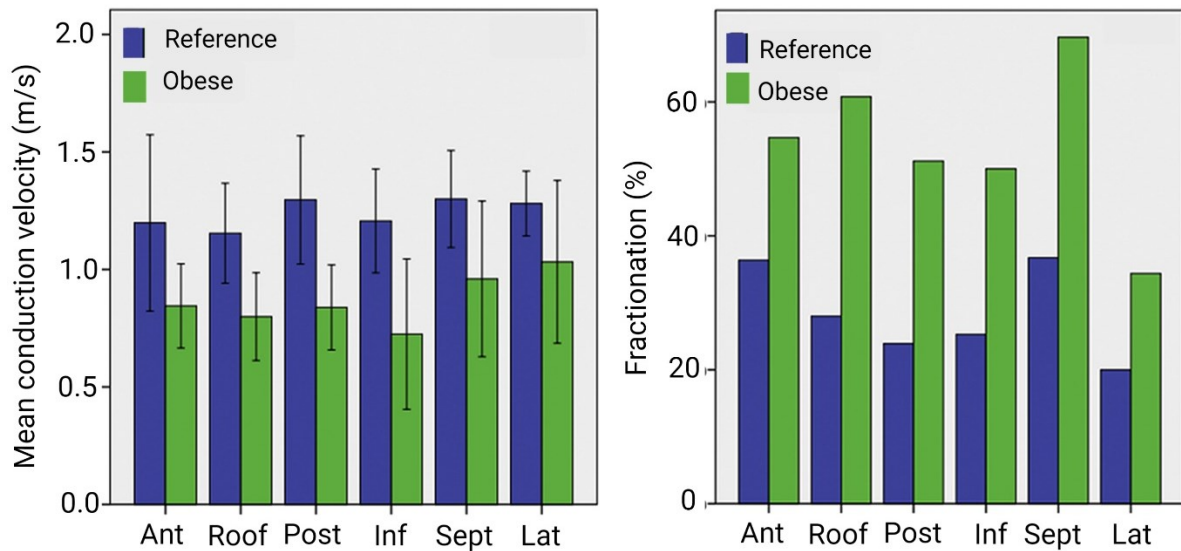


Figure 1.6: Atrial conduction is slower and electrogram fractionation is greater in left atrial tissue of obese vs non-obese individuals. Obese and non-obese individuals with AF underwent electroanatomic mapping. Isochronal activation maps of the left atria were generated from this data and conduction velocity determined in the direction of the wavefront propagation by calculating the difference in local activation times and distance between two points. Fractionated signals were defined as electrograms ≥ 50 ms duration with ≥ 3 deflections crossing the baseline. Electrophysiological differences between the lean and obese groups were observed across different regions of the left atria: Ant, anterior; Post, posterior; Inf, inferior; Sept; septal; Lat, lateral. Figure reproduced from Mahajan et al. (99)

Correspondingly, there is strong prospective and retrospective clinical evidence linking obesity to a higher incidence of AF (100, 101). Meta-analysis of 51 studies including 626,603 participants showed that a 5-unit increase in BMI conferred 29% and 19% higher odds of incident AF in cohort and case-control studies, respectively (100). Odds of post-operative and post-ablation AF were also 10% and 13% greater

per 5-unit increase in BMI, respectively, the latter suggesting that AF recurrence after invasive treatment is greater in those with higher body mass (100). Karasoy et al showed that although AF incidence was low amongst over 270,000 young female patients (mean age 31 ± 5 years), obesity was still an independent predictor of AF even in this age group (102). Furthermore, in a prospective community cohort study comprising over 3000 patients followed for 6 years, BMI independently predicted progression of paroxysmal to permanent AF [OR 1.04 per kg/m^2 (1.03–1.06)] after adjusting for age and sex (103). Thus, there is compelling evidence linking obesity to higher AF risks across a wide age range, as well as in both sexes.

1.9.5. Obesity and ventricular arrhythmic risk

On the 12-lead ECG, the QT interval is taken as the duration from the start of the QRS complex to the end of the T-wave. It is therefore a marker of ventricular activation and repolarisation. Given that action potential duration shortens as heart rate increases, it is corrected for heart rate (QTc) when evaluated in clinical practice. Generally, QTc intervals ≥ 450 msec in males and ≥ 470 msec in females are considered abnormal. QTc dispersion, is calculated as the difference between the longest and shortest QTc interval on a 12-lead ECG and has been used as a non-invasive metric to quantify dispersion of ventricular repolarisation. This refers to the spatial heterogeneity of repolarisation, such that increased dispersion may result in re-entry and thereby facilitate ventricular tachycardia/fibrillation (VT/VF). Although the clinical utility of QTc dispersion over QTc interval in predicting adverse arrhythmic and cardiovascular outcomes has been debated (104), both have nonetheless been shown to prolong with obesity and shorten with weight reduction (105, 106, 107, 108, 109, 110, 111, 112, 113, 114). One of the most comprehensive

analyses of the effects of the obesity on ventricular repolarisation was reported in a meta-analysis by Omran et al, who demonstrated that QTc was significantly longer in obese versus non-obese individuals (mean difference 21.74ms, 95%CI: 18.76 to 32.32ms). Similarly, pooled estimates for QTc dispersion were greater in obesity (mean difference 15.17ms, 95%CI: 13.59 to 16.74ms) (105). Another meta-analysis of 22 studies showed that QTc shortened and QTc dispersion decreased after lifestyle- or surgically-induced weight loss (QTc: mean difference -13.46ms, 95%CI: -15.60 to -11.32ms; QTc dispersion: mean difference -13.46ms, 95%CI: -15.60 to -11.32ms) (106, 111).

Consistent with abnormal ventricular repolarisation, obesity is the most common non-ischaemic cause of sudden cardiac death (SCD) (115). However, this risk may not be attributable to obesity alone given the variable definitions of SCD that may also include fatal cardiovascular events resulting from concurrent morbidity. Nevertheless, one meta-analysis of 14 prospective studies reported relative risks for SCD of 1.16 (95%CI: 1.05-1.28) per 5-unit increment in BMI, 1.82 (95%CI: 1.61-2.07) per 0.1 unit increase in waist-hip ratio, and 1.03 (95%CI: 0.93-1.15) per 10cm increase in waist circumference (115). Similarly, amongst participants of the ARIC study, increasing BMI, WG and WHR were independent risk factors for SCD after adjusting for age, sex, ethnicity and educational attainment. This association was stronger for WHR than for BMI and persisted even after further adjustment for traditional cardiovascular risk factors such as hypertension, diabetes, dyslipidaemia, concurrent ischaemic heart disease and heart failure, thus implicating central adiposity as an adverse prognostic marker (116). These studies support the

evidence for visceral adiposity being more detrimental than its subcutaneous counterpart (117).

In the MADIT trial, obese individuals received a higher rate of appropriate shock therapy from implantable cardiac defibrillators (45). The higher burden of ventricular ectopy, particularly in the context of left ventricular hypertrophy (44), may partly explain the higher incidence of VA and SCD in obesity, whereby critically timed extrasystoles can trigger re-entrant arrhythmias in a remodelled substrate (118). Furthermore, amongst over 1.2 million in-hospital cardiac arrests in the USA, 2.1% occurred amongst morbidly obese patients in whom the mortality was significantly greater compared to their non-obese counterparts and was also associated with prolonged hospitalisation (119). It is likely that the risks of VA and SCD in obesity are mediated by increasing adiposity as well as concurrent cardiometabolic comorbidity that independently cause electrophysiological remodelling. Although these studies demonstrate the obesity is linked to abnormal ventricular repolarisation, the extent to which this is due to increasing adiposity or conferred by concurrent ill-health, or modulated by individuals' genetics and if it translates into a higher prevalence of ventricular arrhythmias in the UK population are all presently unknown.

1.9.6. Obesity as a cardiometabolic risk factor

The economic costs of obesity are borne out of the wide range of associated adverse healthcare outcomes, including higher risks of cardiovascular, metabolic and musculoskeletal disorders as well as some cancers Organization (120).

Importantly, obese individuals are at higher risk of cardiovascular disease compared to normal weight counterparts, even in the absence of metabolic abnormalities (121). Observational data identifying associations between obesity and adverse health outcomes has recently been backed up with the advent of Mendelian randomisation (MR) studies that can more robustly establish causal links between the two (122). For example, a large meta-analysis comprising a total of 501 observational cohort studies and 12 MR studies showed that increasing BMI was associated with higher risks of coronary heart disease, heart failure, AF, ischaemic and haemorrhagic stroke, aortic valve stenosis, pulmonary embolism, and venous thrombo-embolism (123). The strength of causal associations was greatest for hypertension, with increasing weight conferring a 49% higher risk (relative risk 1.49; 95% CI 1.40-1.60). Similar findings have been reported amongst a large sample of the UK population in the UK Biobank, wherein a genetic predisposition for increasing BMI conferred higher risks for aortic stenosis, heart failure, deep venous thrombosis, hypertension, peripheral artery disease, coronary artery disease, AF, and pulmonary embolism by 6–13% (124). Additionally, there was an inverse link between fat-free mass and AF, ischaemic stroke and aortic aneurysms (124). Given that the costs of cardiovascular disease alone in the UK was over £29 billion in 2004, of which 60% was related to healthcare provision and services and almost 25% due to productivity losses from mortality and morbidity, obesity is a modifiable risk factor that warrants timely intervention.

1.9.7. Metabolic syndrome

The observation that obesity is often associated with cardiometabolic complications, such as insulin resistance, dyslipidaemia and hypertension was documented almost

a century ago (125). In 1988 this cluster was first referred to as “syndrome X” by Gerald Reaven in his Banting Lecture at the American Diabetes Association (126). He proposed that this syndrome, comprising insulin resistance, glucose intolerance or hyperinsulinaemia, increased very-low density lipoprotein and decreased high-density lipoprotein cholesterol, and hypertension, were common in obese patients and contributed to the higher risk of coronary artery disease in this population. More than a decade later the World Health Organisation (WHO) redefined syndrome X as metabolic syndrome (127) and has since undergone several iterations by other organisations, including the European Group for Study of Insulin Resistance (EGIR), National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) and International Diabetes Federation (IDF) (128). These are summarised in Table 1.1. The American Association of Clinical Endocrinologists (AACE) suggested the term insulin resistance syndrome based on the presence of glucose intolerance, abnormal uric acid metabolic, dyslipidaemia, hypertension, and serum biomarkers for thrombotic vulnerability, inflammation and endothelial dysfunction (129). This definition, however, is cumbersome and clinically difficult to implement and therefore has not been widely adopted. Although Table 1.1 demonstrates significant overlap between definitions of metabolic syndrome, there was a risk that their inconsistencies would introduce disparities in treatment. In response to this, a joint interim statement of the IDF Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity suggested using the IDF global consensus definition, but without obesity as a mandatory criterion (71). Instead, the presence of any three or more of the five parameters could be considered as diagnostic of metabolic

syndrome. Importantly, the ethnicity-based cut-offs for WG suggested by the IDF were retained in this joint statement (>90cm for males of Asian or Chinese descent vs >94cm for Caucasian/African/Middle Eastern populations).

With respect to WG, studies have shown that it is a better indicator of visceral adiposity and fasting and post-glucose insulin levels compared to other anthropometric indices, such as waist:hip ratio (WHR) (69). In combination, therefore, BMI and WG provide a useful indication about whether an individual has predominantly central visceral adiposity or whole body “general” obesity. This distinction is clinically important given that abdominal visceral adiposity has been associated with a poorer cardiometabolic status compared to subcutaneous fat (69, 117, 130, 131, 132, 133, 134) and better indicator of mortality than BMI (135). For instance, amongst over 3000 participants of the Framingham Heart Study (48% female) with no history of cardiovascular disease, the risks of metabolic syndrome were greater with every standard deviation increment in visceral adipose tissue (VAT) compared to subcutaneous adipose tissue (SAT) in females (OR 4.7 vs 3.0, $p<0.0001$) and males (OR 4.2 vs 2.5), and these persisted even after adjusting for BMI and WG (117). Interestingly, however, VAT was found to be less heritable than SAT, suggesting that modifiable risk factors such as lifestyle choices were important determinants of VAT volume and consequently health-related outcomes (117).

More recently, a genome-wide association study (GWAS) identified 209 single nucleotide polymorphisms (SNPs), of which 102 were novel, that were associated with visceral adiposity using data from over 300,000 individuals in the UK. The study

also showed that visceral fat was causally linked to greater risks of hypertension, ischaemic heart disease, diabetes and dyslipidaemia (136). Correspondingly, in another study using data from the same sample of the British population, 14 SNPs were associated with subcutaneous adiposity that were linked to lower risks of ischaemic heart disease, diabetes and hypertension (137).

Criterion	WHO	EGIR	NCEP ATP III	IDF
Blood pressure	>140/90mmHg	>140/90mmHg	>130/85mmHg	>130/85mmHg
Triglycerides	$\geq 150\text{mg/dl}$ +/- HDL <35mg/dl (M); <39mg/dl (F)	$\geq 150\text{mg/dl}$ +/- HDL <39mg/dl	$\geq 150\text{mg/dl}$ +/- HDL <40mg/dl (M); <50mg/dl (F)	$\geq 150\text{mg/dl}$ +/- HDL <40mg/dl (M); <50mg/dl (F)
Obesity	BMI >30kg/m ² +/- WHR>0.9 (M); >0.85 (F)	WG >94cm (M); >80cm (F)	WG>102cm (M); >88cm (F)	WG >94cm (M); >80cm (F)
Microalbuminuria	ACR >30µgm/mg	-	-	-
Plasma glucose	IFG or IGT or diabetes	IFG or IGT	Fasting glucose >110mg/dl	Fasting glucose >100mg/dl or type 2 diabetes

Diagnosis	Any 2 or more with IFG/IGT/diabetes	Any 2 or more with plasma insulin >75 th percentile	Any 3 or more	Any 2 with obesity
Table 1.1: Definitions of metabolic syndrome. Bold text denotes mandatory requirement for diagnosis. ACR, albumin:creatinine ratio; BMI, body mass index; EGIR, European Group for Study of Insulin Resistance; F, female; HDL, high-density lipoprotein; IDF, International Diabetes Federation; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; M, male; NCEP ATP III, National Cholesterol Education Program Adult Treatment Panel III; LDL, low-density lipoprotein;; WG, waist girth; WHO, World Health Organisation; WHR, waist:hip ratio.				

1.9.8. Obesity phenotypes

According to WHO, EGIR, NCEP ATP III and IDF joint interim statement definitions, it is possible for metabolic syndrome to occur in non-obese patients. It is also true that obese individuals may have no other features of metabolic syndrome. These possibilities have resulted in the concept of obesity phenotypes and raised the question whether obesity can be healthy, i.e. if metabolically healthy obesity exists as clinical phenotype. This was partly borne of the observations that the relationship between cardiovascular mortality and BMI was a J-shaped curved, with overweight or mildly obese individuals a lower mortality than underweight or morbidly obese individuals. Amongst the first to describe this observation was Fleischmann et al in a cohort of patients on renal dialysis in the USA (138). Later, the term “obesity paradox” was popularised after Gruberg et al showed that mortality rates in overweight or mildly obese patients undergoing percutaneous coronary intervention was lower than in other BMI groups (139). Over the next decade, several studies showed that all-cause mortality in patients with cardiovascular disease was lower in overweight individuals compared to their under-weight, normal-weight and obese counterparts, even after adjusting for age, sex, ethnicity, medication and clinical variables (140).

The question whether metabolically healthy obesity exists remains unresolved. On the one hand, amongst over 6000 individuals in the Multi-Ethnic Study of Atherosclerosis (MESA) study almost half of the metabolically healthy obese patients developed metabolic ill-health by IDF criteria over a median of 12 years follow-up compared to metabolically healthy normal weight individuals, and was associated with a higher odds of cardiovascular disease that were proportional to the duration of

metabolic complications (141). Metabolically healthy obesity may therefore represent a transient subclinical phenotype and a harbinger of complications associated with chronic excess adiposity. Yet, others have suggested that obese individuals may be considered healthy depending on their fitness level. For instance, amongst over 43,000 adults (almost 25% female) the risk of all-cause mortality, cardiovascular disease and cancer mortality amongst metabolically healthy obese and metabolically healthy non-obese were similar after adjusting for cardiovascular fitness quantified by a surrogate measure of maximal oxygen uptake, suggesting that the former is a benign phenotype compared to metabolically unhealthy obesity (142). On the other hand, a meta-analysis of 10 studies showing hazard ratio of cardiovascular mortality and morbidity in metabolically healthy obese vs metabolically healthy non-obese individuals remain greater by 33% (95% CI: 0.83–2.11) after adjusting for cardiovascular fitness (143), has led others to question whether obesity can ever be considered healthy (143, 144). In fact, more recently, with the advent of MR studies it has been suggested that the obesity paradox reported in previous observational studies may in fact be the result of confounding of catabolic status and poor cardiovascular outcomes, both of which occur in chronic disease such as heart failure, and that there is in fact a positive linear association between weight and mortality in patients with heart disease and diabetes (145).

1.10. Adipose tissue

Although the higher risks of cardiac arrhythmias in obesity may be partly explained by concurrent cardiometabolic ill-health, growing evidence suggests that increasing adiposity independently contributes to this risk. In particular, epicardial adipose tissue (EAT), which is the visceral fat depot of the heart, has been implicated as a

transducer of inflammation to the underlying myocardium and a mediator of cardiac arrhythmias (1, 146). The following sections outline the classification of adipose tissue, the role of EAT under physiological conditions and its pathophysiological effects on cardiac electrophysiology which renders the myocardial substrate proarrhythmic.

1.10.1. Classification and anatomy of adipose tissue

Adipose tissue can be histologically categorised as white, brown or beige (Figure 1.7). White adipose tissue (WAT) is widely distributed throughout the body as either the subcutaneous or visceral fat. Its main function is that of energy storage, and hence its adipocytes consist of a single lipid droplet of triglycerides, giving it a characteristic yellow colour, with minimal space for mitochondria which may be thin, elongated and often variable in number (147). WAT also secretes a range of hormones, cytokines, complement and growth factors which have both endocrine and paracrine effects on adjacent and distant tissues (148). On the other hand, brown adipose tissue (BAT) is commonly located in cervical, supraclavicular, axillary, paravertebral, mediastinal, and upper abdominal regions, and has an antagonistic role to WAT; it dissipates energy through thermogenesis (147). The cellular composition of BAT reflects its function, in that there is abundance of mitochondria within the adipocytes giving it a brown hue, and triglycerides are stored as small vacuoles. BAT has a denser microcirculation and sympathetic innervation owing to a greater oxygen demand and its need to respond to thermogenesis (147). Due to this unique property, BAT is widely found in small mammals and new-borns in which the high surface area to volume ratio predisposes them to heat loss. In adulthood, BAT

has a minimal role in thermoregulation as core body temperature is regulated via other mechanisms, such as muscle shivering and insulation with WAT.

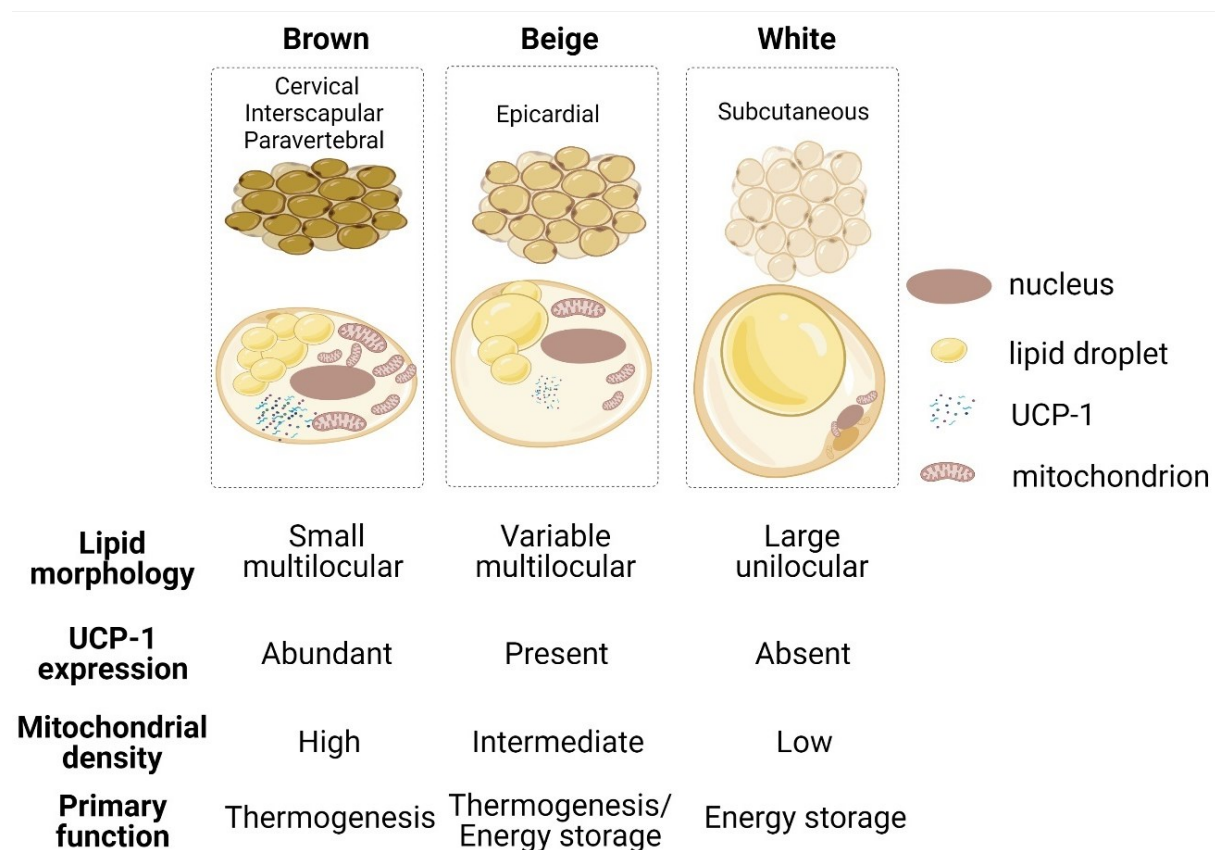


Figure 1.7: Histological characteristics of adipose tissue. Brown adipose tissue (BAT) is mainly located in cervical, interscapular, supraclavicular and paravertebral regions. Brown adipocytes contain several small vacuolated lipid droplets, multiple mitochondria that give its characteristic colour, and express uncoupling protein-1 (UCP-1) in relative abundance. These features reflect its primary function in non-shivering thermogenesis. By contrast, white adipose tissue (WAT) is more widely distributed subcutaneously and visceraally. WAT adipocytes typically contain a single large lipid droplet with few mitochondria, minimal UCP-1 and serve as an energy store. Beige adipose tissue, of which epicardial fat is example, is an intermediate phenotype characterised by multiple loculated lipid

droplets with a mitochondrial density and UCP-I expression that is greater than WAT but less than BAT. Beige adipose tissue contributes to thermogenesis as well as serving as an energy store. Figure reproduced from Patel et al (1) under Creative Commons Attribution Licence (CC-BY) 4.0.

1.10.2. Anatomy of epicardial adipose tissue

EAT is the visceral fat depot of the heart localised deep to the visceral pericardium. It lies in direct contact with the underlying myocardium due to a lack of a fascial boundary (Figure 1.8). It covers up to 80% of the heart surface and contributes to 20% of the whole cardiac mass despite being primarily distributed in the atrioventricular and interventricular grooves (149, 150). EAT is unique amongst other cardiac adipose tissue depots as it shares the same embryological origin and circulation with the myocardium: both structures are derived from the splanchnic mesoderm and receive their blood supply from the coronary arteries (151). The anatomical proximity of EAT to myocardium and shared circulation has led to a growing body of evidence supporting a paracrine role of EAT in the development of cardiac diseases. In this regard, the inconsistent definition of cardiac adiposity in the literature is a limitation to extrapolating the evidence between fat volume and health outcomes. *Pericardial* fat is often used interchangeably with EAT, although the latter strictly refers to adipose tissue deep to the parietal pericardium (152). On the other hand, pericardial fat is the combination of EAT and adipose tissue external to the parietal pericardium, i.e. *paracardial* fat (1).

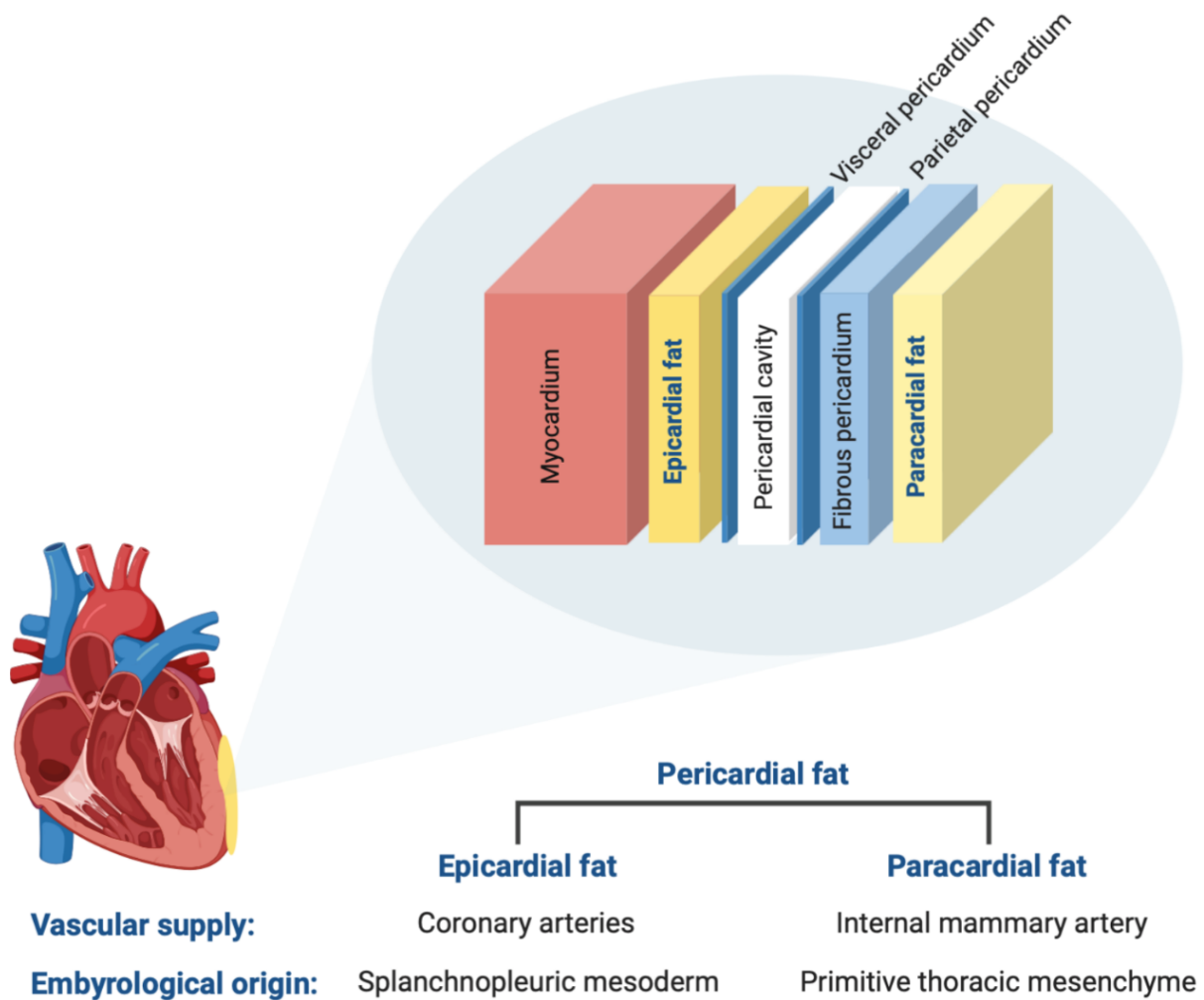


Figure 1.8: Anatomy of epicardial adipose tissue. Epicardial adipose tissue (EAT) lies deep to the visceral pericardium (i.e. the epicardium) and in direct contact with myocardial tissue. It derives from the splanchnopleuric mesoderm and draws its vascular supply from the coronary arteries. By contrast, paracardial fat refers to adipose tissue external to the parietal pericardium. Its origin is from the primitive thoracic mesenchyme and is perfused by the internal mammary artery. Pericardial fat refers to EAT and paracardial adipose tissue. Figure reproduced from Patel et al (1) under Creative Commons Attribution Licence (CC-BY) 4.0.

1.10.3. Physiological function of epicardial adipose tissue

EAT is a type of WAT and has a physiological role of protecting the myocardium against potential stressors. Due to its elastic and compressible properties, it protects the coronary arteries from mechanical forces generated by arterial pulsation and myocardial contraction (153). Unlike other fat depots, EAT has a uniquely higher rate of lipogenesis and lipolysis and therefore act as a local energy store of free fatty acids (FFA) while also as a buffer against the lipotoxic effects of excess FFA (154, 155). It also functions as an endocrine organ secreting a range of adipokines (151, 156). In this way, EAT has shown to modulate proliferation of vascular smooth muscle cells and vascular contractility while also exhibiting anti-apoptotic and anti-oxidant effects (157, 158, 159). Uniquely amongst WAT depots, EAT exhibits BAT-like features, in that it expresses uncoupling protein-1 (UCP-1) which has been implicated in non-shivering thermogenesis by uncoupling mitochondrial oxidative phosphorylation (Figure 1.7). Interestingly, low levels of UCP-1 have been linked with AF (160) and it is possible that EAT may protect against hypothermia-induced fatal arrhythmia (161).

1.10.4. Pathophysiological effects of epicardial adipose tissue

Under pathological conditions EAT undergoes a phenotypic shift from a protective neighbour to an inflammatory substrate. For example, EAT in patients with coronary artery disease (CAD) exhibit a greater pro-inflammatory profile than in non-CAD counterparts. Both mRNA expression and protein levels of pro-inflammatory cytokines such as tumour necrosis factors alpha (TNF- α), interleukin (IL)-6 and monocyte chemoattractant protein (MCP-1) have shown to be upregulated, and anti-inflammatory adiponectin downregulated after adjustment for concurrent

hypertension, diabetes, dyslipidaemia and BMI (162, 163, 164, 165). Consistent with the rise in MCP-1, histological analysis of EAT from CAD patients exhibited infiltration by M1 macrophages and CD68+ cells (166). The shift towards a proinflammatory secretome has also been observed in type 2 diabetes mellitus (T2DM), with a reduction in adiponectin and increase in expression of MCP-1 and CD68+ cells compared to non-diabetic counterparts (167). The transcriptome of EAT in patients with T2DM has also been shown to primarily consist of genes coding for cytokine production and leucocyte recruitment, such as TNF- α , IL-6 and chemokine receptors CXCR1 and CXCR2 (168). By contrast, a study by Teijeira-Fernandez et al. demonstrated no difference in the adipokine profiles of EAT in T2DM and non-T2DM patients, although this may be explained by an older and more overweight non-diabetic cohort in which subclinical insulin resistance may have masked any differences in adipokine profiles between diabetic and non-diabetic groups (169).

1.10.5. Epicardial adipose tissue as a mediator of cardiac arrhythmias

Given that EAT as a metabolically active depot that is intimately related to the myocardium, there are several lines of evidence that implicate EAT with arrhythmogenesis which have been summarised by Hatem and Sanders (170). This association was first demonstrated by Thanassoulis et al in the Framingham Heart study in which AF risk increased by almost 30% for every standard deviation increment in volume of pericardial fat (171). Furthermore, AF burden correlated with the pericardial fat volume; pericardial fat in persistent AF was 23% greater than in paroxysmal AF (172). A meta-analysis of 63 observational studies encompassing 352, 275 participants also reported that one SD increment in the volume of EAT was associated with a 2.2-fold higher risk of persistent AF compared to paroxysmal AF

(101). Furthermore, EAT has demonstrated to be a better predictor of AF risk than other measures of adiposity, including BMI, body surface area, waist circumference, waist/hip ratio, intrathoracic fat and abdominal VAT (101, 171, 172, 173). EAT volume has also shown to be as much as 34% greater in patients with recurrent AF than those in sinus rhythm (174) with several other studies implicating EAT as an independent predictor of AF recurrence following radiofrequency- and cryoballoon-based ablation (174, 175, 176, 177, 178). Furthermore, EAT volume correlates with serum CRP in those with recurrent AF suggesting inflammation as the mediator between EAT and arrhythmic risk(178). EAT volume has also been associated with ventricular arrhythmogenesis. For instance, EAT thickness positively correlates with the ventricular ectopy burden (179, 180) and is predictive of QT interval prolongation (defined as >450ms), VT/VF risk in the context of heart failure and VT recurrence post-ablation (181, 182, 183).

1.10.6. Differential physiological effects of epicardial and subcutaneous adipose tissue

Studies have shown that EAT exhibits a more pro-inflammatory profile compared to other fat depots (184, 185, 186). Mazurek et al. first demonstrated mRNA and protein levels of IL-1 β , IL-6 and TNF-alpha were greater in EAT vs SAT in paired samples harvested from CAD patients.(184). EAT was also associated with a higher concentration of macrophages, mast cells and T-lymphocytes. Similarly, Baker et al. showed 5-fold lower concentrations of adiponectin and 3-fold higher resistin, as well as a greater expression of IL-6, leptin and CD45 in EAT compared to SAT (186). Although these findings are consistent with Mazurek et al, EAT and SAT biopsies in this study were unpaired, i.e. harvested from different patient cohorts, which may

confound comparisons. Addressing this by harvesting paired EAT and SAT during cardiothoracic surgery, Fain et al. showed that EAT expresses a greater concentration of inflammatory adipokines, such as IL-1 β , MCP-1, leptin and TNF- α compared to sternal, abdominal and leg SAT (185) Adipocytes isolated from EAT and cultured in vitro also demonstrate an adipokine imbalance that favours proinflammatory pathways (187).

More recently, Nalliah et al. showed a differential effect of murine pericardial vs subcutaneous fat on cell electrophysiology in vitro (188). HL-1 atrial cardiomyocytes incubated with pericardial fat conditioned media for 24 hours recorded slower conduction velocities and longer activation times than cells exposed to inguinal SAT. Analyses of their respective secretomes showed that pericardial fat had an abundance of proteins associated with disruption of inter-myocyte adhesion, modulation of cellular metabolism and inflammation compared to the relatively anti-inflammatory proteome of inguinal SAT (188). This was consistent with observations of Cx40 lateralisation in cells incubated with pericardial vs subcutaneous fat and explains the conduction slowing observed in the study. The relatively pro-inflammatory proteome of pericardial fat is also consistent with the paracrine effect of cardiac adiposity in arrhythmogenesis.

Electrotonic interactions between adipocytes and myocytes may also alter electrophysiological function of the myocardium. For example, Ten Sande et al. showed that monolayers of neonatal rat ventricular myocytes (NRVM) incubated with human and rat adipose-derived stromal cells exhibited slower conduction than cells treated with their respective conditioned media (189). However, given that adipose

tissue comprises of adipocytes and stromal cells, both of which synthesise and secrete adipokines, it is possible that stromal cell-derived conditioned media may have underestimated the paracrine effect of adipose tissue in this study.

Interestingly, porcine adipose-stromal cell conditioned media exerted similar degree of conduction slowing as the stromal-adipocyte co-culture in the same experimental settings, supporting a species-specific adverse paracrine effect of adipose tissue on adjacent myocardium (189). Despite these important insights, the differential effects of human EAT and SAT on cell electrophysiology have not been reported.

1.10.7. Mechanisms underlying epicardial fat-related arrhythmias

The mechanisms by which EAT may induce a proarrhythmic substrate are shown in Figure 1.9 and discussed in the following sections.

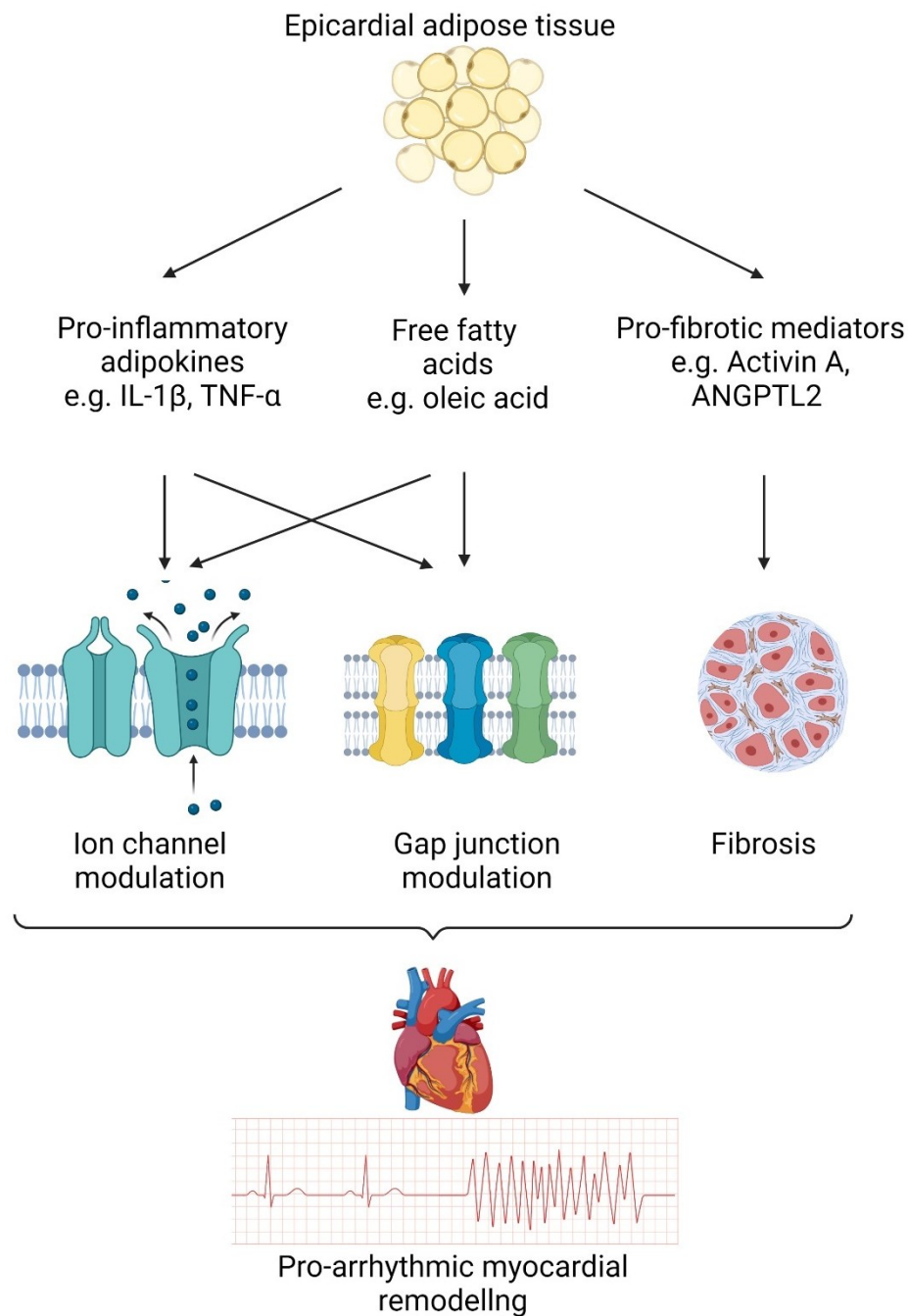


Figure 1.9: Epicardial fat-related electrophysiological remodelling. Epicardial adipose tissue (EAT) is a rich source of pro-inflammatory cytokines and free fatty acids that modulate ion channels and gap junction proteins. It is also a source of profibrotic mediators which may induce structural remodelling. These mechanisms result in delayed conduction and increased repolarisation heterogeneity within the

myocardium that renders it vulnerable to arrhythmias. Figure reproduced from Patel et al (1) under Creative Commons Attribution Licence (CC-BY) 4.0.

1.10.7.1. Cytokine-mediated ion channel modulation

Several *in vitro* studies support a paracrine effect of EAT in generating an arrhythmic substrate. For instance, Lin et al. studied the electrophysiological changes in rabbit LA atrial myocytes induced by either epicardial, retrosternal, abdominal adipocytes or adipocyte-conditioned media for 2-4 hours (190). Compared to control, adipocyte-incubated myocytes demonstrated prolonged APD at 90% repolarisation (APD₉₀), suggesting a shared arrhythmogenic potential amongst different fat depots.

Moreover, EAT adipocyte-incubated myocytes displayed modulation of ionic currents, which included increased late sodium (I_{Na}) and I_{CaL} currents, and decreased delayed rectifier potassium I_{Kr} and inward rectifier potassium currents (I_{K1}), all of which were consistent with prolonged APD (190). Furthermore, myocytes incubated in adipocyte-conditioned media exhibited prolonged APD, supporting a paracrine mechanism of action (190). Although this effect was modest in comparison to adipocyte-myocyte co-culture, the duration of incubation with conditioned media was relatively short and may have underestimated this effect. Moreover, microscopic observation of adipocyte-myocyte co-culture rarely revealed infrequent direct contact between adipocytes and myocytes suggesting that a paracrine mechanism of action underpinning electrophysiological modulation was more likely than an electrotonic interaction between the two cell types. A similar APD prolonging effect in rabbit LA myocytes was observed in the context of heart failure (HF) whereby incubation of LA myocytes with HF EAT resulted in early and delayed afterdepolarisations, as well as increased spontaneous activity. (191) Ventricular myocytes incubated with EAT also

demonstrate APD prolongation at 90% (APD90), 50% (APD50) and 20% (APD20) repolarisation than cells not exposed to EAT, in addition to having a more positive resting membrane potential whereby threshold for depolarisation is reached earlier and facilitating triggered activity (192). Given its inhomogeneous distribution around the myocardium, these results suggest that EAT may induce APD heterogeneity and thereby facilitate an arrhythmogenic substrate that enables re-entry (192).

The electrophysiological effects of EAT highlighted above may be explained by cytokines, such as IL-1 β , TNF- α and IL-6, which have been shown to alter ion channel function and thereby also explain the increased arrhythmogenicity associated with increasing adiposity (193) (Figure 1.10). For instance, IL-1 β causes APD90 prolongation that may be explained by increased $I_{Ca^{2+}}$ current (194).

Reduced transient outward potassium current (I_{to}), and altered Ca^{2+} homeostasis in the sarcoplasmic reticulum that increases Ca^{2+} spark and prolongs APD has been shown to increase propensity for spontaneous depolarisation and triggered activity (195). TNF- α and IL-1 β synergistically increase sarcoplasmic Ca^{2+} leak and its Ca^{2+} content which increases the frequency of spontaneous Ca^{2+} waves (196).

Transgenic mice overexpressing TNF- α exhibit elevated diastolic Ca^{2+} concentration and a prolonged Ca^{2+} transient, resulting in slow heterogeneous conduction and consequently a functional line of conduction block (197). IL-6 treatment in guinea pig ventricular myocytes has also been shown to alter APD, an effect that was reversed with an IL-6 antagonist (198).

Much of the above evidence for cytokine-mediated ion channel modulation comes from murine models. Although the currents that contribute to rapid depolarisation

(phase 0) and resting membrane potential (phase 4) - I_{Na} and I_{K1} , respectively - are common amongst mice and humans, those determining AP morphology differ significantly. I_{CaL} is weaker in murine cardiomyocytes resulting in an almost absent plateau phase (phase 2) (199, 200). In mice, repolarisation is driven by I_{to} , $I_{K,slow}$ and a non-inactivating steady state current (I_{ss}), whereas in humans it is primarily mediated by I_{Kr} and I_{Ks} currents (199, 200, 201). Potassium currents equivalent to $I_{K,slow}$ and I_{ss} have not yet been identified in the human ventricles, and I_{Kr} and I_{Ks} have minimal roles in the murine cardiomyocytes. Thus, murine studies showing APD prolongation via modulation of $I_{K,slow}$ would not be expected to be replicated in human cardiomyocytes. The effect of cytokines on I_{Kr} and I_{Ks} is also less well-characterised (200, 202). This may limit the extrapolation of the effects of EAT on ion channel function in humans.

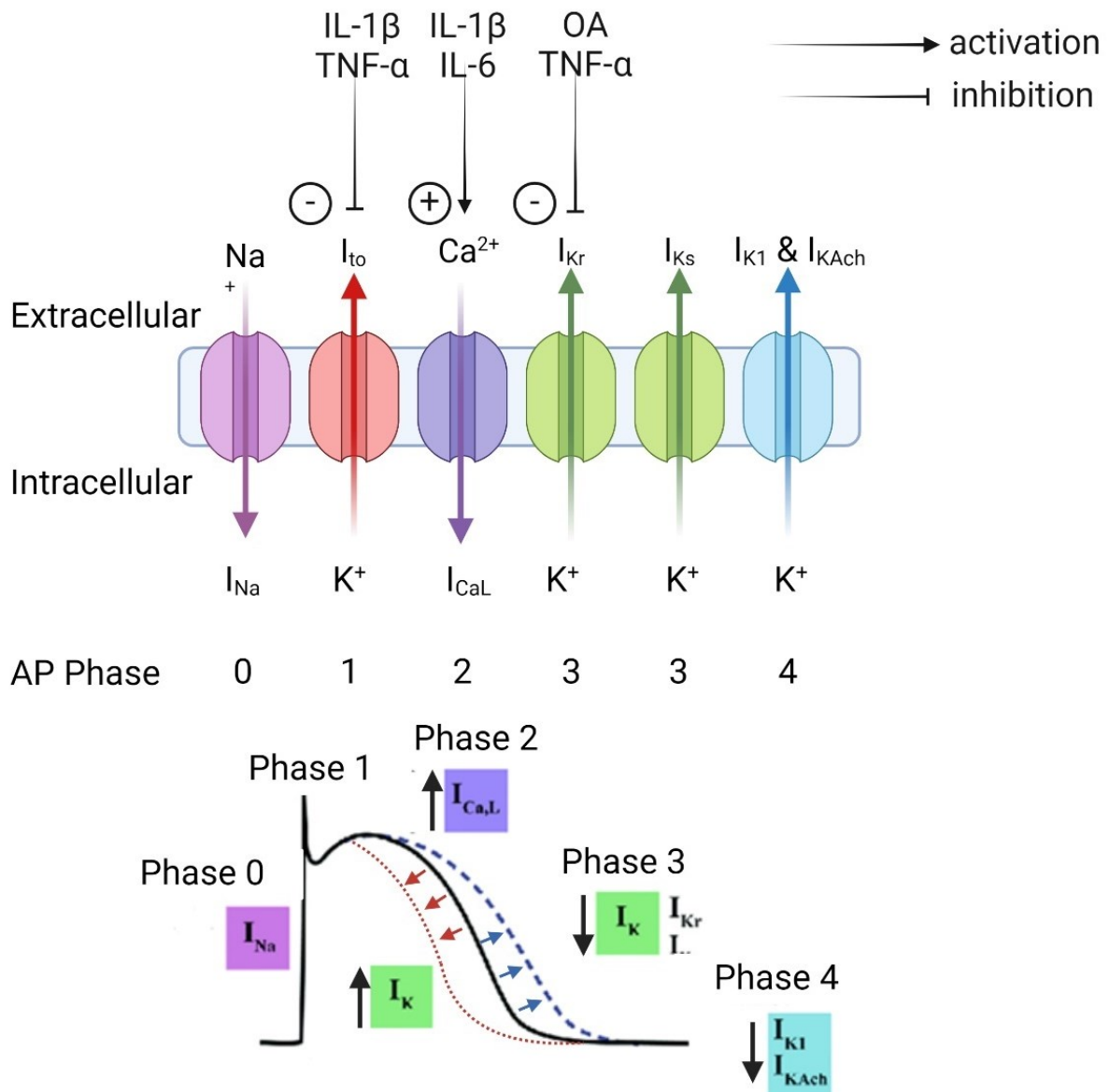


Figure 1.10: Cytokines from epicardial fat modulate ion channels. EAT secretes IL-1β, TNF-α, IL-6 and oleic acid (OA) which exert a paracrine effect on the ion channels expressed in the membranes of myocardial cells. The activation of L-type calcium channels (I_{CaL}) and inhibition of fast (I_{Kr}) and slow (I_{Ks}) rectifying potassium channels results in action potential prolongation (dashed blue line). By contrast, activation of the rectifying potassium currents may shorten

action potential duration (dashed red line). Figure reproduced from Patel et al (1) under Creative Commons Attribution Licence (CC-BY) 4.0.

1.10.7.2. Free fatty acid-mediated ion channel modulation

In addition to inflammatory cytokines, FFA secreted by EAT exert an arrhythmogenic influence on the underlying myocardium via direct modulation of APD. EAT, like other fat depots, is a rich source of saturated, monounsaturated and polyunsaturated FFA, with a greater content of eicosapentanoic and arachidonic acids compared to SAT (203). These have been shown to modulate APD in guinea pig and ovine left atrial myocytes and rat ventricular myocytes by enhancing repolarisation currents (204, 205, 206), as well as increase risk of arrhythmias by promoting DADs owing to dysregulation of intracellular calcium homeostasis (207).

FFA also modulate connexin expression with oleic acid and arachidonic acid (AA) shown to inhibit gap junctional coupling within hours of exposure in neonatal rat cardiac myocytes (208, 209). This was rapidly reversed with washing the cells in a FFA-free solution, suggesting the effect is most likely to be due to an extracellular component activating membrane receptors (208, 209) and subsequently initiating an intracellular signalling pathway mediated by protein kinases via G protein-coupled receptors (210, 211). 24-hour incubation with oleic acid has shown to activate protein kinase C in rat cardiomyocytes which consequently induced post-translational phosphorylation of connexins and hence the disintegration of gap junctions (212). Furthermore, protein kinase A, PI3K, AKT, Src and MEK1/2 have been shown to similarly modulate connexin functionality following FFA exposure (213, 214, 215,

216). FFA-activated protein kinases also modulate adherens junctions (217) which comprise catenin and cadherin proteins that maintain the integrity of intercalated discs (218). Neonatal rat cardiomyocytes treated with oleic acid results in activation of the PKC α /PKC ϵ -Fyn signalling cascades and consequently tyrosine phosphorylation of β -catenin and p120 catenin (217). This in turn results in decreased binding of β -catenin with α -catenin, and p120 catenin with N-cadherin, causing adherens junctions to become unstable and gap junction disassembly due to disruption of catenin-ZO-1-Cx43 complex (219).

1.10.7.3. Fibrotic remodelling

Fibrotic remodelling in the myocardium provides a substrate for re-entry as it produces tortuous paths of conduction and concurrently slows macroscopic conduction through the myocardium. Mahajan et al demonstrated atrial fibrosis increases with adiposity and provided evidence for EAT as a source of profibrotic cytokines that are responsible for structural remodelling (220). Indeed, Nalliah et al also demonstrated the association between EAT volume and degree of myocardial fibrosis and Abe et al provided histological evidence of a greater degree of fibrosis in LA adjacent to EAT compared to tissue that was not associated with EAT (16.5% vs 8.4%), and that this may be explained by paracrine mechanisms (188, 221). Severity of atrial fibrosis positively correlated with EAT fibrosis, meanwhile fibrotic EAT was infiltrated with inflammatory macrophages that were positioned between the adipocytes and the myocardium, suggesting involvement of local inflammation in the induction of fibrotic remodelling (221). IL-1 β , IL-6, TNF- α and monocyte chemoattractant protein-1 (MCP-1), which are actively secreted by EAT, have been associated with atrial collagen deposition and the degree of atrial fibrosis was

positively correlated with fibrotic markers such as platelet-derived growth factor-BB and MMP2 (221). ANGPTL2, which is also found in EAT, also positively correlated with the local concentrations of MMP2 and MMP9, as well as atrial collagen level, implicating its mediation of myocardial fibrosis (221, 222). Other studies have demonstrated that MMP2 and MMP7 can induce interstitial fibrosis, and that MMP-2 is positively correlated with EAT volume as well as AF severity (223, 224). Moreover, connective tissue growth factor (cTGF) is more highly expressed in EAT of AF patients compared to those in sinus rhythm (225). Local concentrations of cTGF also strongly correlated with atrial fibrosis and was an independent predictor of AF occurrence, suggesting EAT may be the source of cTGF facilitating atrial remodelling (225).

Activin A, a member of the TGF- β superfamily, is also abundantly synthesised in EAT and has been associated with fibrotic remodelling of the atria (226). Organoculture of rat atria treated with Activin A for a week at a concentration of 2ng/ml resulted in increased production of collagen types I, III and VI and marked global fibrosis (226). Furthermore, preincubation with anti-Activin A antibodies prevented fibrotic remodelling induced by EAT-conditioned media. EAT-conditioned media and Activin A induced comparable changes in atrial gene expression, specifically increased TGF- β 1 and TGF- β 2, suggesting that EAT-induced atrial fibrosis in this study was primarily facilitated by Activin A (226).

1.10.7.4. Fatty infiltration

Aside from paracrine effects, epicardial adipocyte infiltration into the myocardium may separate myocardial fibres resulting in conduction slowing or block that would

facilitate re-entrant arrhythmias. This is not dissimilar to the fibro-fatty infiltration observed with inherited cardiomyopathies (227). For example, Venteclef et al showed that myocardial fibrosis was more profound in areas of EAT filtration into atrial and ventricular tissue *in vitro*, and that this occurred at the interface between adipocytes and the surrounding myocardium as well as neighbouring tissue (226). These effects were unique to EAT and were not observed with SAT. Mahajan et al showed EAT infiltration into the posterior left atria of chronically obese sheep was associated reduced endocardial voltage (220). In addition to conduction slowing, greater conduction heterogeneity and fractionated electrograms, the atria of obese sheep were also more dilated and pressure-overloaded compared to their non-obese counterparts (220). Similar conduction abnormalities associated with obesity have been shown to co-localise with EAT in humans and may be attributed to atrial fatty infiltration (99). As well as greater EAT volume, atria of obese adults exhibit a global reduction in conduction velocity, greater electrogram fractionation and reduced voltage which correlate more strongly with EAT volume than global measures of obesity, such as BMI (99). Vulnerability for atrial fibrillation as a consequence of epicardial fatty filtration has also been demonstrated in obese canines with or without rapid atrial pacing to induce AF (228). Interestingly, epicardial fatty infiltration occurred following rapid atrial pacing to induce AF, even in non-obese animals, and correlated with interstitial fibrosis (228). A lower negative resting membrane potential, smaller action potential amplitude and longer repolarisation time as well as augmented transient outward potassium current and reduced L-type calcium and inward rectifier potassium currents have all been observed with cardiomyocytes connected to epicardial adipose tissue, and may explain the vulnerability for arrhythmias as a consequence of fatty infiltration (192).

1.10.8. Regression of epicardial adipose tissue with weight loss

Given the evidence supporting EAT as a source of a pro-arrhythmic secretome, it follows that its regression may reduce arrhythmic risk. EAT volume has been shown to decrease with weight reduction achieved by lifestyle modification and surgical intervention (229). The EISNER (Early Identification of Subclinical Atherosclerosis by Non-invasive Imaging Research) registry demonstrated an association between weight loss and EAT volume, such that a 2.3% reduction in EAT was recorded amongst participants achieving >5% body weight loss (230). However, less than 5% whole body weight loss was not associated with significant changes in EAT volume (231). Specific weight loss programmes via low-calorie diet targeted at obese patients also demonstrated similar pattern of EAT change (232, 233). Iacobellis et al showed that a 6-month 900 kcal/day diet resulted in 20% whole body weight loss and reduced EAT thickness by 32% (232). Kim et al showed a more modest calorie restriction of 1547 kcal/day over 12 weeks was sufficient to result in significant changes in body weight (-11.0%) and EAT thickness (-17.2%) as well as visceral abdominal adiposity (233). An hour of aerobic exercise achieving 60-70% of maximal predicted heart rate three times a week for 12 weeks in obese individuals has been shown to result in almost 9% reduction in EAT thickness (234). Weight loss following bariatric surgery has been shown to regress EAT volumes by as much as 24%, although results may vary according to intervention (14.6% with Roux-en-Y gastric bypass vs 5.3% with sleeve gastrectomy) (235, 236).

1.11. Obesity management

The following sections summarises the current UK guideline on the management of obesity and review the evidence for weight reduction strategies for mitigating arrhythmic risks.

1.11.1. NICE guidelines on obesity

The growing burden of obesity and its clinical sequelae discussed hitherto prompted the UK's National Institute of Healthcare and Clinical Excellence (NICE) to set out clinical guidelines (C189) for its management (68). In addition to providing suggestions for the practicalities of delivering care in specialist settings, such as special seating and equipment, it provides recommendations for lifestyle, pharmacological and surgical interventions to manage obesity. Given that this thesis contains data collected and analysed from UK inhabitants, including those that would have received or continue to receive care based on these guidelines, salient recommendations for obese adults are summarised herein for context, and also represented in Figure 1.11.

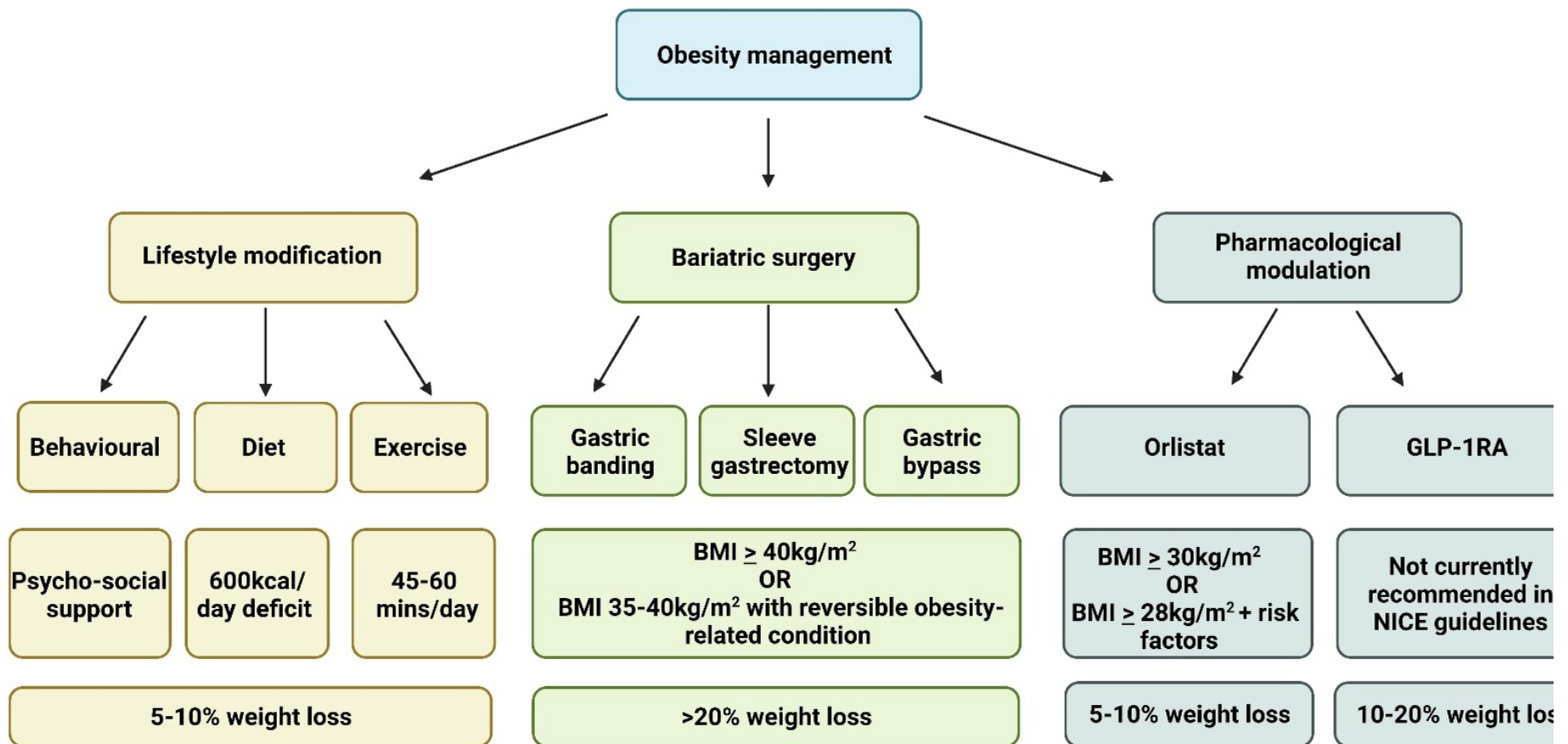


Figure 1.11: Summary of NICE guidelines for the management of obesity in the UK.

Overweight Caucasian adults (BMI 25-29.9kg/m²) with a low WG (<90cm males, <80cm females) can be offered general advice on maintaining healthy weight and lifestyle. All other Caucasian adults that are overweight or obese (BMI>30kg/m²) with a high WG (94cm for males, 80cm for females) or very high WG (102cm for males, 88cm for females) should be recommended diet and physical activity to lose weight. Amongst these, pharmacological modulation may be considered in those who are overweight or obese with related co-morbidities, irrespective of their WG. Surgical intervention should be considered in individuals in obese grade II who have obesity-related co-morbidities and all individuals with grade III obesity. Given that the equivalent risks of cardiometabolic disease occur at a lower BMI in some ethnic minorities, a BMI of 23kg/m² and 27.5kg/m² have been recommended as thresholds for identifying increased risk and high risk, respectively, for individuals of Asian, Chinese, Middle Eastern and African descent in NICE guideline PH46 (70).

The graded approach to obesity management is reflected in the tiered system of care in the UK. All obese adults are recommended to receive behavioural interventions to address the psychosocial stimuli that encourage weight gain. This should be supplemented with daily physical activity of moderate intensity lasting up to 45-60 minutes, increasing to 90 minutes if required, that can be incorporated into a daily routine. Although calorie deficit is complementary to physical activity, low-calorie diets (800-1600kcal/day) are likely to result in nutritional deficiencies. For this reason, very low calorie-diets (<800kcal/day) are not routinely recommended without the support of a specialist team. Instead, patients are usually advised to aim for 600kcal/day deficit in relation to their calorie intake that would otherwise result in maintenance of their current weight. Pharmacological modulation, specifically with

orlistat, is only recommended after and as an adjunct to lifestyle adaptations, particularly in patients in whom weight loss has plateaued without achieving their target weight. In this respect, drug treatment for obesity is recommended to maintain weight loss rather than to continue losing weight. Orlistat may be used to aid weight loss prior to bariatric surgery or weight maintenance after surgery. Bariatric surgery is recommended for obese individuals with BMI $\geq 35\text{kg/m}^2$ with co-morbidities, for example type diabetes or hypertension, or $\geq 40\text{kg/m}^2$ with or without co-morbidities who have not responded to lifestyle or pharmacological interventions and are able to undergo anaesthesia and surgery. Specifically, an expedited assessment for bariatric surgery is recommended in obese patients with BMI $\geq 35\text{kg/m}^2$ with recent onset type 2 diabetes. The tier 3 service is a multi-disciplinary team of specialists that includes a clinician, specialist nurse, dietitian, psychologist or psychiatrist, and physiotherapist or physical activity specialist. Access to these services, and consequently bariatric surgery, is currently non-uniform across the UK and only available in relatively small number of specialist services. Follow-up after bariatric surgery should continue for at least 2 years and encompass monitoring for nutritional deficiencies and co-morbidities as well as lifestyle and dietary recommendations as recommended for patients not eligible for bariatric surgery.

1.11.2. Bariatric surgery

Bariatric surgery is a term used to described surgical procedures that modify the gastrointestinal tract to induce weight loss. This can be achieved by shortening a length of the tract to limit absorption, reducing the volume of the stomach to limit calorie intake, or a combination of both. Given that bariatric surgery features in the UK guidelines for management of obesity, the following sections detail the history of

this procedure and its effects on cardiovascular outcomes. The evidence for weight reduction as an intervention to mitigate arrhythmic risk is summarised thereafter.

1.11.2.1. History of bariatric surgery

The history and development of bariatric surgery over the past half century has been described in detail by others (237), and is summarised here forth. The modern concept of bariatric surgery began when in Sweden in 1952 when Viktor Henrikson resected just over a metre of small bowel from a young obese female patient. A year later in the USA, Richard Varco performed a bypass procedure by anastomosing the proximal jejunum to the distal ileum. This was followed by a more extensive ileal resection by Kremem et al in 1954. Although intended to induce malabsorption to facilitate weight loss, these intestinal bypass procedures were associated with diarrhoea, protein deficit and metabolic complications which resulted in their discontinuation. Later, the term “gastric bypass” was coined in 1967 when Edward Mason resected the stomach and performed anastomosis of the proximal and distal gastrointestinal tracts. The notion that weight loss could be achieved with malabsorptive surgical procedures continued in the form of biliopancreatic diversion which was first reported in 1979 and comprised of a partial distal gastrectomy and intestinal bypass. In the 1990s, the distal gastrectomy was replaced by a proximal one. Unfortunately, these variations of the original intestinal bypass surgeries continued to be associated with complications of malabsorption that were poorly tolerated by patients despite achieving weight loss.

During the decades that malabsorptive surgical procedures were being studied, restrictive options were also being explored in parallel. These focussed on promoting early satiety by reducing the gastric volume, and thereby restricting calorie intake,

rather than causing malabsorption. In 1973, Printen and Mason published a study titled, "Gastric surgery for relief of morbid obesity", in which the stomach was partitioned horizontally so that the greater curvature remained physiologically active. Paradoxically, this procedure led to weight gain due to dilatation of the stomach. Almost a decade later, this procedure was adapted by Mason in which the stomach was partitioned vertically, and a band placed around the gastric outlet to prevent dilatation. Unfortunately, this resulted in modest weight reduction and was associated with poor tolerance to solids. Around the same time, Wilkinson and Peloso approached the same problem by partitioning the stomach proximally using a band and this was made possible laparoscopically by Kuzmak in 1986. Even in these instances, patients reported only mild weight loss and complications such as prolapsing bands, oesophageal ulceration and dilatation and strictures were common. The implementation of sleeve gastrectomy as the stand-alone procedure it known as today was popularised by Michel Gagner. Prior to this, patients would typically undergo a two-staged surgical procedure which first involved sleeve gastrectomy followed several months later by a bypass component. Gagner observed that patients achieved sufficiently weight loss with sleeve gastrectomy alone and did not require or wish to undergo an additional bypass component several months later. Thus, in present day sleeve gastrectomies the greater curvature of the stomach is resected thereby significantly reducing the volume of the stomach. The lesser curvature is retained leaving a small-volume conduit between gastro-oesophageal sphincter to the duodenum. In the historical context of bariatric surgery described herein, it is unsurprising that this has become the favoured surgical procedure over other options; the greater curvature is excised which avoids gastric dilatation and paradoxical weight gain, and there is no intestinal bypass component

which mitigates malabsorptive complications and improves tolerability resulting in fewer re-do procedures (238).

Gastric bypass surgeries combine restrictive and malabsorptive procedures.

Amongst the first of these was described by Mason and Ito which comprised gastric partition with a loop gastrojejunostomy in the late 1960s. Thereafter, this procedure underwent several permutations that included the Roux-en-Y gastric bypass by Griffen et al. which was first performed laparoscopically by Wittgrove, Clark and Tremblay in 1994. The Roux-en-Y gastric bypass consists of fashioning a small gastric pouch from the superior portion of the stomach and anastomosing it to the jejunum, thus bypassing the stomach and duodenum entirely. Although anatomically it combines restrictive and malabsorptive procedures, the metabolic effects of Roux-en-Y surgery have been attributed to its restrictive effects on calorie intake and modulation of the gastrointestinal neurohormonal axes. Today, the three most frequently performed surgical procedures worldwide are laparoscopic sleeve gastrectomy, Roux-en-Y gastric bypass and laparoscopic adjustable gastric banding, of which sleeve gastrectomy is the most common (Figure 1.12).

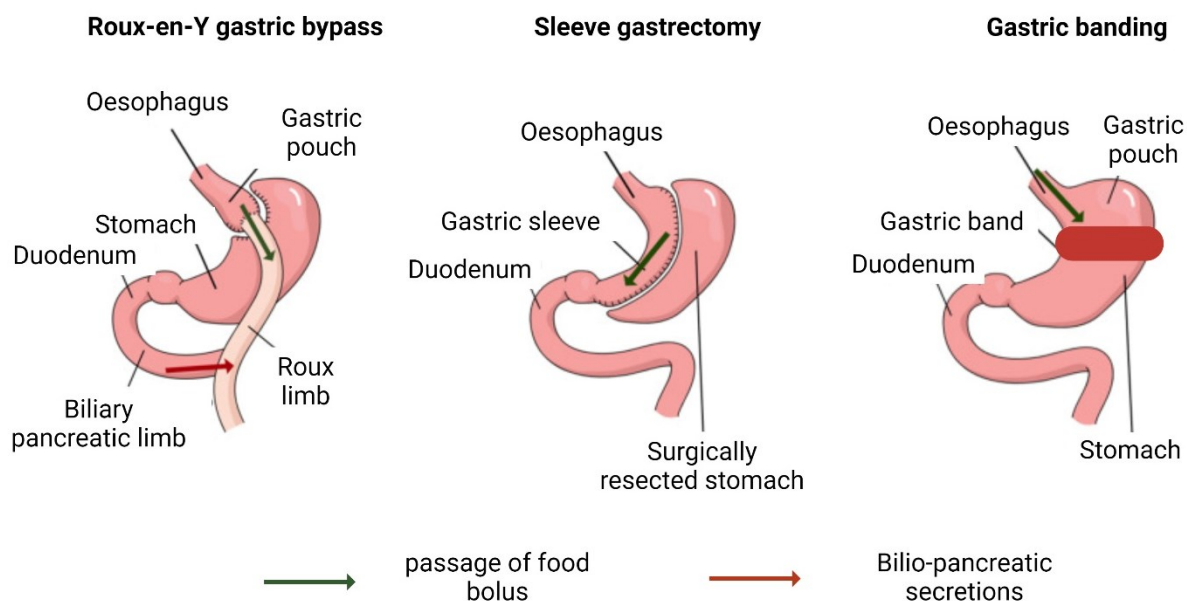


Figure 1.12: Common bariatric surgical procedures. Roux-en-Y gastric bypass circumvents a large portion of the stomach to connect the oesophagus to the small intestine. Sleeve gastrectomy renders the stomach smaller in volume. Gastric banding is the most conservative of the options shown, by introducing an adjustable band around the neck of the stomach and leaves abdominal anatomy intact.

1.11.2.2. Bariatric surgery and cardiovascular outcomes

Bariatric surgery is a cost-effective intervention for moderately to severely obese people compared with non-surgical interventions (239) and confers a number of cardiovascular health benefits. In a meta-analysis of 39 cohort studies reporting long term healthcare outcomes, bariatric surgery was associated with a significant reduction in all-cause mortality (HR 0.55; 95%CI: 0.49-0.62) and cardiovascular mortality (HR 0.59, 95%CI: 0.47-0.73) (240). There were also reductions in incidences of heart failure (HR 0.50, 95%CI: 0.38-0.66), myocardial infarction (HR 0.58, 95%CI: 0.43-0.76) and stroke (HR 0.64, 95%CI: 0.53-0.77) (240). These findings were corroborated in two smaller meta-analyses, one comprising of 11

studies with data from 74,000 obese patients undergoing bariatric surgery compared to almost 1.7million receiving non-surgical care (241) and the other of 10 studies published between 2016 and 2021 evaluating outcomes post-bariatric surgery (242). The favourable cardiovascular outcomes following bariatric surgery are independent of the type of surgery (Roux-en-Y vs sleeve gastrectomy) (243).

In addition to improvement in cardiovascular outcomes, bariatric surgery induces remission of diabetes, dyslipidaemia, hypertension and sleep apnoea, even in older (<60 years) patients (244). Indeed, in another study, remission of diabetes was as high as 92% amongst 48 patients undergoing Roux-en-Y gastric bypass by 60 months post-surgery and no new diagnoses of diabetes were recorded amongst 57 pre-diabetic patients undergoing the same procedure(245). Amongst 2287 diabetic obese patients receiving bariatric surgery, incidence of adverse cardiovascular outcomes (mortality, coronary artery events, stroke, heart failure, AF and renal disease) were much less frequent compared to the patients receiving standard care for diabetes and obesity (246).

1.11.3. Effect of weight reduction on cardiac arrhythmias

Although obesity has been associated with a higher risk of sudden cardiac death in large multicentre, prospective cohort studies (115, 116, 247, 248), no studies to date have quantified any reduction in risk following weight loss. This is perhaps unsurprising given the practical challenges in anticipating terminal events and contribution of concurrent morbidity. However, given also that obesity is also associated with a higher risk of ventricular tachycardia/fibrillation (45, 249) and

weight loss with ECG changes suggestive of favourable changes in ventricular activation and repolarisation (105, 106, 250, 251), it may be inferred the weight loss may at least partly mitigate SCD risk in obesity. On the other hand, there is increasingly compelling evidence supporting weight reduction for mitigating AF risk and burden. The following sections will summarise this evidence and is also represented in Figure 1.13.

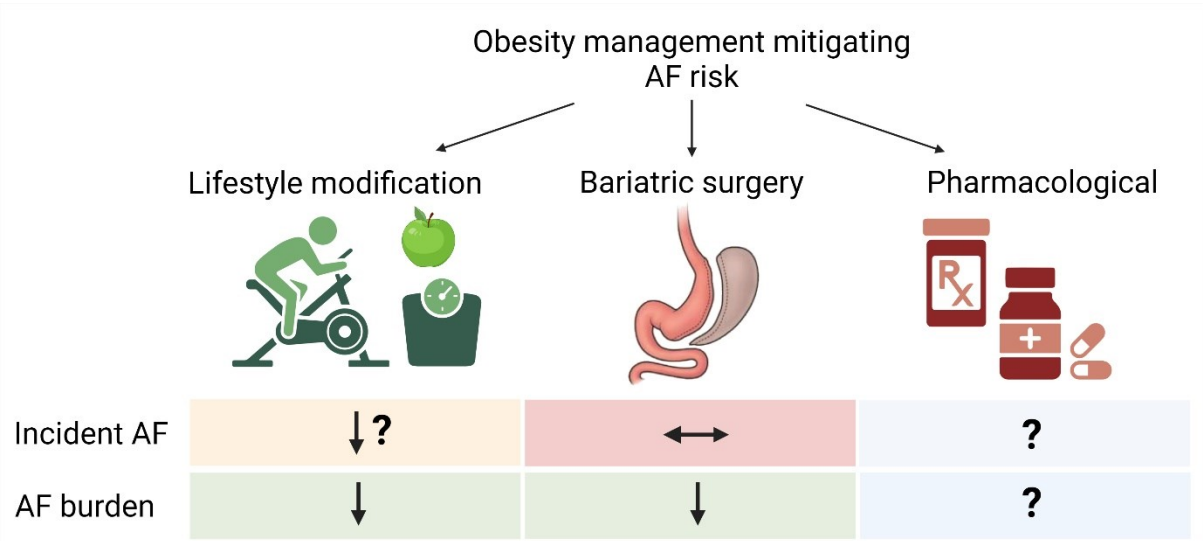


Figure 1.13: Management options for obesity and their effects on AF. Obesity may be managed by a combination of lifestyle, pharmacological and surgical options, each of which mitigate AF risk to a variable degree. Although lifestyle changes and bariatric surgery reduces AF burden in obese patients with pre-existing AF, the evidence for surgical weight reduction on mitigating incident AF risk is conflicting. Lifestyle changes likely reduce incident AF risk. The effects of pharmacological modulation of obesity on incident AF risk and AF burden is not well described.

1.11.3.1. Lifestyle modification

Several Australian observational cohort studies have demonstrated the benefits of non-surgical aggressive risk factor management to address arrhythmic risks in obese patients. In a single centre partially blinded study, 150 overweight and obese ambulatory patients with symptomatic paroxysmal AF were randomised to an active weight management programme or offered general lifestyle advice (control) to assess the impact of weight loss on AF burden and severity using the Atrial Fibrillation Severity Scale (252), which was recorded every 3 months over 15 months follow-up. Secondary outcomes were total AF episodes and cumulative duration measured by 7-day Holter monitoring, echocardiographic left atrial area, and interventricular septal thickness that were recorded at 12 months from baseline. Participants randomised to the active weight management programme experienced on average 14.3kg weight reduction compared to 3.6kg in the control group ($p<0.001$), as well as improvements in AF symptom burden scores (11.8 vs 2.6 points, $p<0.001$) and symptom severity scores (8.4 and 1.7 points, $p<0.001$). These results occurred in the context of a reduction in the number of AF episodes, cumulative AF duration as well as reductions in interventricular septal thickness and left atrial area in those randomised to weight management programme, suggesting reverse structural remodelling of the atria with weight loss.

The ARREST-AF cohort study offered patients with $\text{BMI} \geq 27 \text{ kg/m}^2$ and any one of hypertension, glucose intolerance/diabetes mellitus, hyperlipidaemia, OSA, smoking, or alcohol excess undergoing catheter ablation for symptomatic AF either aggressive risk factor management according to American Heart Association/American College of Cardiology guidelines or standard care (253). The predefined primary outcome was recurrent AF post-ablation and secondary outcomes of interest were AF

frequency, duration and symptoms. Participants were reviewed every 3 months for the first year and then every 6 months thereafter, which included ambulatory 7-day Holter monitoring at each review. 61 patients opted for risk factor management and 88 patients for standard care. Risk factor management resulted in significantly greater weight and blood pressure reductions, and better glycaemic control and lipid profiles. Arrhythmia-free survival was substantially greater in those receiving risk factor management compared to standard care despite the study not being randomised. Risk factor management was also an independent predictor of arrhythmia-free survival after multiple ablation procedures. However, in the context of a comprehensive blood pressure, glycaemic, lipid and lifestyle management, it is not possible to ascertain the extent to which weight loss reduced arrhythmic burden in this study.

In a similar subsequent observational cohort study (LEGACY) of 355 obese patients with AF receiving risk factor management followed-up over a mean of 4 years, Pathak et al showed that the degree of weight loss was related to freedom from AF(254). Individuals maintaining >10% weight loss had a 6-fold greater probability of arrhythmia-free survival compared to patients with 3-9% or <3% weight loss. Importantly, there were no differences in the number of anti-arrhythmic drugs or ablation procedures between these groups at baseline, and interestingly, the degree of weight loss corresponded with participation in weight management clinic suggesting a positive reinforcement effect of losing weight. Arrhythmia-free survival was also associated not only with weight loss but also its subsequent maintenance, such that AF burden and symptom severity was less in those with <2% weight fluctuation compared to those with >5% (254).

The implications for weight loss on AF in the LEGACY cohort was further characterised in the REVERSE-AF study (255). Approximately 40% of individuals with <3% weight loss progressed from paroxysmal to persistent AF and a quarter reversed from persistent to paroxysmal or no AF. Of the individuals experiencing 3-9% weight loss, a third progressed from paroxysmal to persistent AF and almost half reversed from persistent to paroxysmal or no AF. Amongst individuals recording >10% weight loss, only 3% progressed to persistent AF, and almost 90% reversed from persistent to paroxysmal or no AF. Taken together, these results suggest that the degree of weight loss as well as its maintenance are important lifestyle changes that assist in alleviating arrhythmic burden. The results of the LEGACY study were validated in a Mediterranean population that also showed weight loss has a dose-dependent effect on AF-freedom in overweight and obese individuals and that weight gain is associated with AF recurrence (256). Furthermore, baseline cardiorespiratory fitness is also predictive of long-term AF-freedom with improvement in cardiorespiratory fitness conferring 12% incremental gain to weight loss in freedom from total AF burden (257). The dose-response effect of exercise on AF burden was also demonstrated in a retrospective cohort study of 69,885 patients undergoing treadmill stress tests, equivalent to a 7% lower risk for every 1 metabolic equivalent (MET) increase in cardiorespiratory fitness over a median follow-up period of 5.4 years, that was more pronounced in obese individuals and independent of baseline fitness (258). In short, these studies provide compelling evidence in support of weight reduction to alleviate arrhythmic burden, particularly in obese patients.

1.11.3.2. *Pharmacological modulation*

Orlistat inhibits gastric and pancreatic lipases and is the only named pharmacological agent in NICE guidelines for the management of obesity Health and Excellence (259). In these recommendations, orlistat is suggested for patients with BMI >28kg/m² and cardiometabolic risk factors, or BMI>30kg/m², in whom lifestyle modification has resulted in insufficient weight loss to reach target weight, and should only be continued beyond 3 months if it results in >5% initial body weight (259). Anti-arrhythmic effects of orlistat have not been described. However, Ardissino et al showed that amongst 36,876 obese patients completing a course of orlistat followed up over a median of 6 years in the UK had lower rates of myocardial infarction (HR 0.77, 95% CI 0.66-0.88, p<0.001), stroke (HR 0.68, 95% CI 0.56-0.84, p<0.001) and incident heart failure (HR 0.79, 95%CI 0.67-0.94, p=0.007) (260).

Recently, glucagon-like peptide 1 receptor agonists (GLP-1RA) have emerged as novel pharmacological weight reduction agents in obesity, even in the absence of diabetes (261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273). Amongst these, the first to be approved by the Food and Drug Administration (FDA) and European Medicine Agency (EMA) was 3mg liraglutide once daily, which was followed by 2.4mg semaglutide once weekly by the FDA in June 2021. Although these drugs appear to have bridged the weight reduction gap between lifestyle and surgical weight reduction strategies, which typically result in <10% and >20% weight loss respectively (274), their anti-arrhythmic effects are yet to proven. One systematic review and meta-analysis of 31 randomised trials comprising approximately 18,000 diabetic patients on GLP-1RA vs 15,000 on standard treatment concluded that exenatide, liraglutide, lixisenatide, dulaglutide, or

semaglutide did not influence AF incidence (275). Similarly, another meta-analysis showed that despite reductions in major cardiovascular events, and cardiovascular and all-cause mortality, GLP-1RA had a neutral effect on AF risk. Nevertheless, given that experimental evidence suggests that liraglutide attenuates atrial electrical remodelling in mice models of chronic intermittent hypoxia (276), whether risk of incident AF is mitigated by GLP-1RA merits investigation in future clinical trials.

1.11.3.3. Bariatric surgery

Despite compelling evidence suggesting weight loss reduces arrhythmic burden in those with pre-existing atrial arrhythmias (277), there is conflicting evidence for the effect of bariatric surgery on incident AF risk. For example, amongst a national primary and secondary healthcare database of over 11 million patients in the UK, although rate of AF resolution among patients undergoing bariatric surgery was greater compared with age-, sex- and BMI-matched controls, there was a downward but not significant trend for AF incidence: adjusted hazard ratio 0.72, 95% CI: 0.492–1.054; $p=0.091$ (278). This result was echoed in another study of 1000 obese patients in whom Roux-en-Y surgery was not associated with a lower incidence of AF compared to those that were medically managed (HR 0.66, 95%CI: 0.40–1.10, $p=0.11$) (279). In a meta-analysis by van Veldhuisen et al, there was no statistical reduction in incident AF risk (HR 0.82, 95%CI: 0.64-1.06) despite improvements in other cardiovascular outcomes (240). This may be because of the 39 studies included in their meta-analysis only 7 listed AF in their primary or secondary outcomes of which only 3 assessed incident AF as a primary outcome measure following bariatric surgery. Consequently, the absence of any effect of bariatric surgery on incident AF risk may be partly explained by the relative paucity of

studies investigating AF as their primary objective. Amongst these three studies, Höskuldsdóttir et al, assessed hospitalisation for heart failure and/or AF in a register-based cohort study comprising 5321 individuals with diabetes and obesity undergoing Roux-en-Y gastric bypass surgery with the same number of individuals not treated with surgery over a mean of 4.5 years (280). Although risk for hospitalisation for AF was 41% lower in the bariatric surgery group, the study did not evaluate incident AF risk, AF severity or burden post-surgery (280). In another study of 90 obese patients with persistent AF in which 58 undertook weight reduction intervention and 32 served as controls there was no difference in arrhythmia-free survival post-catheter ablation at one year (63.8% vs 59.3%, $p=0.68$) (281). This was despite the weight reduction group recording almost 25kg median weight loss (IQR 19.1-56.7kg). Singh et al showed that overall rate of incident AF was not different between 5087 obese patients undergoing surgery and 9858 receiving standard care (adjusted HR 0.93, 95%CI 0.68-1.27, $p=0.661$), irrespective of whether the procedure was gastric banding or Roux-en-Y gastric bypass (282). Although sub-analysis comparing patients with and without type 2 diabetes showed that AF risk was lower in the former (adjusted HR 0.57, 95% CI 0.32-1.01; $p=0.053$) and paradoxically higher in the latter (adjusted HR 1.24, 0.85-1.81, $p=0.271$), neither of these results were statistically significant (282).

By contrast, in a Swedish prospective multicentre cohort (SOS) study with median 19-year follow-up and comprising over 4000 obese patients of which half underwent bariatric surgery, AF risk was 29% lower in the surgery group versus the control group (HR 0.71; 95%CI: 0.60-0.83, $p<0.001$), although these effects only became apparent after several years follow-up and not all patients were randomly allocated

to bariatric surgery that may have introduced bias (283). In another single centre retrospective cohort study AF incidence was lower in obese patients that had undergone bariatric surgery compared to those that were medically managed (0.8% vs 2.9%, $p=0.0001$) (284). A reduction in AF following bariatric surgery has also been reported in the context of obesity-related co-morbidities. For example, in a longitudinal study involving 827 morbidly obese patients who underwent laparoscopic adjustable gastric banding, incident AF significantly greater in patients with OSA than in those without OSA (1.7% vs 0.5%, $p<0.001$). By contrast, among the Look AHEAD (Action for HEAlth in Diabetes) cohort, weight loss from lifestyle modification in overweight and obese individuals with type 2 diabetes did not reduce incident AF risk (285). The absence of the preventive anti-arrhythmic effect may be due to the relatively modest body weight reductions achieved by lifestyle measures compared to bariatric surgery (approximately 10% vs 20%) and is consistent with observations from studies supporting sustained weight reduction for greater arrhythmia-free survival and improvements in cardiovascular outcomes (252, 254, 286). In summary, although surgical and non-surgical weight reduction is an effective preventative intervention, the evidence for bariatric surgery reducing incident atrial arrhythmic risks is less consistent (111).

1.12. Non-invasive electroanatomic mapping

Despite the compelling evidence for obesity as an independent arrhythmic risk factor and weight reduction in mitigating this risk presented in this chapter, the proarrhythmic substrate in obese individuals without a history of arrhythmias has not been characterised in-depth. Although small-scale clinical studies show that increasing adiposity is associated with adverse changes on 12-lead ECG and in vitro

animal studies that this is likely underpinned by cellular remodelling, these do not provide adequate mechanistic insights into the adverse adaptations of human obese hearts that make them vulnerable to arrhythmias. This is explained by two limitations. First, the 12-lead ECG is a low-resolution modality that cannot provide detailed information on spatial heterogeneities of activation and repolarisation that are known to render the heart susceptible to arrhythmias. Second, obese patients without cardiac arrhythmias do not have a clinical indication to undergo invasive electrophysiological studies by which the limitations of the 12-lead ECG could be addressed. It may be inferred, therefore, that these limitations can be addressed by a technique that enables non-invasive electroanatomic mapping with a greater number of body surface electrodes than with a 12-lead ECG.

1.12.1. Body surface potential mapping

Body surface potential mapping (BSPM) is a non-invasive modality by which electrical signals can be recorded over a larger area of the torso to enable more detailed characterisation of cardiac electrical source than afforded by a 12-lead ECG. This modality therefore represents a compromise between high-resolution invasive and low-resolution non-invasive mapping. It has mainly been the preserve of research studies given that practicalities associated with the application of a large number of electrodes across the torso and the computational analyses required of the recorded electrical signals.

BSPM was first reported by Taccardi more than half a century ago to study the cardiac electrical source in detail (287). Signals acquired by BSPM can be analysed by one of three methods (Figure 1.14). First, signal analysis-only methods can be applied to the raw BSPM data independent of torso geometry. Second, map analysis combines BSPM signals and torso geometry to create electrical maps on the body surface. Third, BSPM and heart-torso geometry can be combined to reconstruct the electrical activity at the source, i.e. the heart. The last option has come to be known as electrocardiographic imaging (ECGi), in which signals from high density of body surface electrodes (for example, 256 electrodes) is combined with patient-specific heart torso geometry and has become the most widely developed of the three. It is, however, a highly complex method that has undergone optimisation and validation spanning more than four decades (288, 289, 290) and continues to be developed by the ECGi Consortium (291) and others (292). The principles underpinning the ECGi method is briefly summarised in the following sections.

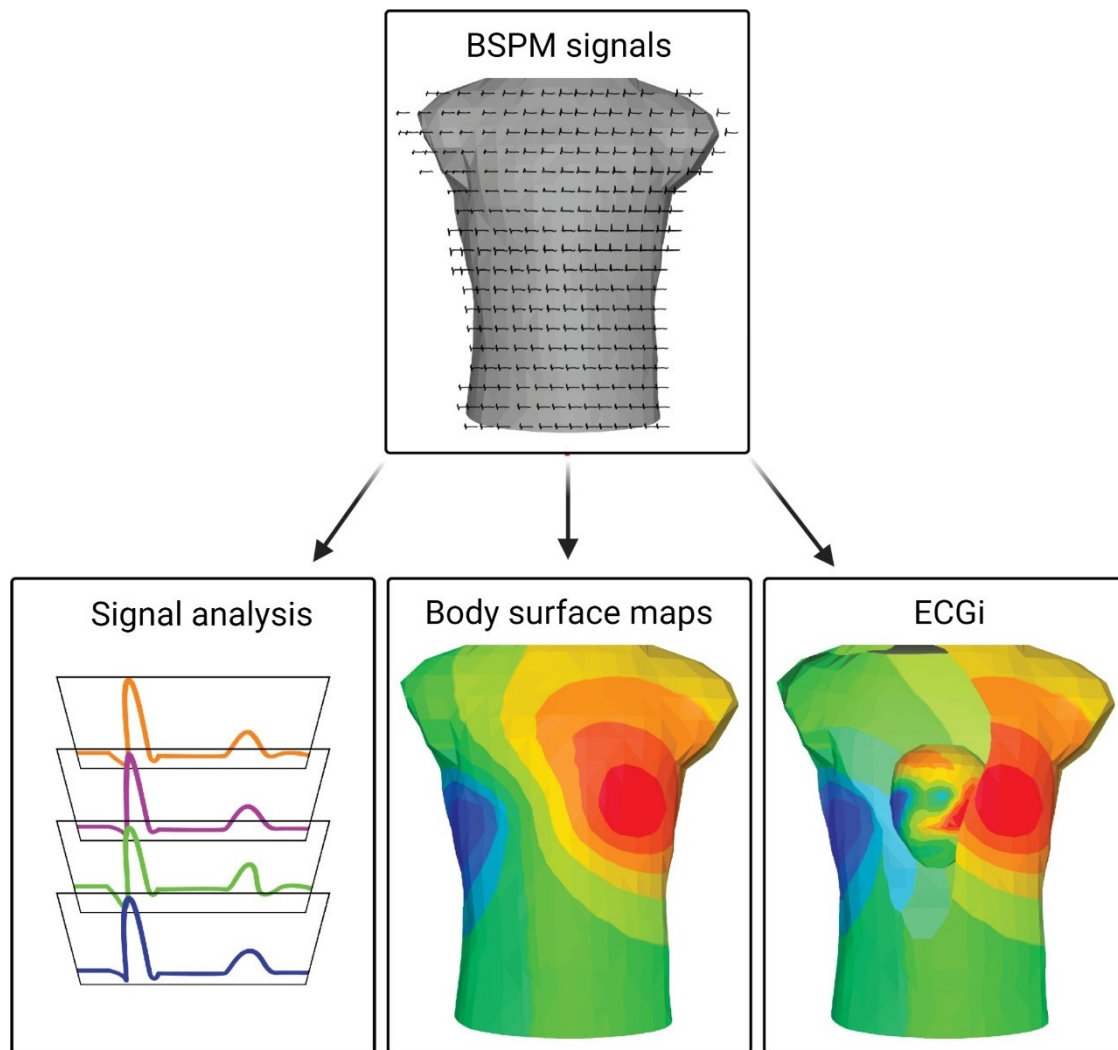


Figure 1.14: Body surface potential mapping. Body surface potentials may be analysed independently (left) or in the context of torso geometry to create body surface maps (centre). Alternatively, epicardial activation and repolarisation patterns can be reconstructed from body surface potentials and heart-torso geometry by solving the inverse problem of electrocardiography (right). Figure adapted from Bergquist et al (293).

1.12.2. Electrocardiographic imaging

One of the challenges of acquiring BSPM data is the practicalities of applying hundreds of electrodes on the torso at a single recording. To aid this, electrodes may

be arranged in strips to facilitate rapid and reproducible application between different torsos guided by anatomical landmarks. Torso electrogram voltages are usually in the magnitude of 1-5 millivolts which makes BSPM highly susceptible to noise that adversely influences reconstruction of cardiac signals. This can be partly mitigated by buffering and amplifying the signals, bandwidth filtering to a physiological range (e.g. 0.2-300Hz) and removing power line noise (typically 50Hz in the UK) during data acquisition (293). Signals are usually recorded at sampling frequencies of 1000-2500Hz and digitised for offline processing. The heart-torso geometry required in ECGi is typically acquired by cross-sectional imaging, either computed tomography (CT) or magnetic resonance imaging (MRI). This data is used to create torso and epicardial meshes using segmentation and meshing software. Recently, studies have shown that it may be possible to reconstruct cardiac geometries without cross-sectional imaging that may mitigate some of the practical and economic limitations of ECGi and facilitate its adoption more widely (294). In addition to heart-torso geometry, computing the bioelectrical sources in the heart requires some *a priori* information relating to location of BSPM electrodes, which can be acquired during CT or MRI, and electrical properties of the tissue between the body surface and the cardiac source.

1.12.3. The inverse problem of electrocardiography

Computation of the electrical activity at the heart in this way involves solving the inverse problem of electrocardiography using LaPlace's and Poisson's equations, but is complicated by its ill-posed and ill-conditioned nature. This refers to the fact that a small degree of noise recorded at the body surface is augmented in a non-linear fashion introducing significant errors in reconstructed signals at the cardiac

source (295). Furthermore, solving the inverse problem using a single set of the BSPM and geometric data may yield several solutions, some of which may be implausible. Thus, a number of constraints have to be applied to limit the solutions in a process called “regularisation” (296, 297, 298). Some of these constraints are targeted at the amplitude and spatial heterogeneities in the cardiac source. For example, Tikhonov zero-order enforces low amplitude signals, Tikhonov first-order small spatial gradients and Tikhonov second-order spatial smoothness (293, 298). The degree to which the output is influenced by the regularisation is determined by the strength (“weight”) ascribed to the regularisation parameter. An over-regularised solution represents a biased representation of the cardiac source that may not discriminate sufficiently between different regions of early and late activation and repolarisation, and an under-regularised solution will be sensitive to artefact and provide an inaccurate representation of the cardiac source.

1.12.4. Cardiac source models

Another important consideration is the cardiac source, i.e the models representing the heart’s electrical activity that explain the BSPM data. Three main models have been used in non-invasive mapping: transmembrane voltage models, surface (extracellular) potential models, and activation-recovery-based models (299). Of these, the transmembrane voltage model most closely approximates the true cardiac electrical source as a dipole, but in vivo validation of this model is difficult as transmembrane voltages are difficult to obtain. The extracellular potential models most commonly represent the atria and ventricular epicardial surfaces thus reconstructing electrical activity for this surface only, although similar can be achieved for endocardial surfaces as well. This model affords the generation of the

electrograms (change in potential over a single anatomical point), as well as activation and repolarisation maps (patterns of change over the surface at any given time) (290). Results using this model can be easily validated from data recorded during invasive studies using a roving catheter to map either the endo- or epi-cardial surfaces. For this reason, this model has been used most widely in ECGi studies. As their name suggests, activation-recovery-based models reconstruct activation and repolarisation patterns without reconstructing electrograms and therefore yield less information about the electrical activity of the heart compared to the previous models.

1.12.5. Applications of electrocardiographic imaging

The reconstruction of cardiac electrical activity using ECGi enables the identification of sites and patterns of abnormal activation and repolarisation, and quantification of their respective spatial gradients. Moreover, it enables a panoramic reconstruction of the electrical activity of the heart on a beat-by-beat basis. It has been implemented in a number of academic and clinical settings to characterise proarrhythmic substrates in vulnerable cohorts (300, 301, 302, 303), study patterns of atrial and ventricular arrhythmias (304, 305, 306, 307, 308, 309, 310, 311, 312), understand the mechanisms underpinning arrhythmias such as repolarisation gradients (33, 38, 313), and guide invasive treatment (314, 315, 316, 317). However, no studies to date have used ECGi to characterise the subclinical electrophysiological remodelling in obese patients who, as reviewed in this chapter, are at risk of atrial and ventricular arrhythmias. Similarly, any reverse remodelling following weight loss in these patients has also not been reported. Addressing these points extends beyond academic interests as any evidence of reverse electrophysiological remodelling in

patients without a history of arrhythmias would support early and aggressive clinical weight management in these patients. Indeed, given that metabolic complications of obesity have been shown to reverse with weight loss (318), evidence supporting the same for adverse electrophysiological adaptations may help to mitigate incident arrhythmias and thereby concurrently alleviate the health and economic burden posed by cardiac arrhythmias and obesity.

1.13. Scope of thesis

1.13.1. Aims and hypotheses

The overarching aim of the work contained in this thesis was to explore the effects of adiposity on cardiac electrophysiology, and thereby understand the mechanisms underpinning adiposity-related arrhythmogenesis and the extent to which it can be mitigated with weight loss.

The main hypotheses were:

1. Increasing adiposity is associated with prolongation of ventricular repolarisation and confers a higher risk of ventricular arrhythmias amongst the UK population
2. Obesity is associated with subclinical proarrhythmic electrophysiological remodelling, even in individuals without a history of arrhythmias, that can be discerned using non-invasive electroanatomic mapping
3. Morbidly obese individuals exhibit conduction slowing in the atria and conduction and repolarisation abnormalities in the ventricles compared to non-obese individuals, and that these can be reversed with weight reduction

4. Epicardial fat is characterised by a more pro-inflammatory secretome compared to subcutaneous fat and causes conduction slowing in vitro.

Specific aims to address the main aim and hypotheses were as follows:

1. To determine the extent to which increasing adiposity independently prolongs ventricular repolarisation in the UK population
2. To determine the degree to which metabolic ill-health alone and/or in combination with obesity contributes to prolongation of ventricular repolarisation
3. To determine whether any cardiometabolic-induced prolongation of ventricular repolarisation translates into a higher prevalence of ventricular arrhythmias at a population-level
4. To optimise ECGi for its application to study cardiac electrophysiology in obese patients
5. To characterise atrial and ventricular electrophysiology in obese and non-obese individuals
6. To characterise atrial and ventricular electrophysiology in obese patients before and after bariatric surgery
7. To characterise the secretomes of human epicardial and subcutaneous fat using quantitative and semi-quantitative biological methods
8. To test the effects of epicardial and subcutaneous fat on cell electrophysiology, specifically conduction velocity

Chapter 2: Methods

2.1. Introduction

This chapter provides a general description of the materials and methods used in the work in this thesis. It also provides explanations of the principles underpinning the methods, and where relevant, details historical and validation studies to contextualise these methods. It first provides information on the UK Biobank and its data acquisition and analysis. Next, it explains the principles, history and methods underpinning electrocardiographic imaging. Thereafter, this chapter describes the laboratory-based techniques used to investigate the effect of adipose tissue on cell electrophysiology, which includes adipose tissue harvest and culture, neonatal rat ventricular myocyte culture, utility of the microelectrode array system to study cell electrophysiology, human adipokine assays and ELISA and immunohistochemistry. Further specific details on methodology are provided in the relevant chapters.

2.2. UK Biobank

2.2.1. Background

This section provides a brief history of the UK Biobank, and summarises the results of studies relevant to obesity using data from the repository to contextualise the results in Chapter 3. Further data acquisition and methodological details are also provided in Chapter 3.

The UK Biobank is one the largest and most comprehensive data repositories worldwide that continues to accumulate data from its cohort. Over half a million UK residents aged between 40 and 69 years at the time of recruitment were invited to participate on a voluntary basis (319). This was with the aim of following them for up

to 30 years to improve the management of major disease. Participants were recruited between 2006 and 2010 underwent detailed baseline phenotyping at one of 22 assessment centres across the UK. This included recording sociodemographic and health status, physical and functional measurements, and collection of blood urine and saliva samples. Resting 12-lead ECG recordings were also recorded in later phases of recruitment. This data was accumulated by a combination of self-reporting via questionnaires and in-person assessments by trained staff at the assessment centres. In 2013, the UK Biobank established linkage to death and cancer registries. Similarly, diagnostic and health outcome data was collated by linkages with secondary care records starting in 2014 with primary care linkages in 2019. These national databases enable follow-up of the UK Biobank participants. Uniquely, all consenting participants also underwent deep genotyping. Indeed, it is with this in mind, that the UK Biobank was conceived. In 2015, the UK Biobank launched an ambitious imaging study, which sought to undertaken imaging in 100,000 individuals, mainly with magnetic resonance imaging of the heart, abdomen and brain, but also encompassing carotid ultrasound and whole-body dual X-ray absorptiometry. Approximately 50,000 participants have undergone imaging, of which 10,000 have been recalled in longitudinal follow-up, and is scheduled to be completed in 2023.

2.2.2. History of the UK Biobank

The UK Biobank was set up after the completion of the Human Genome Project in 2001 with the aim of translating the progress in genotyping into clinical applications. As such, from its conception, it was not a hypothesis-driven research project but rather a response to converting scientific (genomic) progress into practical benefits.

In this regard, the UK Biobank was not unique, as projects with a similar aim of improving personalised medicine were set up across Europe, Asia and the United States in the post-genomic era. The UK Biobank was the product of a workshop between the Medical Research Council (MRC) and the Wellcome Trust that took place in May 1999. At this meeting, it was decided that a new large-scale cohort was justified to gather health-related data that was not available in existing databases or cohorts. In fact, these discussions had suggested establishment of two cohort studies: an adult study to examine the genetic and environmental risks factors for disease in adults, and a separate birth cohort in which the influence of environmental factors on disease development and progression could be studied. A greater emphasis, however, was placed on the former rather than latter because of its potential for improving public health in the short and medium term. It was suggested that the new prospective adult study should collect data to help understand the genetic, lifestyle, environmental and physiological factors underpinning common adult diseases that represent a significant public health burden. These recommendations were eventually approved by Wellcome Trust and MRC in 2000 and marked the beginning of the UK Biobank.

2.2.3. Adiposity and electrophysiology: findings from the UK Biobank

Relevant to the aims of this project, there have been notable results from recent studies using the UK Biobank resource some of which are summarised herein.

Genomic data from the UK Biobank has been used to identify novel loci for visceral adiposity and a Mendelian randomisation study showed visceral adiposity to be associated with higher risk of cardiometabolic diseases (136). Similarly, visceral adiposity, determined by abdominal MRI, has been shown to be associated with

smaller left ventricular end-diastolic volumes and reduced systolic function on cardiac MRI (320). Utilising 12-lead ECG, MRI and health data in the same individuals, neural networks applied to 12-lead ECG can predict different normal weight and overweight or obese individuals categorised by BMI with over 70% accuracy in males and females (321). Furthermore, any differences between predicted and calculated BMI was indicative of underlying abdominal visceral adipose volume and concurrent cardiometabolic disease (321). More recently, convolutional neural networks applied to 12-lead ECG to predict AF risk have also been validated using the UK Biobank (322).

Amongst the UK Biobank cohort, risk of new onset diabetes, cardiovascular disease and heart failure are also greater in metabolically healthy obese compared to metabolically healthy lean individuals, suggesting that the term “metabolically healthy obesity” is a misnomer and not a benign phenotype (323). Indeed, body weight and waist circumference were shown to be independent risk factors for AF. Interestingly, this risk was largely driven by lean, rather than fat, mass in females. By contrast, waist circumference was a stronger determinant of AF risk than fat mass in males but not in women, suggesting that sex-related differences in adiposity and its distribution are important factors for arrhythmic risk (324). These findings are also supported by another study demonstrating differential effects of body composition and morphology on cardiovascular risk (325). Physical activity has also been associated with lower risks of AF and ventricular arrhythmias (326).

One the advantages of the UK Biobank is that has undertaken in-depth genotypic as well as phenotypic characterisation of the same participants enabling the identification of disease-modifying genes and linking them to biological pathways to determine their individual and combined effects on health outcomes. In this regard, the genome-wide association studies utilising genetic data from the UK Biobank have identified novel single nucleotide polymorphisms (SNPs) related to body composition that correlate with obesity phenotypes (327). Similarly, using a polygenic risk score generated from 300 SNPs associated with cardiovascular disease, obesity-promoting unhealthy lifestyles amongst the UK population have been associated with increased all-cause as well as cardiovascular mortality independent of genetic risk, although genetics had a modulatory effect on lifestyle factors for risk of stroke (328). In other study, genetics and lifestyle factors were shown to be independent risk factors for incident coronary artery disease, AF, stroke, hypertension and diabetes (329). A Mendelian randomisation study combining data from UK Biobank and other similar studies from Copenhagen shows that every unit increment in BMI was associated with unadjusted causal risk ratios of 1.39 for heart failure, 1.18 for heart failure-related mortality and 1.02 for all-cause mortality (330). These findings were corroborated in another study in which although Cox models showed a J-shaped relationship between BMI and mortality, Mendelian analyses revealed a positive linear causal link between increasing weight and mortality. In this respect, the obesity in which cardiovascular mortality has been reported to be lower amongst overweight individuals is likely to be a observational phenomenon and one which may not translate into reality The authors therefore argue that a national effort to encourage weight loss amongst overweight and obese individuals in the UK would result in a reduction rather than a paradoxical increase in mortality (145). Indeed,

amongst the UK Biobank cohort Mendelian randomisation evaluating the cost-effectiveness of weight reduction strategies showed that laparoscopic bariatric surgery would increase quality-adjusted life years and decrease healthcare costs with a net saving of up to £20,000 per person over 20 years and up to a total of over £600 million per year (331). Therefore, although bariatric surgery may be associated with higher short-term hospital-related costs (332), it nonetheless confers significant cost-saving benefits in the long term given that inpatient costs for obese patients are greater than for their leaner counterparts (333).

Increasing adiposity has also been associated with a range of other health risks amongst the UK Biobank cohort, including cataracts (334), coronavirus infection (335, 336) that is attenuated with physical activity (337), liver disease (338, 339), osteoporosis (340), malignancies (341, 342, 343), multiple sclerosis (344) and cognitive decline (345).

2.2.4. Data acquisition from the UK Biobank

UK Biobank data was accessed following an on-line application (number 48666) on its website (www.ukbiobank.ac.uk) outlining the above rationale. Following a favourable review of the application, de-identified data was provided to address the research questions. A material transfer agreement to not attempt to identify any participant, to keep the data secure, and to use it only for the purposes of the approved research was signed. Data available in this application was sizeable and included resting 12-lead ECG, abdominal, brain and cardiac MRI, body composition anthropometry, hospital records as well as lifestyle, sociodemographic and

healthcare information. The application was linked to existing datasets acquired by Professor James Ware at Imperial College London (dataset 47602) to access genetic data. Further details on the UK Biobank data utilised for the work contained in this thesis is provided in Chapter 3.

2.3. Electrocardiographic imaging (ECGi)

2.3.1. Background

This section first describes the rationale for using electrocardiographic imaging (ECGi) to investigate the reversibility of the proarrhythmic substrate associated with obesity with weight loss. This is described in the context of the limitations of 12-lead ECG and high-density body surface mapping. Although in principle combining two well-established clinical tools, namely ECG and cross-sectional imaging, may seem intuitively straightforward, in reality, ECGi is a complex research method that has been developed and validated over decades. The history of ECGi is summarised herein, followed by an explanation of the ECGi protocol used for data acquisition in the clinical study.

2.3.2. Rationale for using ECGi

Cardiac excitation refers to the wave of depolarisation that is initiated at the sinus atrial node and, in health, conducted via specialised tissue and via cell-cell coupling, throughout the atria and into the ventricles. A potential field is created between the body surface and the epicardial surface during cardiac excitation. This can be measured by electrodes on the body surface. The 12-lead ECG is the most commonly used method by which cardiac potentials are recorded for clinical

investigation, diagnoses and management. However, the ECG is constrained by its spatial resolution in that although it provides information on the electrical activity, it cannot accurately locate regions of rhythm abnormality. Similarly, it cannot easily discern subtle spatial differences in electrical activity that may render the myocardium proarrhythmic. The 12-lead ECG also assumes standard heart-torso geometries between individuals. Given that the potentials recorded on the body surface are influenced by the conductive properties of the tissue between the body surface and the heart, and that body geometry, composition and the electrical properties of its constituent parts vary between individuals, the 12-lead ECG is subject to considerable inaccuracies.

Body surface mapping refers to the utilisation of a larger number of body surface electrodes to record an ECG. In some instances, this can be up to 256-electrodes applied sequentially across the torso. Although body surface mapping addresses the low resolution limitation of the 12-lead ECG, and has been shown to be efficacious in localising the sites of origin of ventricular tachycardia in hearts following infarction (346), it is nonetheless subject to the other limitations of the 12-lead ECG. Given that each body surface potential is determined by the electrical activity of the entire heart it cannot adequately resolve single or multiple local cardiac electrical events in space. Furthermore, the volume conductor between the heart and body surfaces exerts a smoothening effect on the body surface potentials through which important electrical information may be lost.

The concept of mathematically computing epicardial electrograms from the body surface potentials using geometric measurements was first proposed in the 1970s (347). By combining high resolution body surface mapping with cross-sectional imaging, electrocardiographic imaging (ECGi) overcomes the limitations of 12-lead ECG. It enables panoramic visualisation of the electrical activity of the heart in a single beat, that can identify the earliest and latest regions to depolarise. Similarly, repolarisation maps demonstrate patterns of ventricular repolarisation that cannot be discerned on 12-lead ECG. This is also in contrast to invasive electrophysiological studies that relies on contact mapping over several beats using a roving probe approach. Thus, the electrical activity of the entire heart, both activation and repolarisation, can be studied on a beat-by-beat basis with ECGi. The fact that it utilises patient-specific heart-torso geometry is also advantageous enabling a more accurate reconstruction epicardial potentials that are influenced by the torso-volume conductor. The greater number of electrodes (up to 256 compared to 10 in a standard 12-lead ECG) affords a greater spatial resolution by which epicardial electrograms can be reconstructed. These reasons underpin the rationale for the development of ECGi as a modality by which arrhythmias and arrhythmogenic substrates can be characterised in greater detail than afforded by its constituent parts.

ECGi is a term that was coined in Professor Yoram Rudy's research group which was also responsible for much its development and validation. Several terms have been used to describe the methodology, including ECG mapping or ECM. For consistency, it will be referred to as ECGi henceforth. The following sections provide a methodological description of ECGi, followed by a narrative of its development and

validation, and finally the specific details on the ECGi methods used for the work contained in this thesis.

2.3.3. High density body surface mapping

ECGi begins with high density body surface mapping. Several systems exist to enable this with the number of electrodes varying from 64 to 256 electrodes (304, 307, 348). The minimum number of body surface electrodes to accurately reconstruct epicardial potentials has been subject to debate. Nevertheless, it has been shown that spatial resolution increases with the number of electrodes and, according to one study, the minimum requirement is 74 electrodes (349).

2.3.4. Defining the cardiac source

The next step requires choosing the cardiac model to represent the source of electrical activity. There are three main representations or formulations for this: potential-based, wavefront-based and bidomain models, which have been described by others (290) and are summarised hereafter. The potential-based formulation assumes that there is a relationship between the potentials recorded on the torso and epicardial surfaces which can be defined by the electrical properties of the volume conductor between the two. Body surface potentials are used to reconstruct epicardial potentials from which activation and repolarisation times and patterns can be determined (350). Compared to the other two formulations, the potential-based models have the advantage that epicardial electrogram and potential maps can be validated relatively easily experimentally by comparing it with contact mapping data. On the other hand, it cannot provide information on transmural electrical activity. The

potential-based model was adopted in many of the in vivo developmental and validation ECGi studies. The wavefront model represents the electrical source as a layer of current dipoles. Transmembrane voltage, which is the potential difference across the cell membranes of a cell, is a better approximation of the true cardiac electrical source but given that these cannot be measured readily either experimentally or clinically, any validation is restricted to extracellular signals which is afforded by the potential-based method (290, 351). Finally, the bidomain approach aims to compute activation and repolarisation times without reconstruction of transmembrane voltages or potentials. This is based on the understanding that a dipole is present at the forefront of an activation wavefront. The substrate is determined by the electrical signals recorded on the body surface to determine the point at which depolarisation reaches the epicardium (352, 353, 354).

2.3.5. Acquisition of heart-torso geometries

Cross-sectional imaging provides the means by which heart and torso geometries can be reconstructed accurately on an individual-specific basis. The cardiac source can be reconstructed as an epicardial shell, as required by the potential-based approach, or a bilayer comprising the endocardium and epicardium for the wavefront approach. One of the inherent limitations of ECGi is the assumption that the heart and torso are stationary. Any reconstruction electrical data is projected onto a 3D heart reconstructed from cross-sections obtained at end-diastole even though body surface potentials are recorded throughout the cardiac cycle with breathing artefact.

2.3.6. Solving the inverse problem of electrocardiography

Following high density body surface mapping, choosing an appropriate cardiac source model and acquisition of cross-sectional images, the ECGi process involves solving the inverse problem of electrocardiography. This is best understood by first considering the forward problem of electrocardiography. 12-lead ECG or body surface mapping records the electrical activity of the heart on the torso. The potentials at the source (the heart) are modified by the electrical and conductive properties of the volume conductor as they pass through the torso before being recorded on the body surface. Thus, computing body surface potentials from those of the heart as a function of the electrical properties of the torso volume conductor represents the forward problem of electrocardiography. It can be described by the following equation:

$$\phi T = A \phi E$$

where, ϕT represents body surface potentials, A the transfer matrix of the torso volume conductor, ie. the thorax, and ϕE epicardial potentials.

A describes the geometric information and electrophysiological properties of the thorax, and demonstrates inter- as well as intra-participant variation; the former owing to natural variation in body composition and geometry, and the latter due to the inevitable subtle variations during data acquisition, for example as a consequence of differences in position and movement during ventilation. The transfer matrix linking epicardial to body surface potentials can be obtained by the finite boundary element method, where the volume conductor is approximated by a

3D mesh with surface grids (355). In this regard, inhomogeneities of the lung fields rather than torso size or shape have been shown to influence reconstruction of epicardial potentials (356). Mathematically, ϕT can be resolved with a high degree of accuracy as the problem has a limited number of solutions and is therefore described as well-posed. Conversely, computing epicardial electrograms from the body surface represents the inverse problem of electrocardiography and entails the derivation of ϕE from ϕT , using the LaPlace equation within the torso volume conductor. Mathematically, therefore, the derivation of ϕE requires the inverse of A . However, given that A is not a full-rank (square) matrix and is influenced by the dynamic of ventilation and cardiac contraction, ϕE can only be approximated by using the least squares solution by computing the inverse of A as follows:

$$\tilde{\phi E} = (A^T A)^{-1} A^T \phi T$$

In addition to only approximating ϕE , the inverse problem of electrocardiography has no unique solution as the input, ϕT , are fewer in number than the desired output, ϕE . Therefore, unlike the forward problem, its inverse counterpart is ill-posed. In other words, it is very sensitive to noise recorded in ϕT and inaccuracies in A result in large errors in the computation of ϕE . Given also that different patterns of cardiac electrical activity may be reproduced from a single set of body surface potentials, the inverse problem also yields several solutions some of which may be electrophysiological or anatomically implausible. Therefore, additional constraints are applied to stabilise the problem to select a realistic solution. The application of these constraints is called regularisation. A regularisation parameter, λ , is applied to the minimise the sensitivity of the ϕE to noise in ϕT and any inaccuracies in A and by

delimiting the amplitudes or derivatives of ϕE in space and/or time. Introduction of the regularisation parameter gives the following equation:

$$\tilde{\phi}E = (A^T A + \lambda I)^{-1} A^T \phi T$$

where I is the regularisation operator (LaPlacian).

In this way, regularisation minimises any errors by imposing constraints on the solution. Consequently, as shown in the equation above, ϕE can only be approximated and is continuously dependent on the inputs on the right side of the equation.

The choice of regularisation parameters can have a profound impact on the output, as over-regularisation may yield solutions that are over-smoothened and lack spatio-temporal definition. On the other hand, under-regularisation may yield inaccurate solutions due to noise and artefact. Thus, regularisation has a smoothing effect on activation and repolarisation maps that may result in loss of diagnostically important information if applied inappropriately. The application of these constraints also requires *a priori* knowledge of the solutions' properties which may be limited in clinical settings. Several regularisation parameters can be used to constrain the solutions to the inverse problem. The most common regularisation method used to impose constraints on $\tilde{\phi}E$ is Tikhonov regularisation (298, 357) and its optimal parameter (λ) has previously been determined using the CRESCO method (355), or L-curve (358) or zero-crossing (359). Other regularisation techniques include the general minimal residual (GMRes) algorithm (360) which does not require *a priori* information of the substrate being investigated. Comparisons of Tikhonov and GMRes regularisation methods showed that they were equivalent in the

reconstructions of activation and repolarisation patterns of normal and infarcted hearts, although GMRes may be better to resolve multiple pacing sites (361). Zero-order Tikhonov regularisation is based on the premise that epicardial electrograms are inherently low amplitude. First- and second-order Tikhonov regularisations work on the assumption that activation should occur as a smooth wavefront over the epicardial surface. More recently, Tikhonov-based regularisation methods have been shown to be as robust in fibrillatory conditions as they are in sinus rhythm (362) and remains the most widely used regularisation method (299). Various options for regularisation in ECGi have been summarised by Milanic et al (297).

2.3.7. Development and validation of ECGi

The earliest studies that attempted to reconstruct epicardial electrograms and potentials from those recorded at the body surface used computational, eccentric sphere or cardiac electric generator models (288, 290, 356, 363). Although these provided useful insights into the complexities of solving the inverse problem accurately, there were nonetheless stylised and did not recapitulate human physiology and anatomy. Subsequent studies used to develop ECGi were in animal models to imitate the heart inside the human body. A tank shaped like a human torso was filled with electrolyte solution and a perfused dog heart was placed inside in the position that resembled human anatomy. Over three hundred torso surface electrodes and the equivalent number of electrodes in direct contact with the epicardial surface enabled a comparison of the potentials recorded at each of these surfaces. Epicardial activation in the human heart was mapped by application of inverse solutions to body surface potentials by Huiskamp and Van Oosterom in 1988 (364). In this study, the patterns of activation were consistent with those described

by Durrer et al (365) in which earliest activation occurred over the left then right ventricles, with breakthrough activation in the anterior right ventricle and occurring latest in the right posterobasal right ventricle. The authors noted that the general patterns were faithfully recapitulated despite the regularisation required to minimise the inherent instabilities of the inverse problem that may have resulted in the loss of the nuances of activation patterns. Later, using a canine heart maintained by Langendorff perfusion in a cage of 216 electrodes placed in the position of a human heart in a torso-shaped tank of a 9 year old male child with 400 electrodes on its surface, Messinger-Rapport and Rudy (366) showed that it was possible to reconstruct epicardial electrograms from body surface potentials with a good degree of accuracy whilst the heart was beating in sinus rhythm. The relative errors between measured and reconstructed epicardial electrograms ranged from 0.35 during the QRS complex to 1.0 between the T-wave and its following P-wave. The patterns of activation were also consistent with those reported by Durrer et al (365). By comparing real-life torso-tanks with stylised torsos which were smoothened and defined by a finite set of simple geometries that only approximate actual geometry, they showed that zero-order Tikhonov regularisation adequately minimised the differences in reconstructed epicardial electrograms that were introduced by differences in torso geometries. These findings were consistent with earlier canine experiments in which the torso geometry from one dog applied for the computation of epicardial potentials of another dog of comparable size did not affect reconstruction accuracy (367). The regularisation process also afforded an error of 1cm in the location of the heart within the torso-tank without causing significant errors in the reconstruction of epicardial potentials, which can be readily achieved with modern cross-sectional imaging. Finally, they showed that reconstructions were least

accurate when using fewer than 100 body surface electrodes. These results were confirmed in a subsequent study using realistic-geometry electrolytic tank that showed that inaccuracies in the position of the heart of up to 1cm were well-tolerated and could be addressed by regularisation processes. By contrast, inaccuracies of 2cm or greater resulted in inaccuracies in the position and amplitudes of the reconstructed epicardial potentials (368). In this context, zero-order Tikhonov regularisation performed comparably to first- and second-order equivalents (369). In order to minimise error, Huiskamp and van Oosterom specified that torso size, heart orientation and its position with respect to body surface positions need to be approximated as close to reality as possible to provide accurate solutions to the inverse problem of electrocardiography (370). These studies attempted to solve the inverse problem in a series of timeframes, i.e. a set of potentials fixed at a specific timepoint, even though activation is, in reality, a time-series rather than a series of static processes. Oster and Rudy showed that factoring this temporal feature into Tikhonov regularisation using the Twomey technique resulted in superior degree of accuracy in the reconstruction of epicardial potential than with Tikhonov alone (371).

After establishing that ECGi could reconstruct epicardial potentials in sinus rhythm, Oster et al showed that it could also resolve disparate electric events on the epicardial surface (372). In the same canine-heart human torso-tank model used for studies evaluating sinus rhythm, single ventricular epicardial pacing sites were identified to within 10mm of their true anatomical positions. Dual pacing sites located 17, 35, and 52mm apart were also similarly resolved. Reconstructed electrograms showed a close resemblance to reconstructed electrograms with correlation coefficients >0.8 , with only 5% of electrodes demonstrating correlation coefficients

<0.5. Regions of sparse and crowded activation isochrones, signifying areas of relatively faster and slower conduction respectively, in response to pacing were also reproduced with similar degrees of accuracy. In this way, it was shown that ECGi could identify sites of ventricular ectopy and areas of slow conduction, a hallmark of re-entry, and therefore demonstrated its clinical utility to guide invasive therapy and provide mechanistic insights into arrhythmias (373). Studies in which complex electrical patterns were not as well reconstructed using ECGi may have been subject to errors introduced by inconsistencies in heart-torso geometries as outlined above, confirming the need for data acquisition in a controlled environment and robust regularisation processes that minimise noise and artefact (374). Given that sites of ectopy may be intramural and that propagation of activation occurs from endocardium to epicardium (365), Oster et al subsequently investigated the ability of ECGi to discern intramural activation patterns (375). The left ventricle was paced from at the epicardium, and intramural depths of 3.2mm, 6.4mm, 9.6mm and 12.8mm in different regions of the left ventricle. Knowing that epicardial potential distributions are dictated by fibre orientation in anisotropic muscle (376, 377), whereby an area of negative potential on the epicardial surface signified focal activation and the orientation of its flanking maxima the fibre orientation, ECGi-derived potential patterns reasonably estimated the location and depth of pacing sites, and therefore, sites of ectopy (375). Although the error with which these were identified varied with depth and site of pacing by as much as 25mm, it nonetheless provided an important proof-of-concept.

Following torso-tank studies demonstrating ECGi could reproduce epicardial electrograms in normal hearts, Burnes et al showed that it could do the same in

canine hearts in which the left anterior descending artery had been occluded for 2 hours and ethanol injected in an acute myocardial infarction model (378). Similarly, activation patterns, regions of epicardial breakthrough and ventricular tachycardia could also be recapitulated with ECGi in 4-day old chronic infarct heart(379).

The studies mentioned hitherto were performed in controlled environments using a homogenous volume conductor that do not reflect human anatomy. Subsequent studies therefore investigated the modulatory effect of torso inhomogeneities on the accuracy of epicardial potential reconstruction with ECGi. Early computational models had previously suggested that lungs, blood and fat augment body surface potentials whereas skeletal muscle does the opposite without altering patterns of electrical activity (288, 363, 380). Although these models bear little resemblance to human anatomy and cardiac electrical activity in vivo, these findings were nevertheless confirmed in silico models by Ramanathan and Rudy. Homogenous and inhomogenous torsos exert a smoothing effect on body surface potentials, and that their amplitudes but not patterns are modulated by the characteristics of the volume conductor (381). Subsequently, they applied measured epicardial potentials to variations in human anatomy to examine the effects of inhomogeneities on epicardial reconstructions from torso potentials in ECGi (382). In this way, three torsos were tested: the first contained all inhomogeneities of a normal human torso, the second was a uniform homogenous torso with no anatomical variations, and the third contained stylise lung tissue. Although the homogenous model was inferior in its accuracy in reconstructing epicardial potentials than its inhomogenous and stylised lung equivalents, the electrical patterns, electrogram morphologies and location of sites of pacing were similar in all three. In the inhomogenous,

homogenous and stylised lung models, 77%, 60% and 63% of reconstructed electrograms, respectively, had correlation coefficients >0.8 between measured and reconstructed electrograms. Similarly, the earliest isochrones were reconstructed with 0ms, 5ms and 3ms delays, respectively, and for dual ventricular site pacing, the reconstructed inter-site pacing distances were 6.24cm, 6.75cm and 6.61cm, respectively. Finally, differences in body habitus and sex did not exert any discernible differences (382). The fact that the homogenous model was comparably efficacious as an inhomogenous model was particularly welcome given that reconstruction of the electrical conductivity of all tissue components of the thorax on an individual basis would render ECGi highly impractical and limit its translational utility.

While earlier studies focussed on non-invasively reconstructing activation patterns using ECGi, subsequent studies also focussed on its application to studying repolarisation abnormalities that are associated with life-threatening arrhythmias that cannot be spatially resolved with torso potentials. Unlike activation, repolarisation progresses from the epicardium to the endocardium (383). Spatial repolarisation disparities are underpinned by variations in refractoriness and have been associated with arrhythmic vulnerability since 1940 (19), specifically re-entry (384). QT interval dispersion on an ECG is the difference between the longest and shortest QT interval and although large dispersions signifying global myocardial repolarisation heterogeneity have been observed in the patients with a history of ventricular arrhythmias and congenital long QT syndrome (385, 386, 387), this metric reflects the integrated electrical activity of the entire heart and therefore cannot readily preserve the spatial resolution of its electrical source. Indeed, the spatial properties

of the electrical source may only be accurately reproduced on a remote surface when it is close to it, the orientation of the source is such that it maximises the potential difference between the itself and the remote surface and there is no other electrical activity masking that of the region of interest being recorded on the source (388). It is therefore unsurprising that QT dispersion has been suggested as a predictor of sudden cardiac death amongst patients with ischaemic heart disease, heart failure and long QT syndrome in some studies (387, 389, 390, 391, 392) but not in others (393, 394, 395), and therefore its clinical utility in this regard has been limited (396, 397). This warranted another method by which patients could be non-invasively assessed for arrhythmic vulnerability. Burnes et al showed that changes signifying repolarisation heterogeneity in body surface ECG indices derived from 12- and 64-lead ECGs, such as QT dispersion and T-wave morphology, were incongruent with the those recorded at the epicardial surface in response to warming of the left ventricular epicardial surface to 42C with heat lamp and cooling it to 28C with a cryoablation system in a canine heart placed inside a human torso-tank (388). This experiment was reminiscent of a previous study by Burgess et al in which the effects of myocardial warming on ECG was investigated in canines (398). Indeed, QT dispersion on the torso paradoxically decreased during warming despite an increase in repolarisation dispersion on the epicardial surface (388). In contrast to the inconsistencies observed between body surface ECG indices (T-wave onset and offset and QT dispersion) and epicardial potentials in response to warming and cooling, ECGi-derived reconstructed electrograms were consistent with those recorded on the epicardium. This included the faithful recapitulation of shortening and prolongation of activation-recovery intervals in response to warming and cooling,

respectively, with preservation of the steep gradients between adjacent warm and cool regions (399).

2.3.8. Validation of ECGi in humans

Following these developments, one of the earliest validation studies in humans compared the accuracy of ECGi to capture electrical events with direct epicardial mapping in patients undergoing open heart surgery (400). Reminiscent of studies performed 15 years earlier (368), pacing sites were located to within 1cm radius. Unlike experimental models, there was only moderate correlation (0.72) between recorded and reconstructed electrogram morphologies during sinus rhythm and pacing although the patterns of activation were reproducible. This may be explained by the fact that epicardial and reconstructed potentials were not recorded simultaneously in this study (400). The next validation study was a case study in which ECGi was shown to successfully identify the source of ventricular tachycardia in an athlete. ECGi maps were congruent with activation maps obtained with invasive electroanatomic mapping techniques and identified the site of a single premature ventricular beat within the left ventricular diverticulum (401).

Thereafter, in a qualitative study of 4 individuals, ECGi was used to map activation patterns in different contexts. In sinus rhythm, earliest epicardial breakthrough occurred in the right ventricular free wall, which was predictably delayed in right bundle branch block. Sites of pacing were located to within a range of 7–11mm (309). After showing that ECGi could be applied in real-life clinical contexts, the ECGi equivalent to Durrer's seminal paper describing the activation patterns of the

human heart in sinus rhythm under physiological conditions (365) came from Ramanathan et al in 2006 (402). In this study, 4 healthy males and 3 healthy females with no history of arrhythmias or heart disease underwent ECGi. All were young (age range 21-43 years) and had narrow QRS complexes on ECG (duration range 76-100ms). Consistent with Durrer et al, they showed that the earliest epicardial activation occurs in the anterior paraseptal region of the right ventricle within 25ms of the QRS onset in all 7 cases. Subsequently, within 10ms of right paraseptal breakthrough, there were several breakthrough regions over the anterior paraseptal, posterior and apical regions of the left ventricle, and further breakthroughs over the right ventricle. All breakthroughs were observed within 40ms of activation onset. The epicardial breakthroughs over the right ventricle eventually coalesced signifying complete depolarisation of the right ventricle. In the left ventricle, the activation wave moves from the lateral-apical region towards the posterolateral base which is the last to activate. In three individuals, the right ventricular outflow tract also activated late. With respect to the atria, the earliest activation was observed in the right atrium near the sinoatrial node and propagated to the rest of the right atrium before encroaching into the left atrium. The left atrial appendage was the last to activate. Given the repolarisation does not follow an activation-like propagation, its sequence was relatively static and was unaffected by activation times. Earliest repolarisation occurred over the anterior wall and intensified throughout the T-wave duration. Latest repolarisation occurred over the inferior-basal regions. The activation-recovery interval maps were almost identical to repolarisation maps indicating that local repolarisation and not activation is the major determinant of the repolarisation sequence. These results were also consistent with those from animal models (403)

and were the first description of activation and repolarisation patterns in human hearts obtained by ECGi.

Another important validation study that bridged the gap between explanted heart and in-human ECGi comprised simultaneous invasive and non-invasive electrogram recordings in intact, closed-chest canines (289). In four dogs, heart-torso geometries were reconstructed from computed tomography images, over 100 epicardial electrodes were placed on the ventricles and approximately 200 electrodes on the chest after thoracotomy closure. Reconstructed epicardial potentials closest in space to their recorded epicardial equivalents were compared by correlation. Among over 5,500 pairs of recorded and reconstructed potentials, the median correlation coefficient was 0.71. 90% of reconstructed electrograms for which correlation with its closest epicardial counterpart was less than 0.7 had an another approximating electrogram within 20mm radius and 77% were within 10mm radius. Using the spatiotemporal approach to determine local activation time (292, 404), in which the spatial relationship between neighbouring sites and their respective potentials are considered to minimise the effect of noise or fractionated electrogram, they showed that patterns of activation were reproduced according to descriptions provided by Ramanathan et al (402). These correlations were similar to those reported in humans by Ghanem et al (400) but less than in torso-tank models (405) that may be explained by torso inhomogeneities in a living animal and breathing motion.

Similar studies were performed in closed-chest pigs evaluating the effect of homogenous vs inhomogeneous torsos and boundary element methods vs meshless

approach for reconstruction heart-torso geometries on reconstruction of ventricular epicardial potentials during activation (406). Mean correlation coefficients were approximately 0.6 but with significant spatiotemporal variability in all tested models, although activation patterns were well recapitulated. Consistent with a previous study (407), the meshless method for reconstruction the epicardium was better than boundary element method which was not improved by introducing torso inhomogeneities.

The first fully experimental evaluation of ECGi to map repolarisation was performed more recently by Bear et al, who studied drug-induced repolarisation heterogeneities by simultaneously recording epicardial and torso potentials in Langendorff-perfused pig hearts in a torso-tank as well as in vivo in closed-chest pigs (313). In both instances, there was a strong concordance between ECGi-reconstructed and recorded T-wave morphologies, as well as repolarisation maps and gradients. Reconstructed repolarisation gradients were spatially discordant with those recorded on the epicardial surface only by up to 14mm, and demonstrated that ECGi could faithfully recapitulate contact-derived abnormalities whilst maintaining the spatiotemporal properties of myocardial repolarisation in vivo. The study showed that by virtue of its non-invasive nature, sequential ECGi could be implemented to study differences in repolarisation and its dispersion pre- and post-treatment or to track disease progression.

2.3.9. ECGi: a clinical context

Over the past 10 years, ECGi has been used clinically for mechanistic and diagnostic insights. Amongst these, it has been used to characterise repolarisation abnormalities in inherited disease (36, 37, 300, 301, 408) as well as early repolarisation syndrome (409) . For example, ECGi could accurately identify prolonged repolarisation and steeper gradients in patients with long QT syndrome compared to control subjects, and showed that activation-recovery intervals were longer and also more spatially heterogeneous. These differences were starker amongst symptomatic long QT syndrome patients (36).

Other studies have used ECGi to understand the mechanisms underpinning AF despite the low amplitude potentials associated with fibrillatory waves with a remarkable degree of accuracy (306, 410) and to localise re-entrant and focal atrial tachycardias with accuracies of 85% (23/27 cases) and 100% (21 cases), respectively (411). The role of ECGi in characterising and treating AF has been comprehensively reviewed by Salinet et al (310). It has also been used to guide successful ablative therapy in AF (306) and ventricular tachycardia (305) and reduce procedural time (412). The same is true for guiding therapy for accessory pathways and ventricular pre-excitation (413) as well as premature ventricular complexes and ventricular tachycardia (307). Jamil-Copley et al showed that ECGi could localise the origin of outflow tract ventricular tachycardias pre-ablation and subsequently guide therapy with 100% ablation success rate in 24 patients, of whom 22 remained asymptomatic with fewer than 1000 ectopic beats per day 6 months post-treatment (414). Finally, ECGi has also been used to evaluate the efficacy of cardiac resynchronisation therapy in heart failure in patients with QRS duration exceeding 120ms by comparing right and left ventricular activation times (314). Importantly, this

study also showed that ECGi was superior to 12-lead ECG-based predictions of CRT response that has been corroborated in a more recent study (415).

In this regard, ECGi has been shown to be clinically advantageous in its ability to characterise proarrhythmic substrates which may not be discerned using routine ECG. For instance, Leong et al showed that the patients with a history of aborted SCD had increased dispersion of repolarisation on ECGi compared to patients with inherited channelopathy but no history of malignant ventricular arrhythmia despite no differences in repolarisation on their respective 12-lead ECGs (301). Importantly, these differences were not observed at rest but were unmasked with exercise and postulated to be due to changes in autonomic tone in the post-exercise recovery period.

2.3.10 Limitations of ECGi

Despite the evidence for ECGi to accurately recapitulate activation and repolarisation times and patterns, some studies have demonstrated variability between reconstructed and ground-truth contact data. For instance, in an earlier qualitative validation study comparing activation maps recorded with ECGi and contact mapping systems by Sapp et al, the former showed areas of extremely slow and fast activation that were not observed with the latter (304). This gave rise areas of artefactual conduction block and hindered the ability to locate exit sites for ventricular tachycardia. Similarly, in another study of 55 patients, lines of conduction block defined as >50ms between contiguous points that were recorded in 27/59 contact maps were absent in non-invasively recorded maps, and 33/59 non-invasive maps

that showed conduction block were absent in contact maps (295). Similar discrepancies in activation times, albeit not to the same extent, have also been observed in torso-tank models despite abnormal repolarisation being faithfully reproduced (416). Another study also comparing non-invasive and contact mapping systems also showed activation and repolarisation parameters were reproduced with only moderate accuracy and that inaccuracies were borne from poor electrode contact and geometric reconstruction that limited spatial resolution (417).

In summary, ECGi has been developed and validated to non-invasively reconstruct activation and repolarisation potentials for mechanistic, diagnostic and therapeutic purposes. It's underlying methodological complexities and nuances, however, has meant that currently its utility is the preserve of academic institutions that have the expertise, experience and capabilities of its application and data processing.

2.3.11. Ethical approval for ECGi study

Ethical approval for the study, "Electrophysiological phenotyping of patients at risk of ventricular arrhythmia and sudden cardiac death", was provided by Surrey Research Ethics Committee under the UK Health Research Authority (reference 19/LO/0762) in June 2019. The study sponsor was Imperial College London. Except for those involved in participant recruitment and data collection, all other members of the research team had access to anonymised data only.

2.3.12. Participant recruitment

Pre-operative obese patients awaiting bariatric surgery were prospectively recruited from a pre-operative dietician's clinic at St Mary's Hospital, Paddington (UK) beginning in December 2019. The primary care team introduced the study to patients attending the clinic. Those fulfilling the inclusion criteria were directed to the research team. Upon contact with the research team, the study was explained to prospective participants and were also provided with a participant information sheet. They were given as much time as required to consider the written information, which contained contact information by which patients could contact the research team for any enquiries pertaining to the study. Age- and sex-matched non-obese volunteers were recruited by advertisement at Imperial College London.

All participants partook in the study on a voluntary basis and provided informed written consent prior to undergoing the ECGi protocol. All participants also completed a MRI safety questionnaire immediately before undergoing the scan and female participants of childbearing age were asked to state their pregnancy status and perform a urinary pregnancy test. Participants were given the option of having a chaperone present during the study protocol. Non-obese volunteers were recruited after the obese cohort had been recruited to ensure that they were age- and sex-matched to their obese counterparts. Eligible non-obese participants were recruited on a first-come first-serve basis to avoid bias. Obese patients underwent the ECGi protocol prior to bariatric surgery and were recalled approximately 6-9 months after their surgery whenever practically possible. Non-obese volunteers underwent the ECGi protocol once only.

Three months into recruitment, the coronavirus pandemic resulted in the study being formally paused from March to August 2020. Researchers were requested by the sponsor to pause the study again from December 2020 to April 2021 during the second lockdown measures sanctioned by the UK government. The coronavirus pandemic caused significant postponements to elective surgeries, including bariatric services at Imperial College Healthcare NHS Trust which impacted patient recruitment and their recall post-surgery. As soon as clinical services were able to resume, eligible obese patients were recruited via on-line pre-operative dieticians' clinics. As an estimate, the pandemic resulted in a delay of up to 12 months in data acquisition and the recall of patients post-surgery.

2.3.13. ECGi systems

At the time of study conception, there were three ECGi systems available to use. The development of ECGi and its subsequent validation by Rudy's group resulted in the development of the only commercialised system called CardiInsight™ (Medtronic) which comprises of a 252-electrode electrode vest and non-contrast CT as the imaging modality of choice. The system includes software that performs the inverse solution with inputs provided by the user. Given that this can be constraining in individuals with a large body habitus, a non-commercial research system (ActiveTwo, BioSemi, Amsterdam, Netherlands) that was provided on loan by Professor Pier Lambiase (University College London) was used for the clinical study. This system was previously used to compare the arrhythmogenic substrate in patients with cardiac amyloidosis (300) and comprises of strips of electrodes that are

manually arranged across the participants' torsos and can be re-used. The digital recording equipment that facilitates data acquisition can be purchased as individual parts from BioSemi. Given that epicardial spatial resolution increases with increasing number of body surface electrodes, the BioSemi system has the added advantage of providing 256 electrodes. It therefore has considerable redundancy to enable electrodes recording noise or artefact to be excluded prior to solving the inverse problem without adversely affecting the quality or accuracy of epicardial potentials (349). The BioSemi and CardioInsight™ systems use the extracellular potential-based model as the cardiac source. By contrast, the third system, Cardiac Isochrone Positioning System uses bidomain model, and was not available at the start of the study.

2.3.14. BioSemi (ActiveTwo) ECGi system

The BioSemi (ActiveTwo) system comprises 256 carbon electrodes arranged over 24 rubber strips with a pre-amplifier integrated at the bottom of each strip. Prior to each recording, each electrode was manually prepared by application of 20mmx8mm double-sided adhesive ring and filled with low-impedance high-conduction electrode paste (Elefix, Nihon Kohden) (Figure 2.1). After being secured on the participants' torsos, the electrode strips were connected to a 256-channel amplifier (AD-box) that was connected to a battery pack. The AD-box digitised the 256-signals with 24-bit resolution and consisted of a low noise DC coupled post-amplifier. The AD-box was connected ("daisy chained") to an extra Analogue Input Box (AIB) by optical fibres which ensured that the analogue ECG signals were isolated from other mains-powered electrical equipment in use at the time of recording. The AIB adjusts its sample rate to the signal received from the subject

AD-box, so the sample rates of both boxes are synchronised. A second optical cable connected the AIB to a USB2 receiver that was plugged into a remote laptop computer. Incoming data via the optical cable was detected by a LED indicator on the USB2 receiver. The USB2 receiver was connected to a laptop computer that had a minimum resolution of 1280x1024 and Windows10 (Microsoft) operating system. This apparatus is shown in Figure 2.2. Body surface potentials were visualised in real-time and recorded using ActiView software at a fixed sampling frequency of 2048Hz. Files were saved as .bdf files, saved to an external hard drive in an anonymised format and processed offline. Electrode strips were washed with soap and water, dried and stored safely for subsequent use.

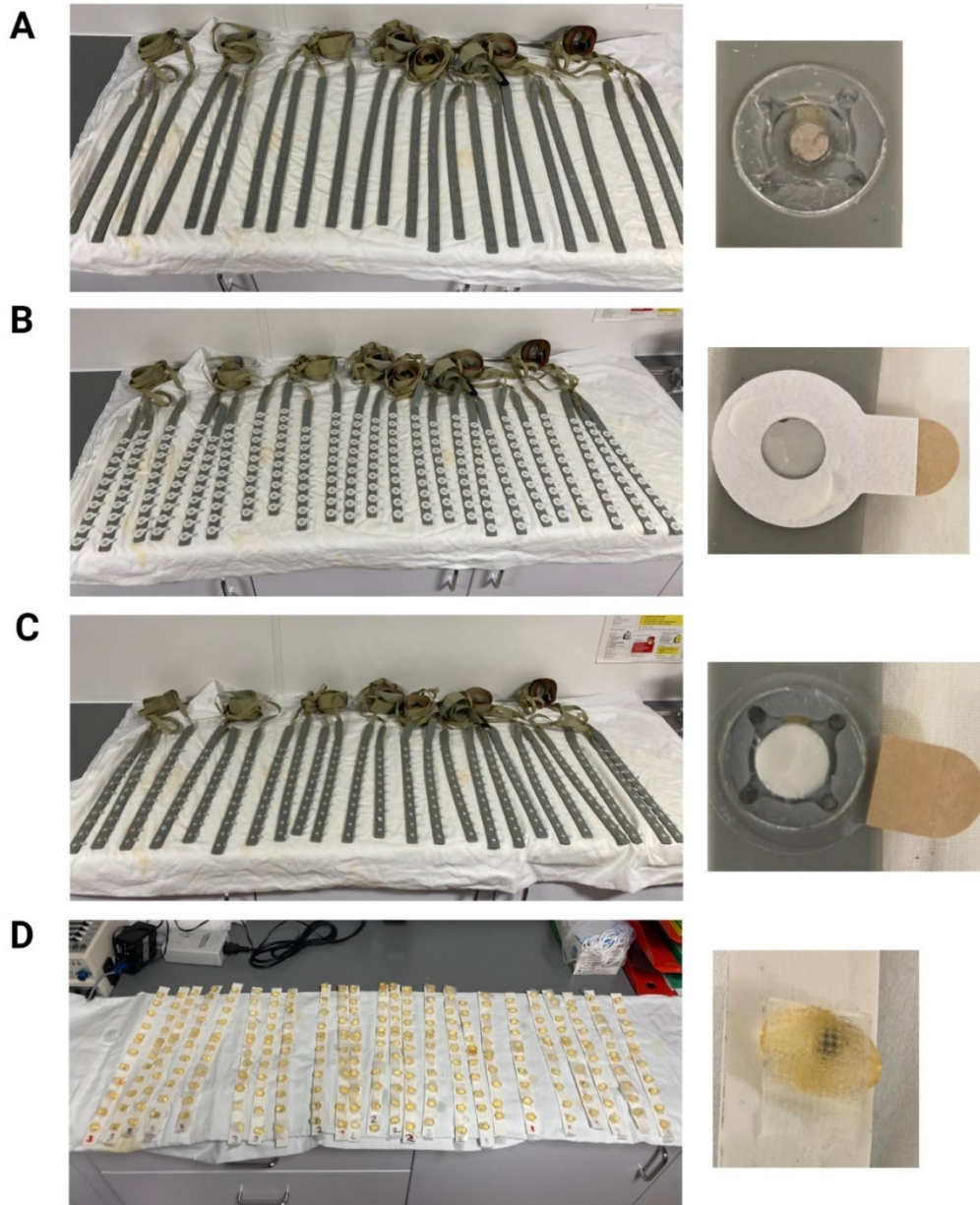


Figure 2.1: Preparation of 256 electrodes for body surface potential mapping.

(A) Strips of electrodes were cleaned, dried and laid out for preparation on a clean work surface. **(B)** Double-sided adhesive rings were placed manually on each of the electrodes and filled with electrode paste to aid recording of body surface unipolar potentials. **(C)** The upper surface of rings were removed to expose an adhesive under-surface that aided skin contact when applied on torsos during data acquisition. **(D)** Custom made strips comprising cod-liver oil capsules were made

to replicate the positions of electrodes to torso-surface and were used during MRI. Inset images provide magnified views of an electrode and its preparation, and a cod-liver oil capsule

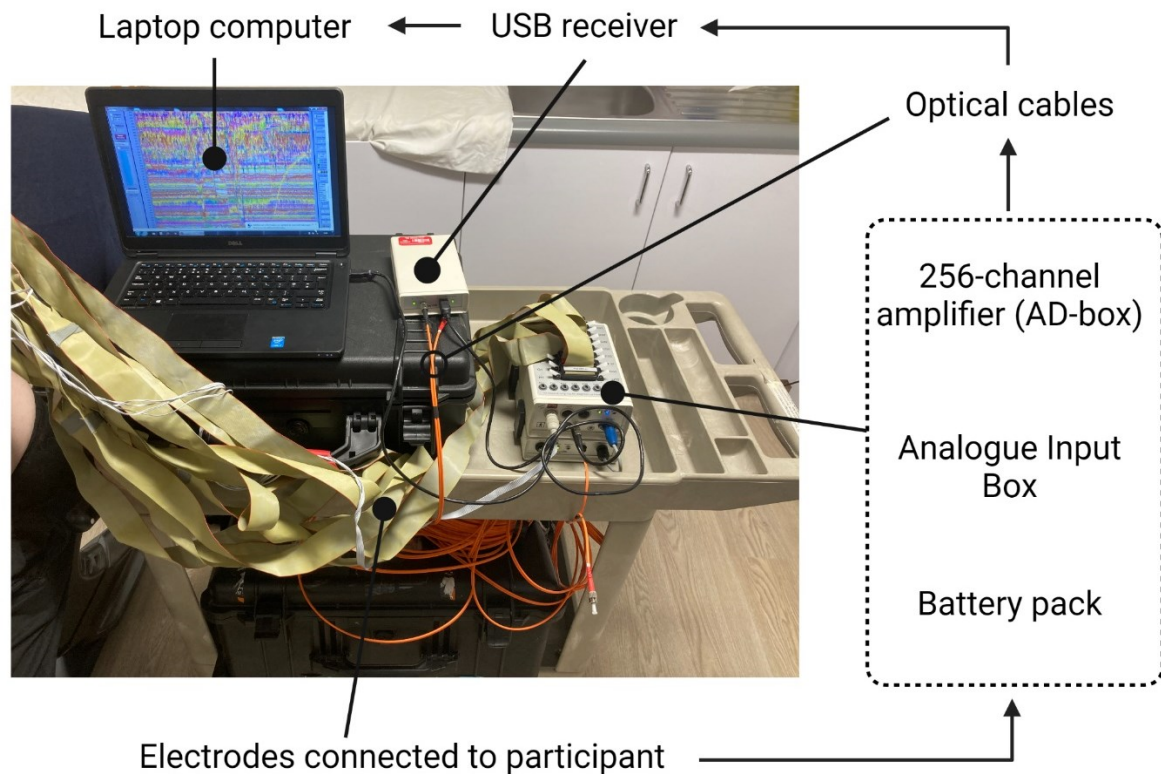


Figure 2.2: Body surface potential recording apparatus. Data from electrodes attached to participants is delivered to the 256-channel amplifier and analogue input box. Optical cables connect these to an USB receiver which is connected to a laptop computer, on which body surface potentials are visualised in real time.

2.3.15. Body surface potential mapping protocol

Each participant had 256 electrodes placed across their chest wall and back whilst seated in an upright position on a static bicycle (Figure 2.3). Strips of electrodes were applied so that they were equidistant from each other on anterior and posterior

aspects of the torso, the top electrodes on the front and back were aligned, and the first electrode on the most medial strips on the anterior chest wall was directly inferior to the sternoclavicular joint. The strips were secured with tape to ensure skin contact. Four limb lead electrodes were also placed as per conventional 12-lead ECG. Resting body surface ECG was recorded for a total of 10 minutes with the participant remaining as still as possible. Participants then undertook incremental bicycle exercise (Cardiostrong recumbent exercise bicycle BC50) at 25Watts per minute until 85% age-predicted heart rate ($220 - \text{age}$) was achieved or were unable to continue exercise whichever was reached first. ECG recordings were saved in the last minute of exercise and a further nine minutes into the post-exercise recovery period. Electrode strips were removed individually and immediately replaced with equivalent customised strips containing cod-liver oil capsules that had been secured by glue and tape (Figure 2.4). These served as magnetic resonance imaging (MRI)-opaque and -safe markers and were positioned in anatomically to exactly matched the location of electrodes over the torso prior to MRI.

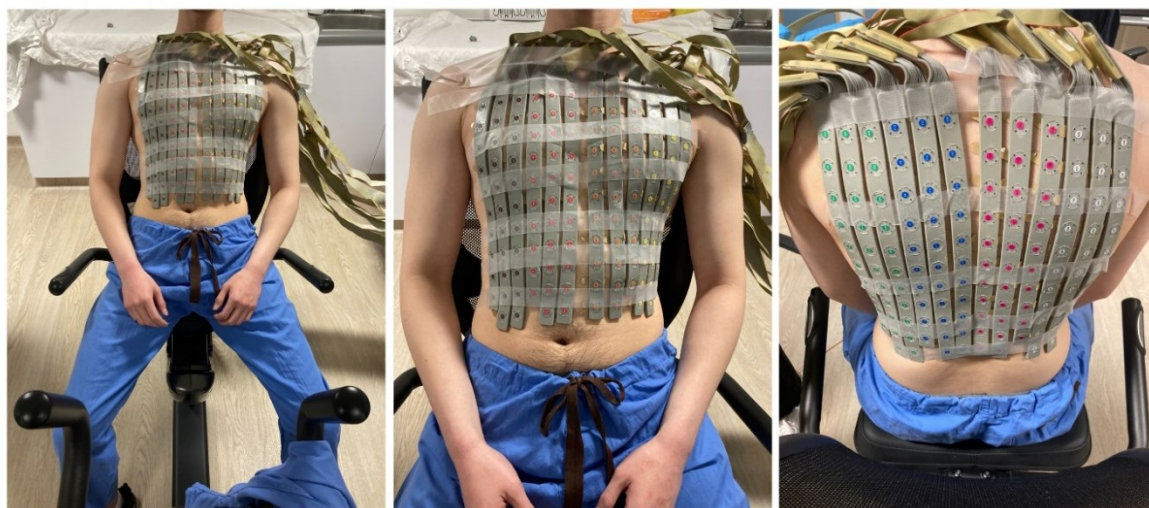


Figure 2.3: Body surface potential mapping. Strips of electrodes were placed sequentially across the torso of participants guided by anatomical landmarks. Photographs taken and included with consent.

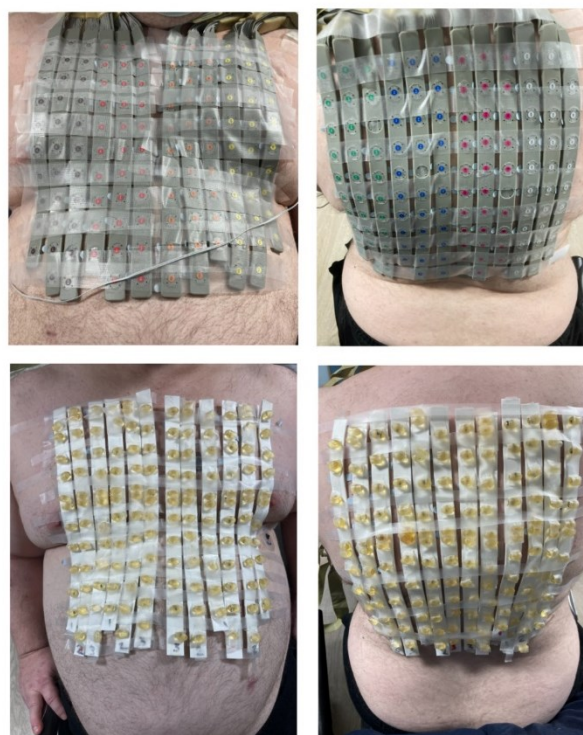


Figure 2.4: Electrode strips with corresponding MRI-safe markers. Following body surface mapping, strips of electrodes (top panel) were replaced with

customised strips containing cod-liver oil capsules (bottom panel) to identify the anatomical location of each electrode. Photographs taken and included with consent.

2.3.16. Cardiac MRI protocol

To minimise radiation exposure in participants in whom there was no clinical indication for computed tomography (CT), magnetic resonance imaging (MRI) was used to obtain cross-sectional data to recreate the 3D geometry of the epicardial surface and torso. This was performed immediately after replacing the electrodes strips with MRI-safe markers. Participants underwent non-contrast cardiothoracic MRI (CMR) on a Siemens MAGNETOM Aera 1.5T scanner (Siemens, Erlangen, Germany) in the Robert Steiner MRI unit at Hammersmith Hospital. The CMR protocol included a respiratory-navigated and electrocardiogram-gated *Half-Fourier Acquisition Single-shot Turbo spin Echo* (HASTE) sequence in the transverse plane. 94 slices were acquired at end-diastole throughout the lung fields ensuring visualisation of all electrode markers. Further imaging parameters included: repetition time (TR)=747ms; echo time (TE)=22ms; flip angle=160°; partial Fourier=4/8; matrix=192x192; slice thickness=4mm; voxel size=1.3x1.3x4mm; sequence time approximately 7.5minutes. Cine imaging was used to measure volumetric and functional data and flow imaging for aortic pulse wave velocity. Images were acquired at end-diastole. Scans were performed by Ben Statton (superindependent radiographer), Elaine Berry (radiographer) and Hannah Bergman (radiographer) in the Robert Steiner MRI unit, Hammersmith hospital.

2.3.17. Epicardial electrogram reconstruction

The following sections outline the methods by which epicardial electrograms for atria and ventricles were reconstructed with body surface ECG and MRI data. Further details are provided in Chapters 5 and 6.

2.3.17.1. Pre-processing of body surface ECG

Body surface potentials were pre-processed using Python (v3.6) with the assistance of Nikesh Bajaj (research associate, National Heart and Lung Institute), who provided computational and coding expertise required for ECGi-related signal pre- and post-processing contained in this thesis.

First, Infinite Impulse Response (IIR) filter was used to remove DC components exceeding 20dB without compromising other frequency components. Savitzky-Golay filter of polynomial order 3 and length 2047 (2048Hz-1) was applied to smoothen baseline wander. A 50Hz low pass filter was applied to remove any noise captured from the mains supply power during acquisition. Following application of filters, the resting 10-minute ECG recording comprising of 256 body surface potentials was split into one-minute segments and RR interval calculate for each segment. The segment with the smallest RR interval standard deviation, an index of RR interval variability, was selected for further pre-processing. Noise or artefact resulting from movement during recording within the one-minute segment were identified by their power (measure of signal strength) and spectral entropy (signal complexity). Body surface signals with power 1.5 times the interquartile range above the third quartile were removed. Similarly, ECG signals with a spectral entropy 1.5 times the interquartile

range below the first quartile and above the third quartile were rejected. The same methods were applied to remove single beats obscured by noise in each lead.

Following removal of noisy signals and beats based on power and spectral entropy, the first 10 consecutive beats in the one-minute segment with smallest RR interval standard deviation were aligned by their R waves to generate a single averaged complex per recording electrode. The average RR interval amongst these 10 beats was calculated and used in data analysis. Signal averaged complexes were generated in the same manner for peak exercise (defined as the first minute immediately following exercise cessation) and for 2- and 4- minute recovery intervals. Any remaining noisy signals were identified manually and discarded. Windows of interest on signal averaged beats (P wave to reconstruct atrial electrograms; QRS complex and T-wave for ventricular electrograms) were manually selected on the signal averaged complexes.

2.3.17.2. Reconstruction of heart and torso geometries

Cross-sectional MRI images were segmented by Ben Statton to generate heart-torso geometries. The series of 94 axial images comprising the MRI scan were loaded onto image analysis software (Amira, ThermoFisher Scientific) to identify the position of each body surface electrode on the torso surface. Volume rendering was used to visualise the images and body surface electrode markers. All markers were manually labelled so that they corresponded to the numerical order of the body surface electrodes.

To obtain cardiac geometry, epicardial surfaces of the ventricles and atria were manually traced on each of the 94 axial images at end-diastole using the Amira software toolkit. Ventricles were segmented from the apex to the base, defined as the tricuspid and mitral annuli. The atria were segmented from the valvular annuli to the pulmonary outflow tracts.

2.3.17.3. Solving the inverse problem

The inverse problem and post-processing steps were performed using a customised code (MATlab, MathWorks, MA) provided by Matthjis Cluitmans, and Job Stoks (Maastricht University), who also provided technical advice when required during the course of the study. Nikesh Bajaj provided additional computational expertise for these methods. To reconstruct epicardial electrograms, the potential-based formulation was chosen as the cardiac source in a homogenous torso volume conductor. Zero-order Tikhonov regularisation was applied to solve in the inverse problem of electrocardiography in this study. Given the sampling frequency of the body surface potentials was 2048Hz, 2048 regularisation parameters (λI) were calculated for every recorded ECG. The median λ was taken to the optimal parameter and applied to the signal. This process was repeated for all body surface potentials that were used to compute epicardial electrograms.

2.3.17.4. Post-processing of reconstructed electrograms

The activation waves of the reconstructed epicardial electrograms are sufficiently discreet and of an adequate amplitude to enable accurate automated annotation of the steepest downslope (activation time). On the contrary, the variable morphology

of epicardial repolarisation waves necessitated post-processing to ensure correct repolarisation time annotation. First, electrograms reconstructed over the valve plane were also excluded. Next, the first derivative of the repolarisation wave was calculated, and only repolarisation times identified within the maximum area of the first derivative were considered in the next steps. This ensured that repolarisation time was annotated within the T-wave and not on other deflections that may constitute noise, artefact, or baseline wander. Thereafter, all remaining epicardial electrograms (approximately 1200 in each case at each time point) were manually reviewed. Flat T waves in which annotation of repolarisation was not possible or incorrect were excluded from analyses. Then, a 5mm spatial filter was applied so that the median of all repolarisation times within 5mm radius of the index node was assigned to the index node to minimise the effect of outliers. Electrograms in which the spatial filter resulted in >5ms difference in repolarisation time were each manually reviewed; any electrograms with flat or ambiguous T wave morphology were excluded. Finally, repolarisation maps and the epicardial electrogram morphology were reviewed in tandem to ensure correct annotation of repolarisation times before calculating the total repolarisation times and gradients. Typically, over a thousand electrograms remained following post-processing from which repolarisation times could be determined.

2.3.17.5. Activation and repolarisation parameters

Local activation time (AT) was taken as time of the steepest downslope ($-dV/dt_{\min}$) of the activation complex in atrial and ventricular electrograms. In the remaining electrograms after post-processing, ventricular repolarization times (RT) was taken as the maximum upslope ($dV/dt_{\max}$) of the T-wave according to the Wyatt method

(418). Activation-recovery interval (ARI), a surrogate for action potential duration (419), was calculated as the difference between local ventricular RT and AT and corrected for heart rate using Bazett's formula (AR_{IC}). Mean AR_{IC} was calculated using local AR_{IC} over the ventricular epicardium. Total atrial and ventricular AT were calculated as the difference between the earliest and latest local AT respectively; total RT was calculated similarly for ventricles. Global dispersions of AT for atria and ventricles, and RT and AR_{IC} for ventricles, were taken as their respective standard deviations. Spatial gradients of activation (ATG) for atria and ventricles, and repolarisation (RTG) for ventricles, were measured as the maximum difference between neighbouring sites within 10mm radius divided by their corresponding distance.

2.4. Adipose tissue culture and characterisation

The following sections describe the methods by which human adipose tissue samples were harvested and cultured to generate adipose-conditioned media and how this was characterised. Danya Agha-Jaffar provided training and practical assistance with these techniques.

2.4.1 Background

Adipose tissue can be classified as white, beige, or brown based on its histological appearance. White adipose tissue is found subcutaneously (SAT) and viscerally (VAT) and is so-called because adipocytes in these fat depots contain a single lipid droplet of triglycerides and very few mitochondria (147). These characteristics reflect its role in energy storage. By contrast, brown adipose tissue is found in larger

volumes in neonates and regresses with age. Histologically, it is mitochondria-rich giving it a brown hue and its triglycerides are stored in several smaller vacuoles. Unlike white adipocytes, they contain greater concentrations of uncoupling protein-1 (UCP-1) which uncouples mitochondrial oxidative phosphorylation to cause thermogenesis. For this reason, its primary function is the induction of non-shivering thermogenesis (147, 420). An inducible intermediate phenotype, called beige or brite adipose tissue, has also been described. Uniquely amongst white visceral adipose depots, epicardial adipose tissue (EAT) exhibits brown-like features by expressing UCP-1 and contains a greater mitochondrial density (1).

The role of adipose tissue in the pathophysiology of cardiovascular disease has recently gained considerable traction. Mazurek et al first showed that EAT has a more inflammatory proteomic signature than SAT in patients with coronary artery disease (184). Similar findings were subsequently reported amongst unpaired EAT and SAT biopsies (186) as well as across sternal, abdominal and leg SAT depots (185). Obesity status also exerts an effect on adipose secretomes independent of concurrent cardiovascular disease, with EAT from overweight and obese individuals expressing higher concentrations of IL-1, IL-6, TNF- α and IFN- γ than SAT or serum (193). Not only does EAT appear to have a unique signature secretome to other depots, there are also intra-depot variations between peri-atrial, peri-ventricular and peri-coronary locations (421). EAT has also been associated with electrocardiographic changes (422) as well as risk of arrhythmias (99) and heart failure (423) and response to cardiac resynchronisation therapy (424).

2.4.2. Adipose tissue harvest

Human adipose tissue biopsies were obtained during cardiothoracic surgical procedures courtesy of Professor Prakash Punjabi and Mr Alessandro Viviano. Patients undergoing cardiothoracic surgery were consented by their primary care team to have parasternal subcutaneous adipose tissue (SAT) and EAT around the left ventricle biopsied during their surgery. Paired EAT and SAT samples from consenting patients were transported over ice in transport media made up of Medium 199 (M199, Gibco, UK) containing Earle's salts, L-glutamine, 2.2 g/l sodium bicarbonate, 25 mM HEPES (all Thermo Fisher Scientific, UK) and supplemented with 1% penicillin-streptomycin (Sigma-Aldrich, UK) to the cell culture facility in the laboratory for immediate processing.

2.4.3. Adipose tissue culture protocol

Adipose tissue culture was performed with assistance from Curtis Se Liebers (undergraduate intercalating BSc medical student) and Joseph Coghlin (Master of Research student) at Imperial College London under direct supervision. All tissue culture practices were performed under sterile conditions in a microbial safety cabinet (BioMat2 Class II) and shown in Figure 2.5. Upon arrival in the laboratory, adipose tissue was cultured according to the protocol outlined by Carswell et al(425). First, the adipose tissue was washed using 500ml Dulbecco's phosphate buffered saline (Thermo Fisher Scientific, UK) to remove blood clots and clean the sample over a 70uM cell strainer (Corning, UK). Any remaining clots and connective tissue were removed using sterile forceps. The tissue was then weighed in 100ml diamond weigh boats (Avantor-VWR, UK) using forceps to handle the biopsy. The tissue was

divided into 100mg aliquots using sterile scissors and each of these aliquots placed into a well of six-well plate (Greiner Bio-One, UK) containing 3ml adipose tissue culture media. This ensured that every 30mg of adipose tissue was cultured in approximately 1ml of culture media. The culture media comprised of serum-free M199 containing Earle's salts, L-glutamine, 2.2 g/l sodium bicarbonate and 25 mM HEPES (ThermoFisher Scientific, UK) that was supplemented with Insulin-Transferrin-Sodium Selenium (ITS), 2.5µM dexamethasone, and 1% penicillin-streptomycin (all Sigma-Aldrich, UK). The 100mg samples in each well were minced into finer pieces using scissors before being placed in a New Brunswick Galaxy 170 S 5%CO₂ incubator at 37C for up to 96 hours. Culture media containing no adipose tissue served as control and was kept in the incubator alongside adipose biopsies contemporaneous during the period of culture. Following incubation, the media in which EAT, SAT or no adipose tissue had been cultured was centrifuged at room temperature before being aliquoted into 1.5ml Eppendorf tubes (Eppendorf, UK) and stored at -80°C until use. In this way, EAT-, SAT- and control-conditioned media were generated. The media was thawed only at the time of use. Freeze-thaw cycles were minimised as far as practically possible. Adipose tissue samples were also stored at -80C in separate Eppendorf tubes.

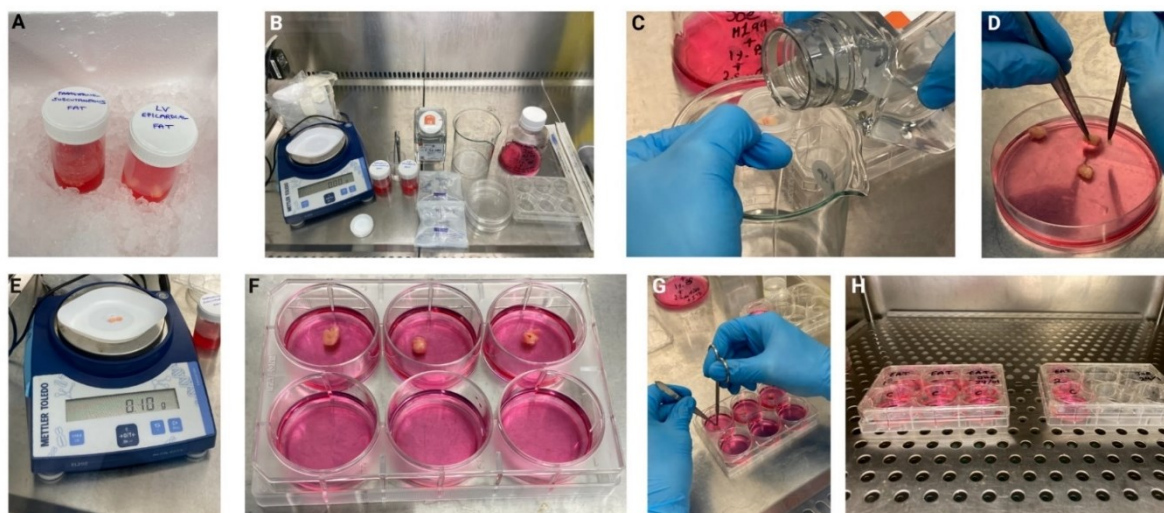


Figure 2.5: Human adipose tissue culture. (A) Paired samples of EAT and SAT were brought on ice to the laboratory tissue culture facility. (B) Apparatus required for processing was prepared in sterile microbial safety cabinet, which included a weighing scale, beakers, culture media, pipettes and culture dishes. (C) Samples were washed with copious volumes of phosphate buffered saline over a strainer. (D) Any debris was removed from samples while keeping them in culture media. (E) Samples were cut to 100mg pieces and weighed. (F) Each piece of EAT and SAT was cultured in 6-well plates and minced with scissors (G), before being placed in a 5%CO₂ incubator at 37C (H).

2.4.4. Adipose tissue viability testing

The CyQUANT™ LDH Cytotoxicity Assay Kit (ThermoFisher Scientific, UK) was used to test adipose tissue viability after incubation by Curtis Se Liebers (undergraduate intercalating BSc medical student) and Joseph Coghlin (Master of Research student) at Imperial College London under direct supervision. This assay provides an indication of cell apoptosis by indirectly quantifying lactate dehydrogenase (LDH) release. LDH is released from apoptotic cells and catalyses

the conversion of lactate to pyruvate coupled while simultaneously reducing NAD⁺ to NADH. Diaphorase enzyme catalyses the reduction of tetrazolium salt (IBT) to formazan, a red dye colour, by simultaneously oxidising NADH back to NAD⁺. In this way, formazan production is directly proportional to the concentration of LDH and the optical density of the sample provides an indication of cell death.

To test sample viability, a small piece of cultured adipose tissue (approximately 10mg) and 100µL of its corresponding conditioned-media were transferred into separate wells in a 96-well flat bottom assay plate (Corning, UK). All wells were first incubated with 10µL of 10x lysis buffer provided in the assay kit for 45 minutes at 37°C 5%CO₂ to cause cell lysis. Thereafter, 50µL reaction mixture, 600 µl assay buffer stock solution (both ThermoFisher Scientific, UK) and 11.4 ml distilled water was added to each well and the plate incubated at room temperature for 30 minutes. After 30 minutes, 50µL stop solution (ThermoFisher Scientific, UK) was added to each well.

For data analysis, the optical density of each well at 680nm wavelength was subtracted from that at 490nm wavelength using the Paradigm detection platform (Beckman Coulter, UK) and SoftMax Pro 7.0.2 (Molecular Devices, USA). The optical densities for adipose tissue and adipose-conditioned media were normalised to optical densities for control media. Normalised optical densities for adipose-conditioned media were divided by the normalised optical densities for lysed tissue. In this way, LDH release in adipose-conditioned media was expressed as a fraction

of maximal LDH release from adipose tissue to quantify the viability of adipose samples from which conditioned media was generated.

2.4.5. Characterisation of adipose tissue secretome

Adipose tissue is a source of numerous cytokines, chemokines and other inflammatory proteins. Fat depots have been shown to have unique secretome signatures that may have important implications for arrhythmogenesis (188, 221).

2.4.5.1. Proteome Profiler Human Adipokine Array

Background

The Proteome Profiler Human Adipokine Array (R&D Systems, USA) is a relatively inexpensive and quick method by which the proteomic profile adipose tissue can be characterised. It is a semi-quantitative assay, in that it does not provide concentrations of proteins contained in the biological sample; instead, it provides an indication of their relative abundance. Thus, the relative expression of 58 human adipokine can be determined in one experiment without the need for numerous Western Blots or enzyme-linked immunoassays which can be time-consuming and expensive. Another advantage of using this assay is that it provides an indication of the range of adipokines present in the biological sample which can guide subsequent tests, like ELISA to quantify their respective concentrations more accurately. This assay has been previously to characterise depot-specific adipokine profiles (426).

Principles

Antibodies to 58 adipokines are contained within spots in duplicate on a nitrocellulose membrane (Figure 2.6). The biological sample of interest, i.e. adipose-conditioned media, is mixed with biotinylated detection antibodies and incubated overnight with the membrane. The membrane is then washed to remove unbound antigen. Streptavidin-HRP and chemiluminescent detection reagents are applied to the membrane and the intensity of the signal over each of the spots, measured in arbitrary units, corresponds to the amount of antigen bound. The signals over the spots are normalised to control spots on the membrane which contain no antibodies. In this way, the assay provides information on the range and relative expressions of adipokines in the biological sample.

Protocol

Each assay comes with 4 nitrocellulose membranes containing spots in duplicate to 58 capture antibodies to specific proteins. These were handled with gloves and flat-tipped tweezers to avoid contamination and spurious results. Wells in which the membranes were treated, washed and incubated with antibodies were provided with the assay. Detection Antibody Cocktail containing lyophilised biotinylated antibodies was reconstituted in 200uL of distilled water prior to use. 40uL of wash buffer was added to 960ml of distilled water for the washing steps. The assay also contained Array Buffer 4, Array Buffer 6 (block buffer) and Streptavidin-HRP. The Chemi Reagent mix comprised of Chemi Reagents 1 and 2 mixed in equal volumes and was prepared within 15 minutes of use and protected from light by storing in drawer. All reagents were brought to room temperature prior to use. Adipose-conditioned media being tested was kept on ice until use.



Figure 2.6: Proteome Profiler Human Adipokine Array assay. The top panel shows the template of the spots on the nitrocellulose membrane in duplicate to identify 58 adipokines. A1-2, F1-2, A23-24 and F23-24 serve as reference spots. The bottom panel shows an example of assay following testing with a sample.

This protocol was performed with assistance from Curtis Se Liebers and Joseph Coghlin under my direct supervision. First, 2ml of Array Buffer 6 was pipetted into a well and the nitrocellulose membrane being used was placed inside with the reference number facing upward. The membrane was incubated for 1 hour on rocking platform and positioned so that the membrane was rocking end-to-end. During this time the sample being tested was prepared: 500uL of adipose-conditioned media was added to 500uL of Array Buffer 4, and this volume was made up to 1.5ml with 500uL of Array Buffer 6. 30uL of the reconstituted Human Adipokine

Detection Antibody Cocktail was then added to the sample. The sample was mixed and incubated for 1 hour at room temperature. After 1 hour, the Array Buffer 6 was aspirated from the well and the biological sample-antibodies preparation was added to the well containing the membrane. The membrane was incubated with this preparation in a cold room (2-8C) on a rocking platform overnight. The next day, the membrane was removed from the incubation mix and placed into a plastic container with 20ml of reconstituted wash buffer. The membrane was washed a total of three times, each time replacing with fresh wash buffer and for 10 minutes, on a rocking platform. In between washes, Streptavidin-HRP was diluted in Array Buffer 6. After the third wash, any excess wash buffer was removed by blotting the edges of the membrane against absorbent paper. 2ml of diluted Streptavidin-HRP was pipetted into a plastic well, the membrane placed in it and covered with a lid to be incubated for 30 minutes on a rocking platform at room temperature. After 30 minutes incubation, the membrane was washed again a total of three times with wash buffer on a rocking platform each time for 10 minutes. After draining excess wash buffer after the third wash, the membrane was immediately placed on a (bottom) plastic sheet protector with the numbered edge facing up. 1ml of the Chemi Reagent Mix was pipetted over the membrane ensuring that its spread over the entire membrane. The membrane was covered with another (top) plastic sheet protector and any air spaces smoothed out to ensure that the Chemi Reagent Mix was evenly distributed over the membrane. The Chemi Reagent Mix was left for 1 minute over the membrane, after which the top plastic sheet protector was peeled away and the membrane was gently blotted with absorbent paper to remove any excess Reagent. The top plastic sheet was used to cover the membrane again taking care to avoid air-trapping. The membrane was immediately transferred to a ChemiDoc MP

Imaging System (BioRad, Germany) and read using chemiluminescence at a 60-second exposure. This revealed spots over the membrane where antigens had bound to the antibodies (Figure 2.7). The images were saved and analysed offline.

Analysis

Images of the membrane were analysed using FIJI open-source software. The image was inverted, and background subtracted using a rolling ball radius of 50 pixels to eliminate any background signal. Circles were drawn manually over each adipokine represented in the membrane (Figure 2.7). The mean pixel density within each circle was calculated in arbitrary units, to give an indication of adipokine concentration within the sample. The average signal of the pair of duplicate spots for each adipokine was calculated and used for analyses comparing EAT- and SAT-conditioned media.

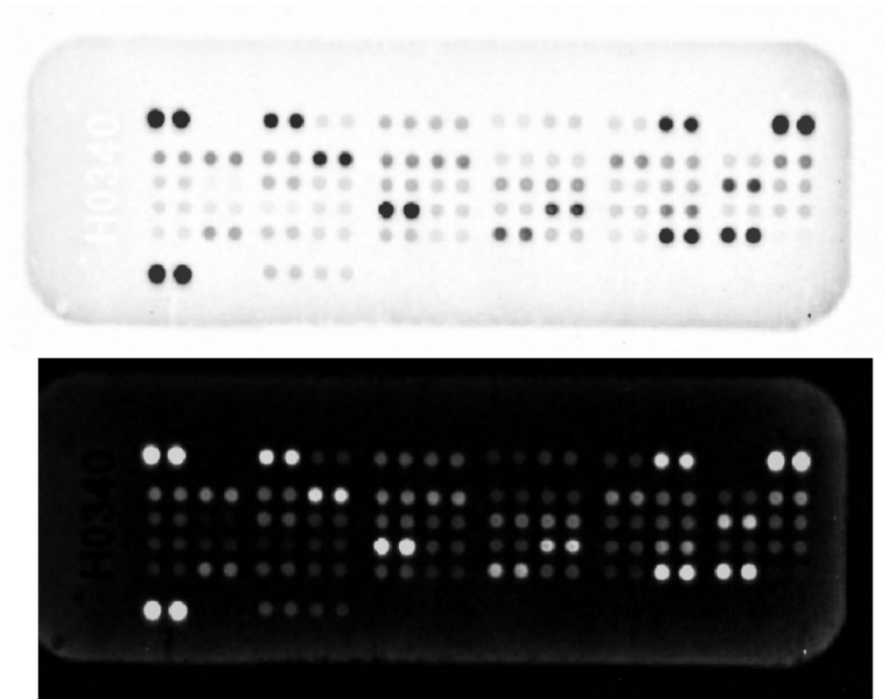


Figure 2.7. Nitrocellulose membrane from the Proteome Profiler Human Adipokine Array. Top image: membrane after chemiluminescence. Bottom image: after inversion and subtraction of the background for offline semi-quantitative analysis.

2.4.5.2. Enzyme-linked immunosorbent assays

After identifying the range of adipokines within adipose-conditioned media, enzyme-linked immunosorbent assays (ELISA) were performed for specific cytokines of interest to more accurately quantify their respective concentrations. The following sections outline the principles and methods for ELISA.

Principles

An ELISA is an immunological assay to quantify antigens, i.e. proteins, of interest in biological samples. They are typically performed in 96-well plates for multiple

samples to be measured in a single experiment. Unlike the human adipokine assay described above, each ELISA is specific to a single protein. One of the most common ELISA techniques is often referred to a “sandwich ELISA” in which two sets of antibodies (primary and secondary) are used to detect antigen and comprises a stepwise process. First, each of the wells in the ELISA plate is coated with a primary, or capture, antibody that is raised specific to the antigen of interest. The wells are then washed using a wash buffer to remove unbound antibody. In some assays the wells may come pre-coated with the primary antibody. Next, the biological sample, for example serum, media or supernatant, is added to each of the wells in which the primary antibody has bound. The antigen of interest in the biological sample will bind to the primary antibody in the plate. To allow sufficient time for binding, the sample is left in the plate according to the manufacturer’s instructions and this may be for several hours. The duration of this incubation period also varies from assay to assay and between antigens of interest. Furthermore, the incubation may require a rocking platform to ensure optimal binding. Biological samples are usually tested in duplicate or triplicate in the plate for statistical analyses and as a contingency precaution in case one well cannot be analysed. Following the prescribed incubation period with the primary antibody, the wells are washed to ensure removal of any unbound antigen and biological sample. The third step involves incubation with a secondary antibody, which binds to the antigen:primary antibody complex. The secondary antibody is labelled with an enzyme, hence the term “enzyme-linked” in ELISA, and is commonly horseradish peroxidase. Again, the secondary antibody is incubated for a duration prescribed by the manufacturer’s instructions. The fourth step involves adding a substrate to the wells which changes colour when it binds to the secondary antibody that has itself bound to the antigen:primary antibody complex. For this

reason, ELISA assays are referred to as chromogenic. During incubation with the substrate, plates are often stored in the dark to avoid interference from ambient lighting that may otherwise alter the enzymatic reaction. Following incubation with substrate, a stop solution may be added to cease further enzymatic reactions. The resultant change in colour intensity is measured on a plate reader as a quantifiable metric of the antigen concentration in the sample. Standard samples, in which the concentrations of the antigen are known, are often incubated simultaneously in the same plate to enable standard curves to be generated. The optical density of the biological samples in the plates are then interpolated on the standard curve to determine the concentrations of the antigen of interest. These principles form the basis of the ELISA assays described in the next section.

Materials and reagents

The Quantikine Human ELISA immunoassays for TNF- α , IL-1 β , IL-6 and Activin A (R&D Systems, Abbingdon, UK) were performed using the manufacturer's instructions. These assays are designed to quantify cytokine concentrations in cell culture supernatants, such as those generated from adipose culture, and also for serum and plasma samples.

All reagents required for the assay were provided by the manufacturer. For TNF- α , this included a 96-well polystyrene microplate in 12x8 configuration coated with TNF- α monoclonal (primary) antibody; a 25x wash buffer concentrate, of which 20ml was diluted in 480ml of distilled water and used in between incubation steps to rinse the plate; 10,000pg/ml recombinant TNF- α standard in a buffered protein base in powder

form, that was serially diluted to create standard concentrations from which the TNF- α concentration of adipose-conditioned media could be determined; Assay Diluent RD1F, a buffered protein base that was added to each well in the microplate; Human TNF- α Conjugate, a TNF- α specific polyclonal (secondary) antibody conjugated to horseradish peroxidase; Substrate Solution that was made by mixing colour reagents A (hydrogen peroxide) and B (tetramethylbenzidine) in equal volumes within 15 minutes of use and kept away from light; and Stop Solution comprising sulphuric acid.

For IL-6, the assay included a microplate coated with a monoclonal (primary) antibody specific for human IL-6, recombinant human IL-6 standard, polyclonal IL-6-specific (secondary) antibody conjugated to horseradish peroxidase, Assay Diluent RD1W and Calibrator Diluent RD5T were used for ELISA. For Activin A, the assay included a microplate coated with Streptavidin, a monoclonal (primary) antibody specific for Activin A conjugated to biotin (primary antibody), recombinant human Activin A standard, a monoclonal (secondary) antibody specific for Activin A conjugated to horseradish peroxidase, Assay Diluent RD1-98 and Calibrator Diluent RD5-54 were used for ELISA.

Protocol

ELISA was performed with assistance from Joseph Coghlin under my direct supervision. The following section describes the ELISA protocol for TNF- α . The ELISA protocols for IL-6 and Activin A followed the same principles outlined below,

but with variations in reagents' volumes and concentrations, that are detailed later in this section.

First, standards with known TNF- α concentrations were made to generate a standard curve, from which the concentrations of TNF- α from the adipose-conditioned media could be determined. This involved reconstituting 10,000pg TNF- α standard provided in powder form in 1ml of distilled water to produce a stock solution of 10,000pg/ml. The standard was gently agitated on a vortex spinner and then allowed to sit at room temperature for 15 minutes. Further TNF- α standard concentrations were made from the 10,000pg/ml stock solution using serial dilutions. For this, 450uL of Calibrator Diluent RD6-12 was pipetted into an Eppendorff tube into which 50uL of 10,000pg/ml stock solution was added. This generated a 500uL TNF- α standard at a concentration of 1000pg/ml and was used as the highest standard in the standard curve. Thereafter, 200ul of 1000pg/ml standard solution was pipetted into another Eppendorff tube containing 200uL Calibrator Diluent RD6-12. In this way, 200uL of the preceding TNF- α standard was add to 200uL Calibrator Diluent RD6-12 in a stepwise manner resulting in six serial dilutions yielding 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5mg/ml, 31.3pg/ml and 15.6pg/ml standards. Calibrator Diluent RD6-12 alone served as the zero (0pg/ml) standard.

For the assay procedure itself, excess microplate strips were removed from the microplate. Next, 50ul of Assay Diluent RD1F was added to each of the remaining wells. The microplate wells were precoated with a E-coli derived recombinant monoclonal (primary) antibody for TNF- α . 50ul of each of the standards, ranging from 0pg/ml to 1000pg/ml created in the serial dilutions method described above,

were added in duplicates. 50uL of adipose-conditioned media were added in the remaining wells in duplicate. Thus, in total, each well contained 100uL and enabled the TNF- α present in the standards or samples to bind to the TNF- α monoclonal antibody precoated in the microplate. The microplate was covered with an adhesive strip, and incubated on a rocking platform at room temperature for 2 hours.

After 2 hours, each well was aspirated and washed 4 times with 400uL of wash buffer using a multichannel pipette. Care was taken to ensure that all liquid phase material was removed in each wash for optimal results. Next, 200uL of Human TNF- α Conjugate, a polyclonal (secondary) antibody specific to TNF- α conjugated to horseradish peroxidase, was added to each well. The microplate was covered with another adhesive strip and incubated for 2 hours at room temperature on a rocking platform. This allowed time for the polyclonal (secondary) antibody to bind to any TNF- α -primary antibody complexes in the microplate. After 2 hours, the microplate was washed again four times as described above, and 200ul of the Substrate Solution was added to each well and incubated for 30 minutes at room temperature in the dark. This enabled hydrogen peroxide in the Substrate Solution to react with the horse peroxidase conjugated with the TNF- α polyclonal antibody resulting a colour change that was proportional to TNF- α concentration in the standard or sample being tested. Stop Solution was added after 30 minutes to inhibit the chemical reaction, resulting in a colour change from blue to yellow. The optical density of each well was determined on a microplate reader at 450nm and 540nm wavelengths. The difference between the readouts at each of these wavelengths was the absorbance values that were used to generate the standard curve for the

standards. The concentrations of TNF- α in each sample was determined by interpolating within the standard curve.

In contrast to the protocol outlined above for TNF- α , for IL-6 the standards made by serial dilution from a stock of 300pg/ml were 100pg/ml, 50pg/ml, 25pg/ml, 12.5pg/ml, 6.25pg/ml and 3.13pg/ml. When testing the viability of adipose tissue, SoftMax Pro 7.0.2 provided a value for each well of the 96-well plate that was equal to the optical density at 680 nm subtracted from that at 490 nm. Data was exported to Microsoft Excel 2016 (Microsoft, USA), where values for wells containing EAT and SAT lysed tissue and wells containing EAT- and SAT-conditioned media were normalised to values for wells containing EAT and SAT control media. Values for normalised EAT- and SAT-conditioned media were divided by values for normalised EAT and SAT lysed tissue to generate values for % maximal LDH release, which gave an indication of the extent of cytotoxicity in the adipose tissue samples. In addition, rather than 50ul, 100ul of Assay Diluent RD1W and standards or samples were added to each well. Incubations with the primary and secondary antibodies were for 2 hours each at room temperature without the use of the rocking platform. Incubation with the Substrate Solution was for 20 minutes at room temperature in the dark.

For Activin A, the standards were made using serial dilutions from a stock of 10,000pg/ml to create 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml. The streptavidin microplate was first precoated with Activin A biotinylated (primary) antibody. Thereafter 100ul of Assay Diluent RD1-98 and standards or samples were added to each well. Incubations with the primary and secondary antibodies were for 3 hours and 1 hour, respectively, at room temperature

on a rocking platform. The Substrate Solution was added for 30 minutes at room temperature in the dark.

Analysis

The duplicate readings for each standard, control and sample were averaged. The averages for the standards were normalised to the zero standard, and the samples were normalised to control, i.e. fat-free media. To determine the concentrations of the cytokines in each sample, the concentrations corresponding to the absorbance values of each standard was first transformed into a logarithmic scale to generate a standard curve. The absorbance of the sample was then determined by interpolation on the logarithmic standard curve and its concentration calculated by inverting its logarithmic value.

2.5. Neonatal rat ventricular myocyte cell culture

This section describes methods by which neonatal rat ventricular myocytes (NRVM) were isolated and cultured, and a description of experimental techniques used to study the effects of adipose-conditioned media on NRVM electrophysiology.

2.5.1. Background

NRVM are an established model in which to study cell electrophysiology(427, 428, 429). Methods to isolate cardiomyocytes were first described in the 1960s by Harary and Farley (430, 431) and these have since been described by others (429, 432, 433). Neonatal rat ventricular cells lend themselves to studying electrophysiology in

vitro for a number of reasons. They are easier and cheaper to isolate and retain their inherent contractile responses for longer than their adult counterparts. Rat cardiomyocyte isolation provides a higher yield of cells that are less prone to over-digestion during the isolation process than seen in mice (429). They can also be more easily cared for and are relatively inexpensive to maintain compared to larger mammals. Rat ventricular myocytes have been used to study the effects of adipose-derived stromal cells (189, 434).

2.5.2. Neonatal rat ventricular myocyte isolation

NRVM were isolated from Sprague-Dawley rats (0 to 3 days old) as previously described(435) All procedures complied to EU Directive 2010/63/EU and were approved by the Imperial College London Ethical Committee. NRVM isolations were performed by Dr Ivan Diakonov and Mrs Danya Agha-Jaffar using a neonatal heart dissociation kit (Miltenyi Biotec GmbH, Germany) according to manufacturer's instructions. In this process, neonatal rat hearts are dissociated into single cell suspensions using mechanical dissociation and enzymatic degradation of the extracellular matrix to yield cardiomyocytes and other cell types, such as fibroblasts.

Neonatal rats were euthanised in accordance with Home Office Schedule 1. This involves anaesthetising neonatal rats with isoflurane followed by cervical dislocation by pressing the neck with a tongue depressor. An audible click was suggestive of cervical dislocation and death confirmed by cessation of circulation by decapitation with scissors. Hearts were excised in under sterile conditions in a microbial safety cabinet. The rats' legs were pinned to a dissection tray. The dissection apparatus was dipped in Hanks Buffered Saline Solution (HBSS) prior to use. The chest was

opened using scissors and scalpel. The heart was excised and placed in a culture dish with HBSS. The atria were dissected, and ventricles transferred to another sterile culture dish. The ventricles were minced into small (approximately 1mm³) cubes and then transferred to Miltenyi tubes. Enzyme mix 1, comprising Enzyme P and Buffer X, were mixed in a 15ml Falcon (UK) tube and incubated at 37C 1%CO₂ for 15 minutes. Thereafter, Enzymes A, D and Y were added to the Enzyme mix 1. This combined enzyme mixture was added to the minced tissue and all components were then transferred into gentleMACS C tube provided in the isolation kit. The tube was inverted and kept in this position during incubation at 37C 1%CO₂ for 18 minutes before being placed in a gentleMACS dissociator and programmed to run the in-built dissociation programme; this step was performed thrice in total. Following termination of the programme on the third occasion, the tube was detached and 7.5ml of M199 media supplemented with 10% neonatal calf serum was added to resuspend the cells. The cell suspension was applied to a 70uM pre-separation filter over a 50ml tube (Falcon, UK). The pre-separation filter was then washed with 3ml the 10% M199 media. The cell suspension was centrifuged at 1000g for 5 minutes and supernatant removed. The cell pellet was resuspended in 9ml 10% M199 per 5 hearts. The cells were counted under light microscopy using a cell counter before being placed in a T75 flasks and preplated in an incubator at 37C 1% CO₂ for 1 hour. During this step, fibroblasts within the cell suspension attached to the flask whereas the NRVM remain in suspension. After 1 hour, the media containing NRVM were extracted into a 50ml tube (Falcon, UK) and centrifuged at 1000g for 5 minutes. The NRVM pellet was resuspended in 10% M199 media (9ml for every 5 neonatal rat hearts) and stored at room temperature on a rotator to maintain suspension until culture.

2.5.3. Neonatal rat ventricular myocyte culture

Training for NRVM culture was provided by Dr Rasheda Chowdhury. NRVM were cultured on human placental type IV collagen (Sigma-Aldrich, UK) in multielectrode arrays (MEA; Multi Systems Channels, Reutlingen, Germany) to study their electrophysiology and Ibidi plates (Ibidi GmbH, Germany) for immunohistochemistry. 5mg collagen was placed in a sterilised jar covered with aluminium foil to limit exposure to light. 2ml of HBSS was added to the collagen, followed by 3ml of glacial acetic acid (Merck, UK). Thereafter 20ml of Hanks' Balanced Salt Solution (HBSS, Thermo Fisher Scientific, UK) was added to make the total volume 25ml. The collagen was stirred for at least one hour to ensure the collagen had fully dissolved before being stored at 4C. The collagen solution was stirred for 1 hour at room temperature prior to use to ensure that the solution was well mixed. Fresh collagen solution was made every 4 weeks to ensure optimal cell culture conditions.

To culture NRVM on MEA, MEA that had been pre-treated with PBS to ensure hydrophilicity of the recording surface were rinsed with distilled water and placed under ultraviolet light for 1 hour prior to use in a microbial safety cabinet. This ensured sterility as well as a dry surface on to which collagen could be applied. 30uL of collagen solution was applied under visual inspection over the electrode matrix and allowed to rest for 5 minutes in a microbial safety cabinet. This allowed the collagen to adhere to the recording surface. Excess collagen was siphoned using a pipette taking care to avoid contact with the MEA surface. The MEA culture chamber was rinsed with 1ml distilled water thrice thereafter to remove any remaining excess collagen. This also served to minimise the volume of acetic acid contained within the collagen solution in the MEA culture chamber that would otherwise impair cell

culture. Following the third rinse with distilled water, the MEA were placed under ultraviolet light for 1 hour for sterilisation prior to NRVM culture.

For NRVM culture, isolated NRVM in suspension were centrifuged (Boeco C-28A centrifuge) at 1000g for 5 minutes. The resulting cell pellet was resuspended in M199 culture media; every 100ml M199 culture media contained Hanks' salts and sodium bicarbonate, 1 ml L-glutamine, 500 µl penicillin-streptomycin, 37.5 µl vitamin B12, 10ml neonatal calf serum. Cells were resuspended to yield a concentration of 200,000 cells per 30uL media. Thereafter, 30ul of the resuspended NRVM solution was pipetted on the collagen over the electrode matrix in the MEA. The NRVM were allowed to rest to facilitate cell adherence for 30 minutes in a cell culture incubator at 37C 1%CO₂. Thereafter, the 30ul drop of NRVM over the recording surface was supplemented with an additional 1ml M199 culture media to ensure that the cells remained submerged in solution during incubation at 37C 1%CO₂. 24 hours post-incubation, the culture media was replaced with M199 culture media that contained 5%, rather than 10%, neonatal calf serum (5% M199). The MEA were manually agitated in orthogonal planes on a flat surface to dislodge unbound or unviable cells. The 5% M199 was then siphoned and replaced with 1ml of fresh 5% M199 and MEA placed into the incubator at 37C 1%CO₂ for another 24 hours. In this way, NRVM were cultured for a total of 48 hours prior to recording baseline conduction velocities using the Multi-Channels Systems operating system.

2.6. Multi-Channel Systems operating system

The following sections outline the apparatus and methods used to record electrical activity in NRVM following culture with EAT- and SAT-conditioned media.

2.6.1. Background

Experimental hardware pertaining to recording electrical activity in NRVM using multielectrode arrays (MEA) and its operating software systems were acquired from Multi-Channel Systems MCS GmbH (Reutlingen, Germany). This system has the benefits of enabling short- and long-term experiments to study electrical propagation patterns in cardiomyocytes in different conditions. Cardiomyocytes are cultured in a MEA (Figure 2.8) on top of a matrix of electrodes and can visually under light microscope. These form an electrical syncytium within 48 hours of culture enabling recording of spontaneous electrical activity or the same in response to pacing. It represents a high throughput in vitro system that can record from multiple electrodes simultaneously in real-time and is therefore ideal to study cell electrophysiology under experimental conditions.

2.6.2. Multielectrode arrays

Transparent (glass-base) MEAs that comprise 60 gold electrodes aligned in 8x8 grid, with no corner electrodes, and interelectrode distance of 700 μ m were used to culture NRVM. Each electrode has a diameter of 100 μ m. The recording surface measures 49mmx49mm. The electrodes are numbered so that the first number represents the column and the second number the row. This standard numbering system is also used in the Multi-Channel Systems data acquisition recording software (MC_Rack).

The MEA feature a plastic ring (internal diameter 26mm, external diameter 30mm, height 6mm) that encircles the electrode matrix to create a culture chamber with approximately 3ml capacity (Figure 2.8).

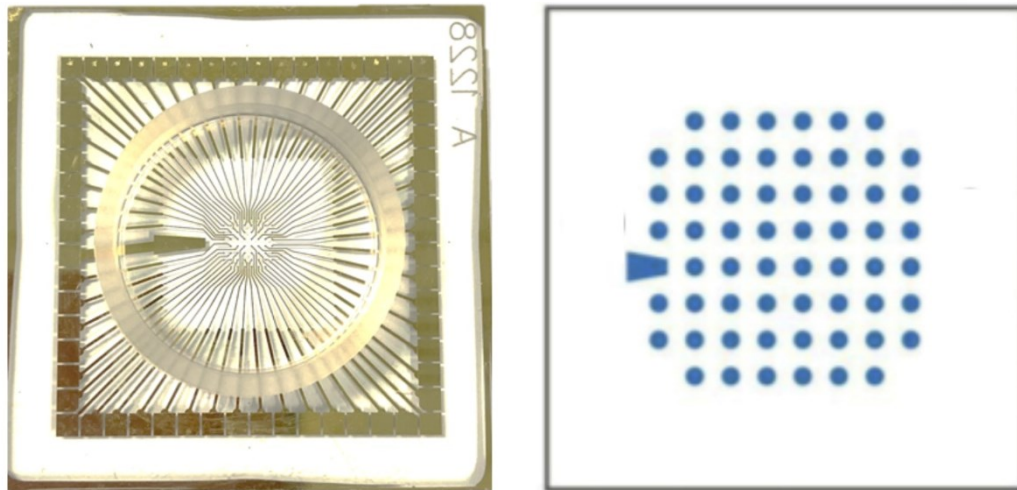


Figure 2.8: Multielectrode array. The multielectrode array (MEA) contains an 8x8 electrode matrix at its centre surrounding by a plastic ring creating a well with a capacity of approximately 3ml (left). The electrodes in the corners of the matrix are absent, resulting in 60 recording electrodes (right).

NRVMs cultured on MEA were tested using a USB-ME64-System shown in Figure 2.9. This comprises an integrated 60-channel MEA amplifier and data acquisition system. The amplifier was placed on a stable and dry workbench surface and connected to a ground cable and the mains power supply. The MEA was placed into the amplifier and lid secured over it. Gold contact pins on the underside of the amplifier lid made contact with the contact pads on the MEA. These pins were kept clean with ethanol to improve signal:noise ratio. The amplifier has an integrated heating system comprising a heating element with a PT100 temperature sensor that

is connected to an external temperature controller (TC01/02) via a D-Sub9 connector. This ensured cells were maintained at a steady-state temperature at 37°C throughout the testing period. A stimulus generator (STG4002) connected to the mains power supply was connected to the amplifier and delivered biphasic rectangular single pulses to the MEA controlled by data acquisition software (MC_Stimulus II) configured for the USB-ME64-System that was downloaded onto a desktop computer. A USB connector connected the computer to the stimulus generator. The amplifier was connected to a separate FA60SBC filter amplifier which further filtered and amplified the raw electrical (analogue) data received from the cells cultured in the MEA. It was connected to an analogue/digital converter that was connected to a PS40W power supply unit and which digitised the raw electrical data in real-time. A USB connector transferred digitised data to the desktop computer via USB High Speed 2.0 cable. Electrograms in response to pacing were observed and recorded in real-time on MC_Rack software on a desktop computer. All components of the operating system, with the exception of the MEA amplifier and computer, were covered with aluminium foil to minimise noise during recording. MC_Stimulus II was used to determine the electrical stimulus protocols and MC_Rack software for data acquisition and display. Software settings were configured to the USB-ME64-System. The sampling rate was fixed at 25kHz per channel. The gain of the filter amplifier was in-built into the hardware and was not adjusted.



- | | |
|-----------------------------------|-------------------------------|
| 1. FA60SBC filter amplifier | 5. MEA Amplifier |
| 2. Analogue to digital converter | 6. STG4002 stimulus generator |
| 3. PS40W power supply | 7. Desktop computer |
| 4. TC01/02 temperature controller | |

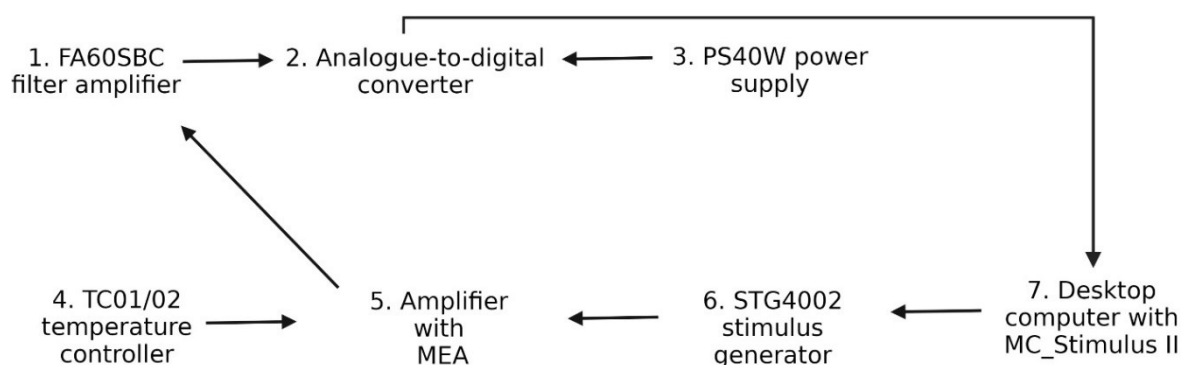


Figure 2.9: Multi-Channels Systems apparatus. The top panel shows the apparatus as labelled in situ under experimental conditions. The bottom panel shows the connections of the constituent parts.

2.6.3. Electrical stimulation protocols

NRVM electrophysiology in response to adipose-conditioned media was tested with the aid of Taesoon Hwang (undergraduate intercalating BSc medical student) under my direct supervision. MC_Stimulus II software (Multi Systems Channels, Germany) was used to determine site of pacing. The stimulation strength and times were

adapted for each recording, although typically cells were paced using a biphasic impulse ($\pm 1000\text{mV}$) for 2ms at 1Hz with a sampling frequency of 25kHz. This resulted in a 998ms window in which to electrically capture and record the cells' electrical activity. An integrated blanking circuit was utilised to disconnect the electrodes from the amplifiers during stimulation to avoid stimulation artefacts. 10-second recordings were saved upon electrical capture of electrograms.

In the event of non-capture or failure to record electrograms with these settings, the software was programmed to pace the cells from a different set of electrodes. If this was unsuccessful, the frequency of pacing was changed in increments of 1Hz up to 4Hz. If electrical capture remained elusive or no electrograms were recorded, voltage was increased up to $\pm 1200\text{mV}$. In this way, any unsuccessful attempts to obtain electrical capture were addressed by changing the direction of pacing, frequency of stimulation and/or voltage, in that order. If these attempts remained unsuccessful and no electrical data was recordable or captured, the MEA was disregarded for the set of experiments.

Cells that recorded a conduction velocity of at least 20cm/s at baseline (435), i.e. prior to treatment with adipose-conditioned media, were used experimentally. For this, the 5% M199 media was removed and quickly replaced with either EAT-, SAT- and control-conditioned media to avoid cells drying in a biosafety cabinet. The conduction velocities of cells in response to EAT-, SAT- and control-conditioned media were tested at 2, 4 and 24 hours into treatment as described above. Cells were kept in the conditioned media at 1%CO₂ 37C incubator between recordings.

Following the final recording at 24 hours into treatment, the conditioned media within the MEA was discarded and replaced with 1ml 1% TergAZyme (Sigma Aldrich, UK), a powdered anionic detergent with protease enzyme, to remove the cells and collagen and placed on a rocking platform overnight. The next day the MEA were rinsed under a copious volume of running water and then stored in distilled water. The water was replaced with PBS and MEA placed in an incubator at 37C for at least 48 hours before next experimental use to ensure that the cell culture surface was hydrophilic.

2.6.4. Cell electrophysiology analysis

Recorded electrical data from cells was saved to an external hard drive and analysed offline using customised code using MatLab (Mathworks, USA) provided by Dr Rasheda Chowdhury. The conduction velocity of NRVM treated with EAT-, SAT- and control media were calculated. Conduction velocity can be calculated by the distance travelled by a wavefront in a unit of time. Given that the recording area of cells on MEA is small, the distance between two electrodes and the time delay between them can be used to determine the speed of activation. The velocity was calculated using a finite differences method as summarised by Cantwell et al (436). The steepest downslope of the unipolar electrogram recorded at each electrode was taken as the local activation time. The finite difference technique uses location activation times to compute gradients of activation and thereby conduction velocity at each electrode. This information was used to determine the average conduction velocity across the MEA.

2.7. Statistical analysis

Shapiro-Wilk test was used to test for normality. Normally distributed continuous variables are presented as arithmetic mean $\pm$ standard deviation (SD). Non-normally distributed data is presented as median and interquartile range (IQR). Normally distributed pairwise comparisons were made using two-tailed paired Student's T-test. Non-normally distributed pairwise comparisons were made with Wilcoxon-signed rank test. Normally distributed unpaired data were compared using unpaired Student's T-test. Non-normally distributed unpaired data were compared using Mann-Whitney U test. Chi-squared test was used to compare categorical variables. Analysis of variance (ANOVA) was used for analyses containing multiple comparisons with Tukey's post-hoc correction if appropriate. $P < 0.05$ was considered significant. Non-significant results are represented by 'ns'. All statistical analyses were performed using Prism 9.3.1 (GraphPad Software, La Jolla, California, USA).

**Chapter 3: Effects of increasing
adiposity on QTc interval and
ventricular arrhythmias amongst the
UK Biobank cohort**

This chapter, including figures and tables, have been published as an original research article in Frontiers Cardiovascular Medicine (Cardiac Rhythmology) (<https://doi.org/10.3389/fcvm.2022.939156>, PMID: 35845082) by Patel et al (437), and sections included herein with permission under Creative Commons Attribution Licence (CC-BY) 4.0.

3.1. Introduction

This chapter includes results from analyses using data from the UK Biobank on the effect of increasing adiposity on ventricular repolarisation and prevalence of ventricular arrhythmias. To contextualise these results, the introduction includes detail on current evidence linking obesity to higher risks of abnormal ventricular electrophysiology. Given that the data used in this Chapter was retrieved from the UK Biobank, the methods section also details information on how this was collected and recorded.

3.1.1. Obesity and arrhythmic risk

The worldwide rate of obesity has tripled within a generation (438). Over 25% of the British population is obese, defined as body mass index (BMI) $>30\text{kg/m}^2$, and in 2019, obesity accounted for over £6billion of the NHS budget (79, 331). Increasing BMI confers higher risks of atrial arrhythmias (100). For instance, AF risk increases by up to 3.5% with every unit increment in BMI (88). The number of patients that this encompasses is forecast to increase over the coming years, not only due to an aging population, but also because of the obesity pandemic and the adverse lifestyle factors driving it (9, 73). Although there is compelling evidence that the higher risks

of atrial arrhythmias in obesity is at least partly explained by increasing volumes of adipose tissue, particularly epicardial fat (99, 188, 220, 439, 440), the evidence for any associations between obesity and ventricular arrhythmogenesis is less robust. Although this may be partly explained by a lower incidence and prevalence of compared to atrial arrhythmias, population-based studies show that idiopathic ventricular arrhythmias are becoming more common (43). As with atrial arrhythmias, even though an aging population is likely to contribute to this rising incidence (43), it is also plausible that obesity is a contributor factor.

3.1.2. ECG indices of ventricular repolarisation

Abnormal ventricular repolarisation on the 12-lead ECG is most easily determined by the QTc interval and its dispersion (105). The QT interval is measured from the start of the QRS complex to the end of the T-wave and encompasses ventricular activation and repolarisation. It is corrected for heart rate (QTc) given that the action potential duration shortens as heart rate increases to facilitate adequate diastolic relaxation and coronary perfusion even in tachycardic states. QTc intervals ≥ 450 msec in males and ≥ 470 msec in females are considered abnormal and may signify abnormal ventricular conduction, repolarisation or both. QTc dispersion, is calculated as the difference between the longest and shortest QTc interval on a 12-lead ECG and has been used as a non-invasive metric to quantify dispersion of ventricular repolarisation, i.e. the spatial heterogeneity of recovery. QTc prolongation or increased dispersion of ventricular repolarisation may can cause early after-depolarisations (EADs) that enable re-entry via the generation of premature ventricular complexes (PVCs). In this regard, the myocardium is particularly vulnerable to arrhythmias in the context of an extrasystole that encounters a

myocardial region with prolonged refractoriness, which could initiate and sustain ventricular tachycardia/fibrillation (VT/VF). For this reason, QTc interval prolongation has been associated with a higher risk of sudden cardiac death (SCD) (441, 442, 443).

3.1.3. Obesity and ventricular electrophysiology

Small-scale studies have shown that obesity prolongs QTc interval and increases its dispersion, and that both reverse with weight reduction. Amongst weight loss strategies, bariatric surgery is more effective at reversing electrocardiographic repolarisation abnormalities than lifestyle or dietary changes. These results have been reported in a comprehensive review and meta-analysis by Omran et al (105, 106), and corroborated by others using different measures of repolarisation, such as T peak-end (110). In the latest meta-analysis on this topic, Sanches et al also show that bariatric surgery results in significant decreases in QTc interval and P-wave dispersion (111). Although compelling in demonstrating the link between obesity and abnormal ventricular repolarisation and reversal with weight loss, current understanding remains limited in two respects. First, it is unclear if and to what extent QTc prolongs as a result of increasing whole-body mass, total body fat or central (i.e. visceral) adiposity, or all three. This is clinically important because although BMI is the most common metric to categorise obesity it is insensitive to adipose volume, particularly in athletic and muscular individuals. Indeed, BMI has been shown to significantly misrepresent visceral adipose volume between normal weight and obese individuals. In one report, a 67 year old male with BMI 25kg/m² had visceral fat volume 2.58L/m² yet a 53 year old male with BMI 30kg/m² recorded only 0.88L/m² on MRI (444). On the other hand, although cross-sectional imaging,

dual-energy x-ray absorptiometry (DEXA) and body impedance can quantify total body fat more accurately, these are not routinely used in healthcare settings for anthropometric assessments and thus this data is often unavailable in patients. Similarly, although central obesity is associated with more adverse healthcare outcomes compared to peripheral adiposity (445), its effects on ventricular repolarisation is unclear. Second, it is unknown if the QTc-prolonging effects of increasing weight reported in small-scale studies is also observed on a larger scale at a population-level and, perhaps more importantly, if abnormal ventricular repolarisation with increasing adiposity associate with higher risks for ventricular arrhythmias.

3.1.4. Metabolic health in obesity

Obesity is often associated with cardiometabolic ill-health, owing to the cardiovascular adaptations resulting from increasing adiposity and its lipotoxic effects on endocrine physiology. These include insulin resistance, dyslipidaemia and hypertension, and their co-existence with abdominal obesity has been termed metabolic syndrome (71). Although there is there are several definitions for metabolic syndrome (128), this clustering of clinical phenotypes has stimulated interest into the metabolic-dependent and -independent effects of obesity on health (446, 447). Whether obesity can be considered “metabolically healthy” in the absence of concurrent hypertension, insulin resistance or dyslipidaemia is debatable (448). Indeed, there have been several studies suggesting that being overweight or obese may in fact be associated with lower morbidity and mortality (138, 449, 450, 451, 452). For example, amongst patients admitted to hospital for sudden cardiac death, BMI<25kg/m² and >40kg/m² were independent predictors of in-hospital

mortality, suggesting a J-shape relationship between BMI and mortality (452). Although this phenomenon has been termed the “obesity paradox”, it remains controversial and may be explained by confounding clinical variables. Indeed, although the J-shape relationship between BMI and mortality risk was also demonstrated amongst 457,746 UK Biobank participants using observational Cox models, a Mendelian randomisation analysis using data from the same individuals suggested that this relationship was in fact linear, with every unit increment in BMI conferring a 5% higher mortality risk (hazard ratio 1.05, 95%CI: 1.03-1.08), suggesting that even mild-to-moderate excess weight is detrimental (145).

Others have argued that metabolically healthy obesity may be considered benign after accounting for cardiorespiratory fitness (143, 453), such that these individuals had lower risk of all-cause mortality as well as non-fatal and fatal cardiovascular disease compared to their metabolically unhealthy obese counterparts, and that these risks were similar to those observed in metabolically healthy lean individuals (142). On the other hand, “metabolic healthy obesity” may be considered a transient subclinical phenotype that evolves into clinically overt metabolic complications, and therefore warrants timely lifestyle and medical intervention to mitigate future risks (454). For example, amongst healthy obese individuals followed up over 10 years, visceral adiposity, HDL cholesterol and fasting plasma insulin were predictors of future metabolic complications (132). This was corroborated in another larger study of over 2000 metabolically healthy obese individuals in whom visceral abdominal adiposity was predictive of future conversion to metabolically unhealthy status (133). Similarly, in another study 40% of 3000 metabolically healthy obese individuals transitioned to another phenotype after at least 3 years, and importantly, of those

that were metabolically unhealthy obese visceral fat loss of more than 5% was associated with reversal of the metabolically unhealthy status according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) metabolic syndrome criteria, suggesting reversibility with weight reduction (455). Thus, even in the absence of metabolic perturbation, obesity is likely to be detrimental for cardiovascular health. Indeed, amongst almost 100,000 female participants in the Nurses' Health Study over a 30-year follow-up period, those who remained metabolically healthy and obese remained at higher risk of cardiovascular disease risk compared with those who maintained normal weight (HR 1.57, 95% CI 1.03-2.38) (456). Although cardiovascular risk was greater in those that had transitioned to a metabolically unhealthy phenotype during follow-up, these observations suggest that chronic obesity in isolation is not a benign phenotype (144). This was supported by a study of 381,363 UK Biobank participants in whom over a median of 11.2 years follow-up, metabolically healthy obese individuals had higher risks of diabetes (HR 4.32; 95%CI 3.83-4.89), atherosclerotic disease (HR 1.18; 95%CI 1.10-1.27) and all-cause mortality (HR 1.22; 95%CI 1.14-1.31) compared to metabolically healthy lean counterparts (323). Although results from other population-based studies may be conflicting with respect to cardiovascular mortality, they nonetheless support a higher risk of incident heart failure and atrial fibrillation in obese individuals (457). Interestingly, risk of cardiovascular outcomes may be sex-specific, whereby rates of myocardial infarction, stroke, and mortality were less common in metabolically healthy obese females compared to those of normal weight, although the opposite was true in males (457). The higher risk of arrhythmias was also evident in a retrospective analysis of 389,321 Korean nationals, amongst which metabolically healthy obese individuals AF incidence rates

was 2.66/1000 person years compared to 0.76/1000 person years for normal weight counterparts (458).

With respect to electrophysiological remodelling, metabolic perturbation is also associated with ventricular repolarisation abnormalities. For example, in rural Chinese population Guo et al showed that QTc prolonged differentially amongst over 10,000 healthy and unhealthy obese individuals (459). Specifically, metabolic ill-health in the presence and absence of obesity was associated with longer QTc interval. However, in contrast to other several other studies (105), obesity alone did not prolong QTc. In this regard, although several studies have investigated the differential effects of obesity phenotypes on cardiovascular outcomes, particularly heart failure and mortality, the effects of obesity and metabolic ill-health on ventricular repolarisation amongst the British population remains relatively unexplored. Consequently, it is also unknown if the effects of obesity and metabolic ill-health on proarrhythmic ventricular remodelling are additive and whether these confer a higher risk of ventricular arrhythmias.

3.1.5. Genetic modulation of ventricular repolarisation

In addition to traditional cardiovascular risk factors, ventricular repolarisation is also influenced by non-modifiable factors. For instance, genome-wide association studies (GWAS) have shown that common genetic variants, also called single nucleotide polymorphisms (SNPs), explain up to 10% of the QTc interval variation in the general population (460, 461, 462). The combined effect of inherited SNPs recognised to modulate QTc interval can be quantified using a polygenic risk score (PRS) (463). Using such a QTc-PRS, Strauss et al showed that resting QTc is indeed modulated

by genetics with implications for drug-induced QTc prolongation and risk of ventricular arrhythmias (464). Furthermore, the QTc-PRS score used by Strauss et al explained more of the variation in QTc interval in a Caucasian population than other clinical parameters (465). On this premise, QTc-PRS have also been used to investigate the degree to which genetics modulate QTc in individuals with a predisposition for QTc-prolongation (466, 467, 468). Thus, an individual's predilection for abnormal ventricular repolarisation is at least partly genetically-determined (469). Given that obesity is becoming increasingly common, yet ventricular arrhythmias are relatively uncommon, it is plausible to hypothesise that the genetic constitution of some individuals may confer protection against the QTc-prolonging effects of adiposity. Yet, despite the clinical evidence suggesting QTc prolongs with increasing adiposity, it remains unknown whether this association is indeed modulated by individuals' genetics. This may be partly explained due to the paucity of the databanks containing anthropometric, ECG and genetic data from a sufficient number of the same individuals to answer this question.

3.1.6. UK Biobank: national data repository

The UK Biobank is data repository containing data from a large sample of the UK population. It contains sociodemographic, healthcare, and uniquely genetic data for its participants (319). A historical perspective of the UK Biobank is provided in Chapter 2. Briefly, it is a longitudinal study initiated to investigate the contributions of genetics, lifestyle and environmental exposures on the natural development of disease. Over 500,000 participants aged 40–69 years were recruited to the UK Biobank over a 4-year period across twenty-two assessments centres in the UK (319). Participants were invited based to partake in the UK Biobank if they resided

within a catchment area of an assessment centre using personal and contact details provided by the NHS to the UK Biobank organisers. Following the initial pilot stage in 2006, the main study began in April 2007 and passed the 500,000 participant target recruitment in July 2010, after which no new participants were enrolled.

The UK Biobank data has been leveraged to show that increasing body weight is associated with higher risks of mortality (145), diabetes (323), cognitive decline (345), hypertension (470), AF (324), aortic valve stenosis, heart failure, deep vein thrombosis, coronary artery disease and pulmonary embolism (124) and adverse outcomes with coronavirus-19 infection (336). Notably, the UK Biobank has two important strengths as a data repository. First, it contains detailed genetic data for its participants that has enabled identification of novel single nucleotide polymorphisms, candidate genes and consequently biological pathways associated with clinical traits and phenotypes. In turn, this has facilitated numerous Mendelian randomisation studies to ascertain causal associations between risk factors and health outcomes. Second, it is a longitudinal study that enables study of the natural history of disease on a population level. Given its wealth of data, the UK Biobank is an ideal resource in which the effects of increasing adiposity and metabolic ill-health on ventricular electrophysiology, and any modulatory effects of genetics, can be studied at a population level.

3.1.7. Aims and hypotheses

Data from the UK Biobank was accessed and analysed to achieve the following aims:

1. To study the links between obesity and metabolic dysfunction, respectively, with QTc interval, amongst a large sample of the UK population
2. To determine whether these associations are modulated by individuals' genetics that may pre-dispose them to QTc prolongation
3. To determine the risks of ventricular arrhythmias amongst UK Biobank participants in the absence or presence of obese and/or metabolic dysfunction.

The hypotheses of this study were:

1. Increasing adiposity prolongs QTc interval independent of sociodemographic factors and co-morbidity
2. Individual's genetics modulate the QTc-prolonging effects of increasing adiposity
3. Ventricular arrhythmias are more common in obese patients, whether in the presence or absence of concurrent metabolic ill-health

3.2. Methods

Given that the UK Biobank data was collected several years prior to the analyses presented herein, the following sections provides details about the data that is available in the UK Biobank, the methods by which it was recorded, and the timelines over which this occurred.

3.2.1. Data collection by the UK Biobank

At the assessment centres, upon providing informed consent, participants completed touchscreen self-questionnaires to provide information about their sociodemographic data, health, lifestyle and environmental exposures, as well as undergoing physical assessments by trained individuals. These included blood pressure readings, anthropometry and body composition measures using impedance. The range of investigations performed on participants was extended during the course of the study to include resting and exercise ECGs. For this reason, participants recruited later in study underwent more extensive tests and recordings. Importantly, although no new participants were recruited after 2010, existing participants were invited several years after their enrolment for tests that were not performed earlier in the UK Biobank. For example, resting 12-lead ECG data began to be collected from participants in the “instance 2” phase of the UK Biobank which began in 2014, several years after the last volunteer was recruited. This was extended to “instance 3” that began in 2019. As of 25 July 2021, there were 23,493 participants for whom 12-lead ECG data was available in the UK Biobank.

3.2.2. Sociodemographic data

Participants’ date of birth and sex were obtained from local health service registries prior to their assessment at one of the UK Biobank centres. This information could be amended by the participants during their visit if required. The Townsend deprivation index (TDI) is a measure of material deprivation that is calculated using data collected in the national census (471). TDI was also calculated immediately prior to participants joining UK Biobank using data from the preceding national census. Each participant is assigned a score corresponding to the output area in

which their residential postcode was located. Participants' ages used in this study were based on their dates of birth and dates of attending the assessment centre in which their ECG was recorded, and rounded to the nearest whole year. Participants' ethnic background was determined by a series of questions on the touchscreen questionnaire. However, this question was removed from the touchscreen protocol on 24 October 2016. Participants that had indicated they were immigrants were asked for the year in which they first came to live in the UK, but this question was also redacted on 23 October 2016.

3.2.3. 12-lead ECG recording

The ECG GE Cardiosoft program was used to digitally record resting 12-lead ECG from participants lying in a semi-prone position, with electrodes positioned in the conventional manner. Electrode-to-skin contact was optimised by shaving if required. All ECGs were calibrated to 1mV=10mm and recorded at a paper speed of 25mm/second. ECGs were recorded by trained members of UK Biobank staff over a standard 10-second period. The RR, QT and QTc intervals, as well as P-wave and QRS durations were derived from the digital ECG recordings.

3.2.4. Anthropometric data

Anthropometric measurements were recorded by trained individuals at the assessment centres using weighing scales and height rulers or measuring tapes. BMI was calculated from height and weight measurements recorded at these centres and omitted for participants for whom either height or weight were not recorded. 499,305 participants had their BMI recorded in "instance 0", i.e. the initial

assessment visit (2006-2010), and was measured again in 20,297 participants in “instance 1” (first repeat assessment visit 2012-2013), 52,360 participants in “instance 2” (2014+) and 5,278 participants in “instance 3” (2019+). Similarly, hip and waist circumferences, and body fat percentage by impedance, were also measured and recorded in instances 0-3. The Tanita BC418MA body composition analyser was used to calculate body fat mass and body fat percentage. This analyser measures body composition by passing a small electric current through the body when the participant places their bare feet on the analyser’s platform over indicated foot markings, whilst ensuring there is no material obstruction to maintain contact with the analyser. The participants also grip two metal handles with their arms by their sides. The analyser measures weight as well as body impedance.

3.2.5. Medical diagnoses

The UK Biobank is linked to national healthcare records. It therefore contains diagnosis codes the participants may have had recorded across all their hospital inpatient notes. These are coded according to the International Classification of Diseases version-10 (ICD-10). Data is available for codes recorded in either the primary or secondary position, as well as the date of diagnosis. In total, over 6 million items of data were available. The ICD-10 codes in this study are presented in Table 3.1.

3.2.6. Serum biochemistry

All participants had blood samples collected at their first assessment centre visit, and approximately 20,000 participants had repeat samples drawn approximately 5 years

later. The biochemistry assays performed in the UK Biobank was informed by their established link to disease or as diagnostic measures. Amongst these were liver function tests, lipid profile, C reactive protein, sex hormones, glycated haemoglobin, urea, creatinine and rheumatoid factor. Importantly, electrolytes with the exception of calcium and phosphate were not measured and are not available in the UK Biobank. The lipid profile included apolipoprotein A and B, HDL and LDL cholesterol, LDL, lipoprotein A and triglycerides. The biochemistry assays performed in participants were not determined by their past medical or drug history.

3.2.7. Drug history

At the assessment centre, participants were required to complete a touchscreen question regarding medication use. For example, they were asked, "Do you regularly take any of the following medications?" and were allowed to select more than one option. The options for this may have been, for example, "Cholesterol lowering medication", "Blood pressure medication", "Insulin", "None of the above", "Do not know" and "Prefer not to answer". Subsequently, participants may have had a verbal interview by a trained nurse on prescription medications if they had answered in the touchscreen questionnaire that they were taking prescribed medication. They were asked to provide any details for any blood pressure lowering, cholesterol lowering, hormone replacement therapy or oral contraceptive drugs. Short-term medications such as antibiotics, over-the-counter medications, supplements or lifestyle agents were not recorded. Importantly, however, doses and formulations were not recorded.

3.2.8. Accessing UK Biobank data

Data from the UK Biobank is accessible to bona fide healthcare researchers upon an application on its website. Data used in this study was accessed under application numbers 48666 and 47602.

3.2.9. Study definitions

To address the first aim of this study, five anthropometric indices that were available for the majority of participants were included in the analysis. These were BMI, body fat percentage (BF), waist:hip ratio (WHR) and waist (WG) and hip (HG) girth.

Obesity was defined as BMI ≥ 30 kg/m², or WG > 94cm in males and > 80cm in females, as per the consensus document published by European and American healthcare bodies (71, 472). Participants that had a primary or secondary diagnosis in their hospital medical records containing ICD-10 codes for any two of dyslipidaemia (hypertriglyceridaemia or hypercholesterolaemia or both), hypertension, or type 1 or 2 diabetes mellitus, were considered “metabolically unhealthy” (MU). Lipid profiles and blood pressure results recorded at the UK Biobank assessment centres were reviewed for participants in whom there was no recorded history of dyslipidaemia or hypertension. Participants whose lipid results or blood pressure recordings fulfilled the criteria for dyslipidaemia or hypertension, according to the International Diabetes Federation (71), were considered metabolically unhealthy in the present study, even in the absence of any recorded medical history. Specifically, the International Diabetes Federation’s definition of metabolic syndrome defines dyslipidaemia as either raised triglycerides (>150mg/dL [1.7mmol/L]) or reduced HDL cholesterol (<40mg/dL [1.03mmol/L] for males and <50mg/dL [1.29mmol/L] in females). Hypertension was defined as systolic blood

pressure ≥ 130 /mmHg or diastolic blood pressure ≥ 85 mmHg. In this way, participants were classified as obese (Ob+), non-obese (Ob-), metabolically unhealthy (MU+) or metabolically healthy (MU-) and categorised into four phenotypes: Ob-MU-, Ob-MU+, Ob+MU- and Ob+MU+. The prevalence of ventricular arrhythmias was determined by an ICD-10 code for any one or more of ventricular tachycardia, (VT) ventricular fibrillation (VF), re-entry ventricular arrhythmia, and catheter ablation of the ventricular wall listed as primary or secondary diagnoses in participants' hospital records. **Table 3.1** provides a list of the ICD-10 codes used to determine the past medical history of participants included in this study.

Comorbidity	ICD-10- Codes	Definitions
Hypertension	I10, I15	Essential (primary) hypertension; Secondary hypertension.
Diabetes	E10, E11, E13, E14	Insulin-dependent diabetes mellitus; Non-insulin-dependent diabetes mellitus; Other specified diabetes mellitus; Unspecified diabetes mellitus.
Dyslipidaemia	E78.0, E78.1, E78.2, E78.4, E78.5, E78.6	Pure hypercholesterolaemia; Pure hyperglyceridaemia; Mixed hyperlipidaemia; Other hyperlipidaemia; Hyperlipidaemia, unspecified; Lipoprotein deficiency.
Coronary artery disease	I20, I21, I22 I24, I25	Angina pectoris; Acute myocardial infarction; Subsequent myocardial infarction; Other acute ischaemic heart diseases; Chronic ischaemic heart disease.

Ventricular Arrhythmias	I47.0, I47.2, I49.0	Re-entry ventricular arrhythmia; Ventricular tachycardia; Ventricular fibrillation and flutter.
	K57.6	Percutaneous transluminal ablation of ventricular wall.

Table 3.1: Definitions of comorbidity based on the main and secondary International Classification of Disease (ICD)-10 summary diagnosis or operative procedures. Reproduced from Patel et al (473) with permission under licence CC-BY 4.0.

3.2.10 Relationship between adiposity and metabolic ill-health on QTc

Any effects of adiposity and/or metabolic ill-health on QTc could only be established in participants in whom these data were available. As discussed earlier in this chapter, not all participants underwent identical phenotypic characterisation. Thus, this analysis was confined to participants that had a recorded QTc by Bazzett's formula from a single resting digital 12-lead ECG and anthropometry in the same window of data acquisition ("instance") in the UK Biobank (2015-2017, n=23,683). Adjusted multiple linear regression analyses were performed in two models to determine the effect of increasing adiposity on QTc. Model 1 was adjusted for age, sex, ethnicity and TDI. Model 2 was further adjusted for hypertension, dyslipidaemia, coronary artery disease, stroke, diabetes, smoking and alcohol consumption as dichotomous variables listed in participants past medical records or self-reported questionnaires. Indices of adiposity were treated as continuous variables to determine their effects on QTc for every unit and standard deviation increment in

adiposity. Similar analysis adjusted for age, sex, ethnicity and TDI was used to assess the effect of obesity phenotypes on QTc.

3.2.11. Derivation of QTc-PRS for QTc interval

Xiao Xu (research fellow, National Heart and Lung Institute) accessed genetic data from the UK Biobank and used this to generate the QTc-PRS. This was achieved using summary statistics from a GWAS of over 70,000 individuals (460). 67 SNPs passed threshold significance at genome-wide association level ($p \leq 5 \times 10^{-8}$), of which 66 were available in the UK biobank genotyping dataset. Table 3.2 lists the SNPs used in the QTc-PRS for this study. The QTc-PRS was generated by Xiao Xu using PRSice (version 2.2.11.b) and the score for each UK biobank individual was calculated by the weighted sum of all 66 SNPs using the equation:

$$PRS_j = \frac{\sum_i (S_i \times G_{ij}) - Mean(PRS)}{SD(PRS)}$$

Where i is i th selected SNP markers used in PRS, j is j th individual, S is β coefficient, G is genotype (coded as 0, 1, and 2) for an additive genetic model. This 66-SNP QTc-PRS explained 3.08% of the variance in 14,501 UK Biobank participants for whom QTc was available. Adding sex and age as covariates, the QTc-PRS accounted for 8.77% of the variance in QTc interval. These results are comparable to those reported in an European Caucasian population (2%-5% and 7-8%, respectively) (465). Thus, a high QTc-PRS represents a greater genetic propensity for a longer than normal QTc interval compared to a low QTc-PRS. In this context, the QTc-PRS could be implemented as a surrogate and inverse measure of an individual's inherent repolarisation reserve, i.e. the extent to which genetics may

protect against any QTc-prolonging effects of increasing adiposity or modifiable risk factors. To study the modulatory effect of genetics on the association between increasing adiposity and QTc amongst individuals that had been genotyped, PRS was added to model 2 to generate model 3 in the regression analysis. Additionally, individuals were stratified into high (>75%), intermediate (25-75%) and low (<25%) QTc-PRS groups, to represent individuals with high, intermediate and low genetic propensity for QTc prolongation. Similarly, the effects of genetics on the association associations between QTc metabolic perturbation was also evaluated.

SNP	CHR	POS	A1	A2	HAPMA P_A1_F REQ	Beta	P
rs12143842	1	162033890	T	C	0.1892	3.5009	1.32E-213
rs3934467	1	162182677	T	C	0.1525	2.7435	2.76E-129
rs880296	1	162128446	G	C	0.2009	2.2456	1.27E-81
rs11153730	6	118667522	T	C	0.4914	-1.6491	2.23E-67
rs16857031	1	116211291 0	G	C	0.1525	2.3653	6.45E-61
rs37055	16	58560775	T	C	0.2895	-1.7297	2.20E-57
rs7122937	11	2486550	T	C	0.2105	1.9273	1.24E-54
rs2072413	7	150647969	T	C	0.2931	-1.6757	1.32E-49
rs6676438	1	161983089	T	C	0.2902	1.5168	2.59E-45
rs846111	1	6279370	C	G	0.2917	1.7317	7.39E-40
rs347273	1	162317513	G	A	0.07589	1.8041	1.87E-38

rs3807375	7	150667210	T	C	0.3795	1.2227	2.88E-33
rs1805120	7	150649531	A	G	0.2054	1.5387	3.55E-33
rs10919070	1	169099037	C	A	0.1417	-1.6781	1.11E-31
rs735951	16	11693536	A	G	0.4688	-1.1529	2.29E-28
rs12271931	11	2478519	G	A	0.1116	-2.7657	8.94E-28
rs6793245	3	38599037	A	G	0.2857	-1.1163	4.43E-27
rs1052536	17	33331575	C	T	0.4955	0.98	6.21E-25
rs12210733	6	118653075	A	G	0.03704	-2.051	1.13E-22
rs12061601	1	169070450	C	T	0.1071	-1.4096	3.10E-21
rs4656345	1	161991237 8	A	G	0.1	-4.7305	1.03E-19
rs457162	6	118535983	T	A	0.01887	-1.8493	2.49E-18
rs545833	1	168689940	T	C	0.2679	0.8975	3.89E-17
rs17460657	1	162261826	C	A	0.03333	-4.6017	2.72E-16
rs3902035	6	119000232	C	T	0.1698	-0.8519	8.00E-16
rs1983546	1	139446183	G	A	0.35	-0.8069	9.74E-16
rs2298632	1	23710475	T	C	0.4955	0.6998	1.38E-14
rs9892651	17	64303793	C	T	0.4688	-0.7441	2.71E-14
rs12997023	2	40752982	C	T	0.05085	-1.6892	4.67E-14
rs7545047	1	162191103	A	G	0.025	-1.7784	8.89E-14
rs12927050	16	11673488	A	C	0.12	-0.973	1.17E-13
rs1811815	11	2475150	A	G	0.2273	-0.9	1.18E-13
rs11710077	3	38657899	T	A	0.1964	0.9224	1.42E-13
rs10775360	17	68325868	T	C	0.3051	-0.7551	1.07E-12

rs12444261	16	11734642	T	G	0.2857	-0.7915	2.14E-12
rs3105593	15	50845018	T	C	0.4732	0.6643	3.06E-12
rs6599234	3	38715300	A	T	0.3	0.6986	2.22E-11
rs11708996	3	38633923	C	G	0.2	-0.9238	2.27E-11
rs164133	1	162381288	C	G	0.1833	0.7195	2.99E-11
rs2273905	14	102974999	T	C	0.2946	0.6102	4.04E-11
rs236523	17	68212642	C	A	0.4955	0.6377	4.77E-11
rs1659127	16	14388305	A	G	0.3	0.7068	6.38E-11
rs13355516	5	137380603	G	A	0.175	-0.8306	6.97E-11
rs6800541	3	38774832	C	T	0.4196	-0.6249	8.14E-11
rs12079745	1	139101060	A	G	0.02586	-1.3408	1.22E-10
rs174577	11	61604814	A	C	0.3393	-0.6523	1.30E-10
rs6544311	2	40353277	A	C	0.3776	0.6457	1.79E-10
rs1296720	16	3873642	C	A	0.2455	0.8297	3.57E-10
rs4716056	6	16278390	G	A	0.3646	0.648	3.95E-10
rs2301696	11	2426984	G	C	0.4	-1.1423	4.26E-10
rs2363719	4	72138216	A	G	0.09375	0.9677	7.84E-10
rs12675772	8	71213705	G	C	0.1	0.9447	1.76E-09
rs11888462	2	201160499	T	G	0.4153	0.5605	4.95E-09
rs183993	4	95130025	G	A	0.35	0.5662	5.19E-09
rs13002675	2	174739352	A	C	0.1937	0.7217	5.38E-09
rs4784934	16	58459926	A	G	0.2091	0.6748	5.55E-09
rs2273042	1	6149122	A	G	0.1308	0.9375	6.80E-09
rs4630352	12	110747419	A	G	0.3227	0.5675	1.09E-08

rs4657172	1	162179632	C	G	0.1583	-0.8141	1.27E-08
rs9489510	6	119043898	G	A	0.3036	0.6085	1.36E-08
rs728926	13	74513122	T	C	0.3438	0.5746	2.06E-08
rs9920	7	116200092	C	T	0.07658	0.7899	2.60E-08
rs2485376	10	104050006	A	G	0.4286	-0.5629	2.69E-08
rs3789530	1	6153406	T	C	0.1205	0.9171	3.29E-08
rs295449	3	47375955	G	A	0.433	-0.549	3.83E-08
rs2916558	8	103847399	A	C	0.3833	0.5563	4.07E-08

Table 3.2: SNPs used to generate PRS. 66 SNPs identified to modulate QT and available in the UK Biobank were used to generate a polygenic risk score as an indicator of repolarisation reserve. Summary statistics from Arking et al (460) were used to derive polygenic risk score. SNP, single nucleotide variants (with corresponding rs identification); CHR, chromosome number; POS: position of each SNP in hg19 reference genome; A1, allele 1; A2, allele 2; HAPMAP_A1_FREQ: A1 allele frequency in Hapmap project; Beta, beta co-efficients from GWAS identifying allele with QT prolongation (460). Reproduced from Patel et al (473) under licence CC-BY 4.0.

3.2.12 Obesity phenotypes and ventricular arrhythmic risks

The association between obesity phenotypes and VA was assessed across the entire UK Biobank cohort (n=502,536), given that recorded VA events were infrequent amongst individuals for whom 12-lead ECG data was available (n=23,683). The mean BMI or WG was used to define obesity (BMI ≥ 30 kg/m² or WG > 94cm in males and > 80cm in females) (71) in instances where this data had been recorded more than once.

3.2.13. Statistical analyses

Given the large volume of data from the UK Biobank, analyses presented herein was accomplished with the computational and coding assistance of Xinyang Li (post-doctoral research fellow, National Heart and Lung Institute). Analyses was performed via the high-performance computing service available at Imperial College London using coding language written by Xinyang Li on Python version 3.6 (Python Software Foundation). The associations between indices of adiposity and QTc were modelled using multivariable linear regression models (models 1-3 as detailed above). Logistic regression adjusted for age, sex, ethnicity and TDI was used to assess associations between obesity phenotypes and QTc and ventricular arrhythmias, respectively. Continuous variables provided herein are expressed as mean $\pm$ standard deviation (SD) and confidence intervals (CI) as 95% ranges. $P < 0.05$ was considered significant.

3.3. Results

3.3.1. Baseline characteristics

23,683 participants (11,563 male, mean age 61.0 ± 7.5 years, 97.1% Caucasian) had ECG, anthropometric, sociodemographic and ICD-10 information data available.

Table 3.2 provides the baseline characteristics for participants in whom the effects of adiposity and clinical phenotypes on QTc were investigated.

	Total	Ob-MU-	Ob+MU-	Ob-MU+	Ob+MU+
N	23683	10500	8818	1571	2794
Sex					
Male	11563 (48.8%)	5219 (49.7%)	3695 (41.9%)	1099 (70.0%)	1550 (55.5%)
Female	12120 (51.2%)	5281 (50.3%)	5123 (58.1%)	472 (30.0%)	1244 (44.5%)
Age	61.0 $\pm$ 7.5	59.9 $\pm$ 7.5	60.4 $\pm$ 7.4	65.0 $\pm$ 6.5	64.4 $\pm$ 6.6
Ethnicity					
Non-Ca	647 (2.7%)	313 (3.0%)	190 (2.2%)	63 (4.0%)	81 (2.9%)
Ca	22996 (97.1%)	10169 (96.8%)	8613 (97.7%)	1504 (95.7%)	2710 (97.0%)
Unknown	40 (0.2%)	18 (0.2%)	15 (0.2%)	4 (0.3%)	3 (0.1%)
TDI	-2.0 $\pm$ 2.7	-2.2 $\pm$ 2.6	-1.9 $\pm$ 2.7	-2.1 $\pm$ 2.7	-1.8 $\pm$ 2.8
QTc	420.4 $\pm$ 26.6	416.5 $\pm$ 25.0	422.9 $\pm$ 27.5	420.6 $\pm$ 26.7	427.2 $\pm$ 27.0
Heart rate	60 $\pm$ 9	58 $\pm$ 8	62 $\pm$ 8	60 $\pm$ 9	63 $\pm$ 10
Adiposity					
BMI	26.7 $\pm$ 4.5	23.8 $\pm$ 2.4	29.2 $\pm$ 4.1	24.6 $\pm$ 2.4	30.6 $\pm$ 4.5
WG	88.4 $\pm$ 12.6	79.8 $\pm$ 8.5	95.7 $\pm$ 10.1	83.7 $\pm$ 7.8	100.1 $\pm$ 11.1
HG	101.4 $\pm$ 8.8	96.1 $\pm$ 5.4	106.6 $\pm$ 8.1	96.5 $\pm$ 5.0	107.8 $\pm$ 8.9

<i>Continued overleaf</i>					
	Total	Ob-MU-	Ob+MU-	Ob-MU+	Ob+MU+
WHR	0.87 $\pm$ 0.09	0.83 $\pm$ 0.08	0.90 $\pm$ 0.08	0.87 $\pm$ 0.07	0.93 $\pm$ 0.08
BF	30.1 $\pm$ 8.2	26.3 $\pm$ 7.0	34.0 $\pm$ 7.5	26.1 $\pm$ 6.4	34.3 $\pm$ 7.9
Table 3.3: Characteristics for UK Biobank participants in whom the effects of adiposity and clinical phenotype on QTc interval was assessed. Values presented as mean ($\pm$ standard deviation). Percentages of the cohort in each category presented in parentheses. Ob, obesity; MU, metabolically unhealthy; +, presence; - absence; Ca, Caucasian; TDI, Townsend deprivation index [units]; BMI, body mass index [kg/m ²]; BF, body fat [%]; WHR, waist:hip ratio [unit]; HG, hip girth [cm]; WG, waist girth [cm]. Age presented in years and QTc in milliseconds. Reproduced from Patel et al (473) under licence CC-BY 4.0.					

3.3.2. Increasing adiposity is associated with QTc interval prolongation

There was a positive association between QTc interval and adiposity, quantified by the listed anthropometric measurements (Figure 3.1). For the pooled analysis (n=23,486), correcting for sociodemographic factors (model 1), QTc interval increased by 0.83ms/kg/m² (BMI, CI 0.76-0.91ms/kg/m²), 0.50ms/%body fat (CI 0.44-0.55ms/%), 42.10ms/unit WHR (CI 36.95-47.26ms/unit), 0.38ms/cm hip girth (CI 0.34-0.42ms/cm) and 0.35ms/cm WG (CI 0.32-0.38ms/cm; all p<0.001).

QTc interval increases, albeit to a lesser degree, with increasing adiposity when additionally adjusted for co-morbidity and lifestyle factors (model 2): 0.76ms/kg/m² (BMI, CI 0.68-0.83ms/kg/m²), 0.45ms/% body fat (CI 0.39-0.50ms/%), 37.34ms/unit

WHR (CI 32.10-42.58ms/unit), 0.35ms/cm HG (CI 0.31-0.39ms/cm) and 0.32ms/cm WG (CI 0.29-0.35ms/cm; all $p < 0.001$). Similar observations were noted in sex-stratified analyses (Figure 3.1).

3.3.3. Genetically-determined QT interval does not modulate the associations between adiposity and QTc

Amongst those individuals that had ECG, anthropometry and genotype data available ($n=10,223$), there was a positive correlation between QTc and PRS ($r=0.17$, $p < 0.001$), such that QTc increased by 4.50ms (CI 4.00-5.02ms) per unit increment in the PRS for genetically-determined QT interval in the pooled cohort. QTc increased by 4.33ms (CI 3.64-5.04ms) and 4.74ms (CI 4.03-5.46ms) per unit increment in PRS in females and males, respectively (both $p < 0.001$). QTc was observed to be greater with every unit and standard deviation increment in adiposity in males and females, after adjusting for the PRS, in addition to socio-demographic, co-morbidity and lifestyle factors (model 3, Figure 3.2). However, the interactions between PRS and the adiposity-QTc relationships were not statistically significant (Figure 3.2). Furthermore, there was no consistent evidence to show that the associations between indices of adiposity and QTc were modulated by PRS when stratified by PRS in males or females (Figure 3.3).

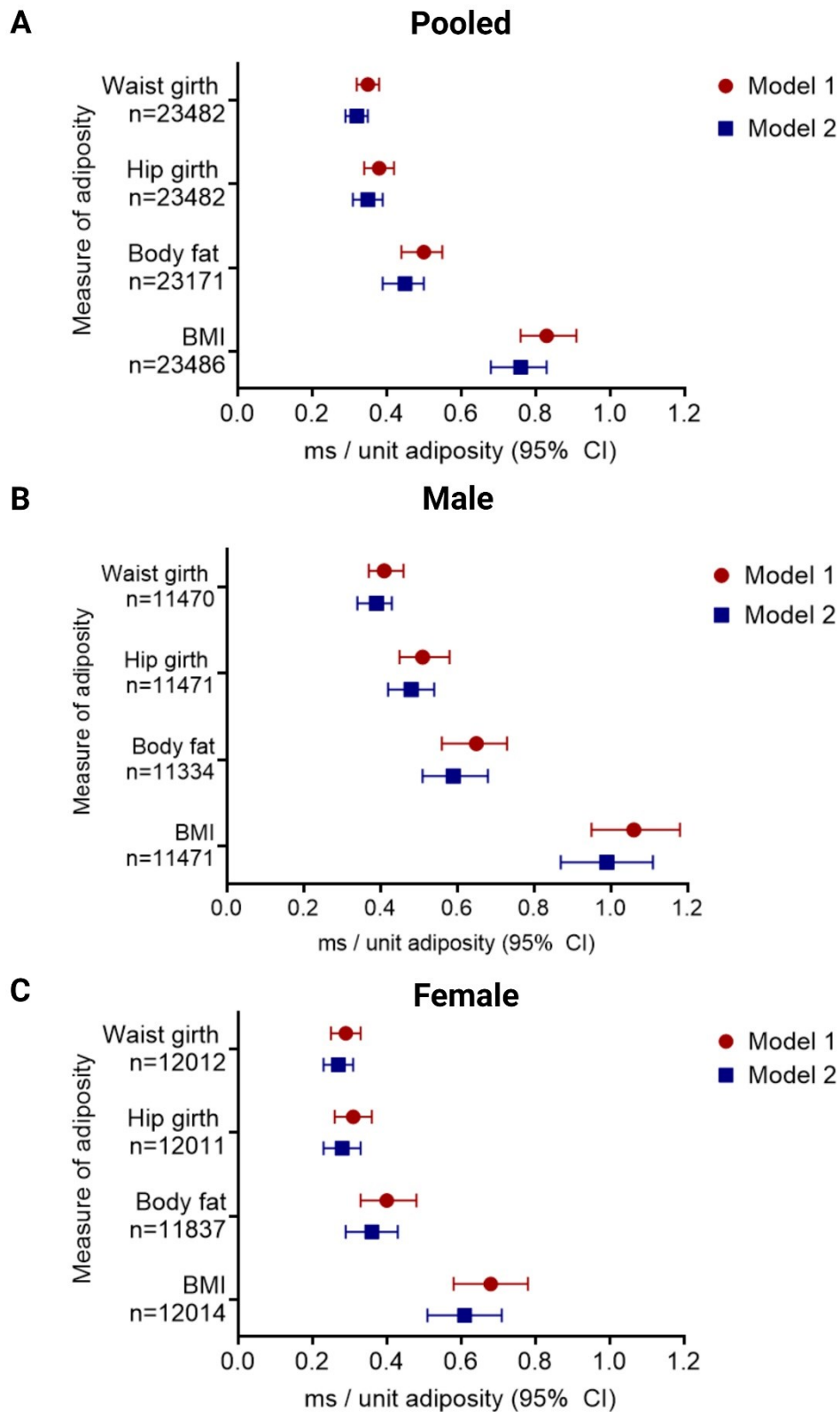


Figure 3.1: Increasing adiposity is associated with increased QTc interval, after adjusting for sociodemographic, co-morbidity and lifestyle factors. The change in

QTc per unit and per standard deviation (SD) increment in adiposity (quantified by BMI, body mass index [kg/m^2]; BF, body fat [%]; WHR, waist;hip ratio [unit]; HG, hip girth [cm]; WG, waist girth [cm]) are shown for the pooled cohort **(A)** and stratified by sex **(B-C)**. Model 1 adjusted for sociodemographic factors; model 2 adjusted for lifestyle and comorbidities. All results presented reached significance $p < 0.001$. Results for QTc change per unit increment in WHR is provided in the main text. Reproduced from Patel et al (473) with permission under licence CC-BY 4.0.

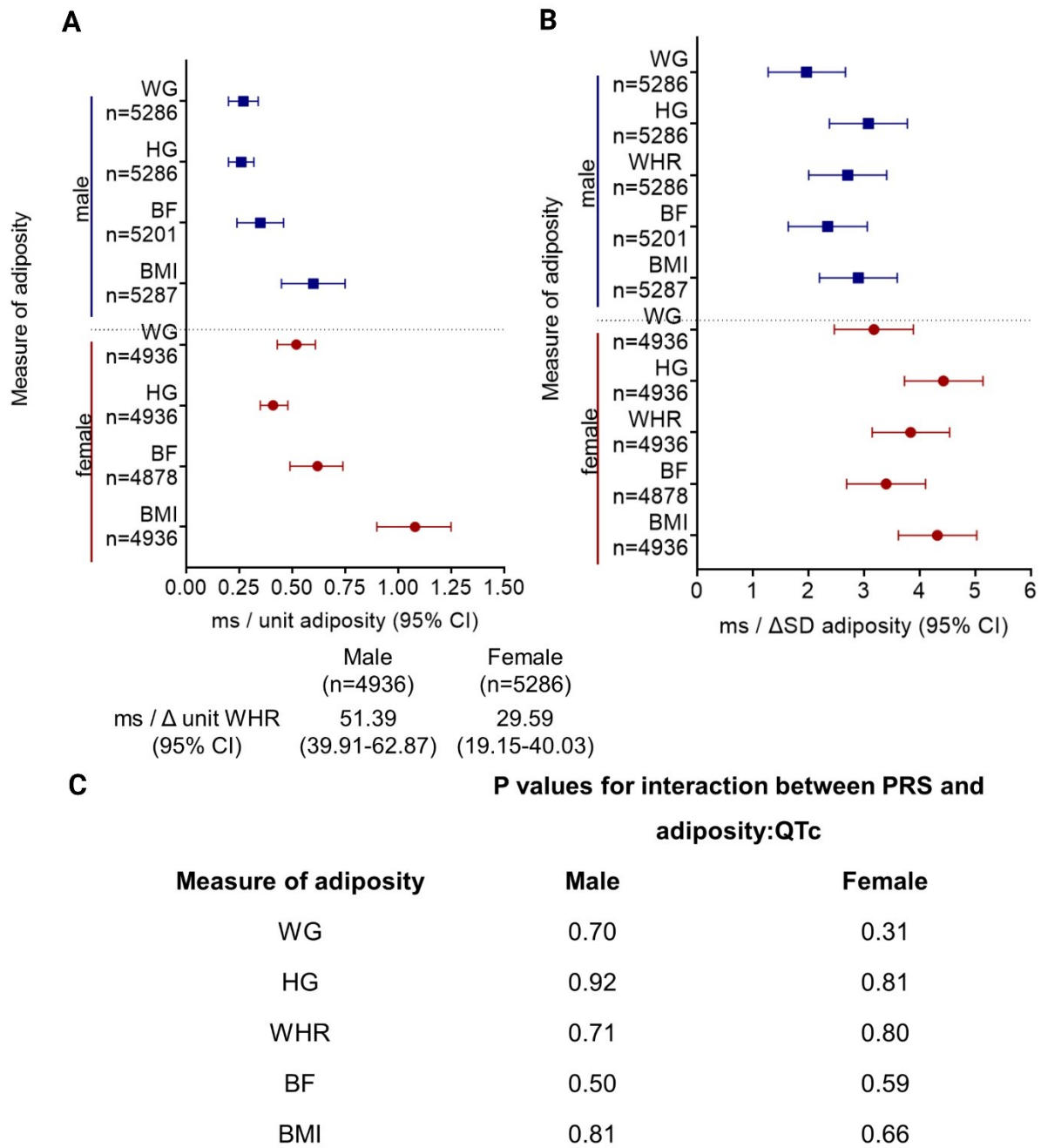


Figure 3.2: Polygenic risk score for genetically-determined QT interval does not modulate the association between increasing adiposity and QTc interval. The change in QTc per unit **(A)** and per standard deviation (SD) **(B)** increment in adiposity (quantified by BMI, body mass index [kg/m²]; BF, body fat [%]; WHR, waist:hip ratio [unit]; HG, hip girth [cm]; WG, waist girth [cm]) are shown stratified by sex. Model 3 is adjusted for PRS in addition to sociodemographic and lifestyle factors and comorbidities as in

model 2 (Figure 3.1). All results presented for model 3 in **A** and **B** reached significance $p < 0.001$. **C**: There was no significant interaction between PRS and the adiposity:QTc relationships (P-interaction, PRS with adiposity:QTc) in either sex, suggesting genetics do not modulate the relationship between adiposity and QTc. Reproduced from Patel et al (473) with permission under licence CC-BY 4.0.

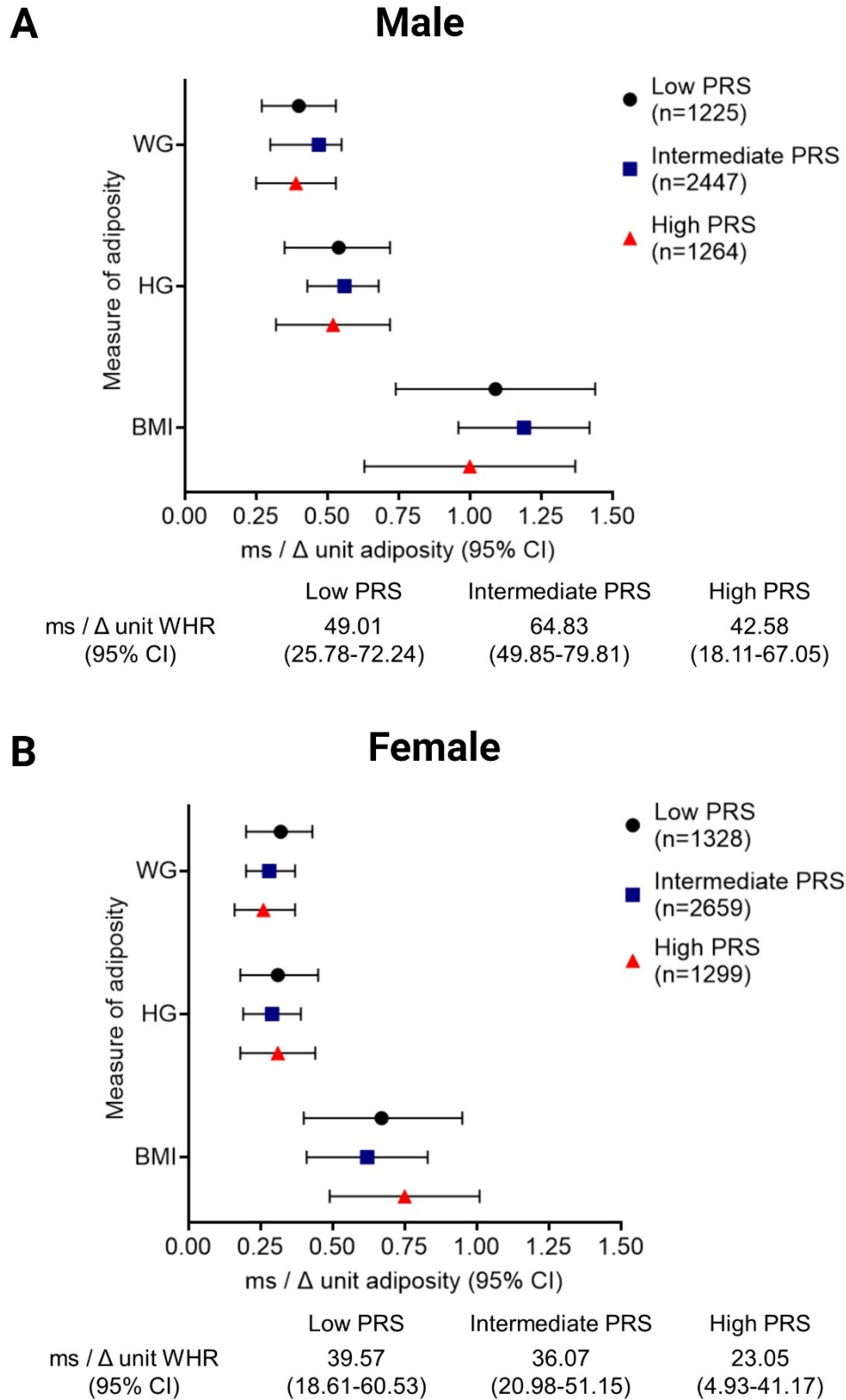


Figure 3.3: QTc prolongs comparably with increasing adiposity in males and females stratified by PRS. The change in QTc per unit and per SD increment of

adiposity (quantified by BMI, body mass index [kg/m^2]; HG, hip girth [cm]; WG, waist girth [cm]; WHR, waist:hip ratio [unit]) are shown stratified according to low (<25%), intermediate (25-75%) and high (>75%) PRS, representing high, intermediate and low repolarisation reserve, respectively for males **(A)** and females **(B)**. All associations between QTc and adiposity measures reached statistical significance $p < 0.001$. Reproduced from Patel et al (473) with permission under licence CC-BY 4.0.

3.3.4. Metabolic perturbation and obesity exert additive effects on QTc interval

Referenced to Ob-MU-, and after adjusting for sociodemographic factors, both obesity and metabolic perturbation were independently associated with longer QTc intervals in the pooled cohort (Ob+MU- +5.56ms, CI 4.48-6.64ms; Ob-MU+ +3.77ms, CI 1.66-5.87ms, both $p < 0.001$; Figure 3.4). The effects of obesity and metabolic perturbation on QT interval were additive, with concurrent obesity and metabolic perturbation (Ob+MU+) was associated with the longest QTc (+9.60ms, CI 7.79-11.22ms, $p < 0.001$). Similar observations were noted in sex-stratified analyses for males ($n=11,471$) and females ($n=12,014$) (Figure 3.4). These associations were not modulated by PRS for genetically-determined QT interval (all $p > 0.05$, Figure 3.4).

3.3.5. Metabolic perturbation exerts a greater effect than obesity on VA risk

Table 3.4 shows the characteristics for the UK Biobank cohort ($n=502,536$, male 22,3477, mean age 62.5 ± 8.1 years, 94.1% Caucasian) stratified by clinical phenotypes in which VA risk was assessed. Odds ratios (OR) for VA were calculated after addressing the imbalance between numbers of individuals with and without a history of VA; individuals without VA were down-sampled and randomly selected to achieve a ratio of 4:1 (no VA:VA).

	Total	Ob-MU-	Ob+MU-	Ob-MU+	Ob+MU+
N	502536	173308	193669	35355	100204
Sex					
Male	223477	76968	76033	21041	49435
Female	264811	95634	108374	13951	46852
Age	62.5 $\pm$ 8.1	60.3 $\pm$ 8.2	61.7 $\pm$ 8.0	66.3 $\pm$ 6.9	66.2 $\pm$ 6.8
Ethnicity					
Caucasian	472793	163880	182939	32666	93308
Non-Caucasian	22996	10169	8613	1504	2710
Non-specified	2717	920	886	314	597
TDI	-1.3 $\pm$ 3.1	-1.5 $\pm$ 3.0	-1.3 $\pm$ 3.1	-1.3 $\pm$ 3.2	-0.9 $\pm$ 3.2
No VA	483014	171382	183533	33899	94200
Total VA	3279	456	543	592	1688
<i>Re-entry VA</i>	<i>52</i>	<i>13</i>	<i>15</i>	<i>11</i>	<i>13</i>
<i>VT</i>	<i>2059</i>	<i>383</i>	<i>464</i>	<i>244</i>	<i>968</i>
<i>VF</i>	<i>1168</i>	<i>60</i>	<i>64</i>	<i>337</i>	<i>707</i>

Table 3.4: Characteristics for the UK Biobank participants stratified by clinical phenotypes in whom VA risk was assessed. Values presented as mean ($\pm$ standard deviation). Ob, obesity; MU, metabolically unhealthy; +, presence; - absence; TDI, Townsend deprivation index [units]; VA, ventricular arrhythmias. Age presented in years. Reproduced from Patel et al (473) under licence CC-BY 4.0.

In both males and females, obesity alone (Ob+MU-) was not associated with a higher incidence of total VA (comprising re-entry VA, VT and VF) compared to non-

obese healthy (Ob-MU-) individuals (**Table 3.5**). On the contrary, metabolic ill-health alone (Ob-MU+) was associated with more VA compared to Ob-MU- (males OR 5.96, CI 4.70-7.55), females OR 5.10, CI 3.34-7.80; both $p < 0.001$). Compared to Ob-MU-, metabolically unhealthy obese (Ob+MU+) males were more likely to have documented VA (OR 6.99, CI 5.72-8.54, $p < 0.001$); in Ob+MU+ females the OR for VA was 3.56 (CI 2.66-4.77; $p < 0.001$). History of VA was more frequent in Ob+MU+ males compared to metabolically unhealthy non-obese (Ob-MU+) males (OR 1.25, CI 1.05-1.49, $p = 0.036$), but not in females (OR 1.16, CI 0.80-1.68, $p = 0.315$). In males, obesity was associated with an increased VA incidence in the context of metabolic ill-health.

In subgroup analyses in comparison to Ob-MU-, Ob+MU- and Ob+MU+ were not associated with higher risk of re-entry VA (OR 1.08, CI 0.52-2.26, $p = 0.84$ and OR 1.82, CI 0.84-3.92, $p = 0.13$, respectively). However, Ob-MU+ was associated with a higher risk of re-entry VA (OR 4.28, CI 1.92-9.55, $p = 0.004$). With regard to VT, compared to Ob-MU-, Ob+MU- was not associated with higher risks of VT (OR 1.13, CI 0.99-1.30, $p = 0.074$). However, Ob-MU+ and Ob+MU+ were both associated with higher risks of VT (OR 3.22, CI 2.74-3.78 and OR 4.60, CI 4.08-5.18, respectively, both $p < 0.001$). Similar observations were noted for VF: Ob-MU+ was not associated with greater risks of VF than Ob-MU- (OR 0.99, CI 0.70-1.42, $p = 0.98$). VF risk was however, greater in Ob-MU+ (OR 28.40, CI 21.57-37.38, $p < 0.001$) and Ob+MU+ (OR 21.44, CI 16.47-27.91, $p < 0.001$) in comparison to Ob-MU-. These results were also consistent in sex-stratified analyses.

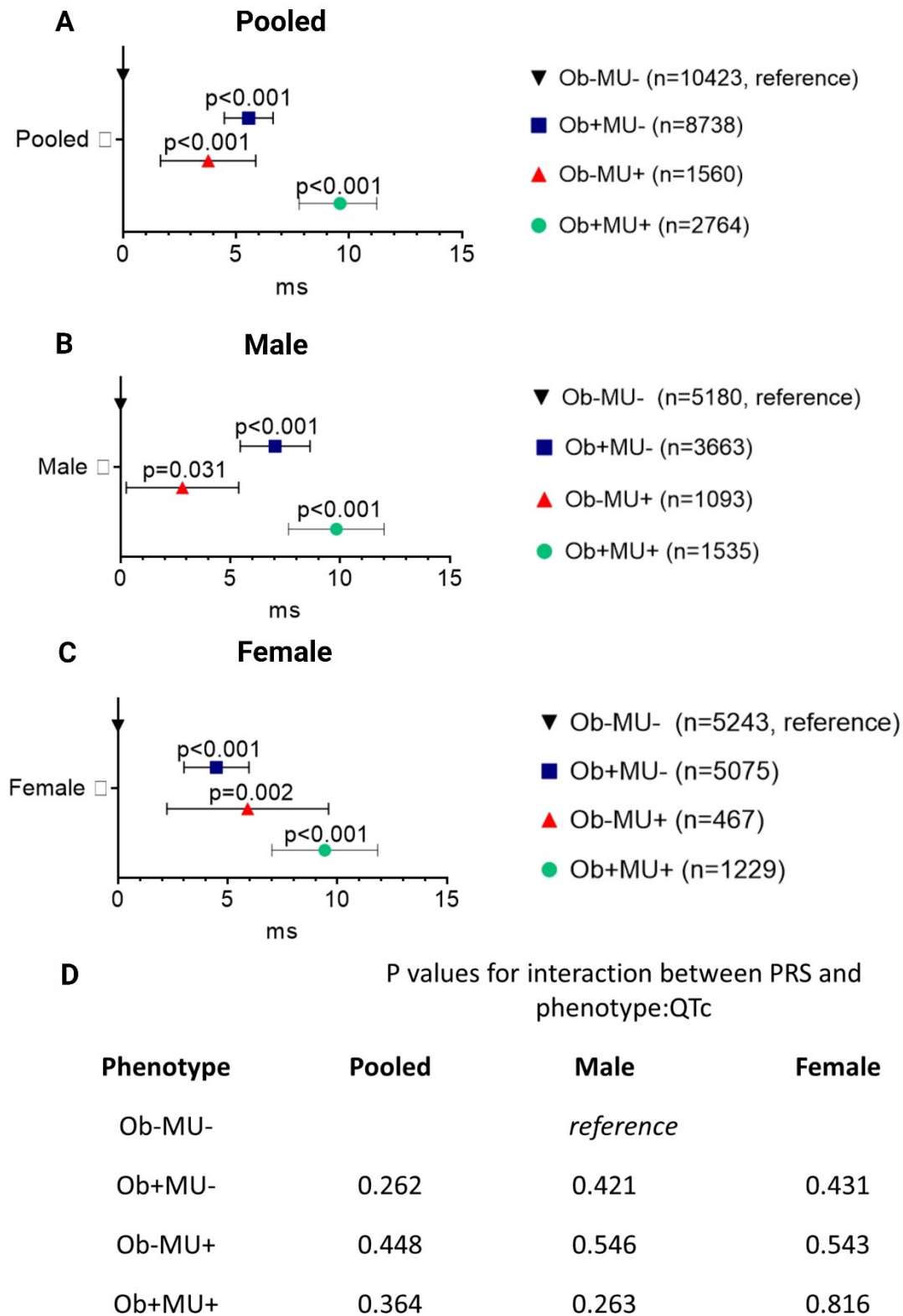


Figure 3.4: Obesity and metabolic perturbation are independently associated with longer QTc interval and their effects are additive when they co-exist. Differences in

QTc interval (Δ QTc) referenced to non-obese metabolically healthy (Ob-MU-) group for each clinical phenotype, in pooled **(A)** and sex-stratified **(B-C)** analysis. P values for the interactions between PRS and phenotype:QTc are shown in the in-set table **(D)** Ob, obese; MU, metabolically unhealthy; +, presence; -, absence; CI, confidence interval; PRS, polygenic risk score. Reproduced from Patel et al (473) under licence CC-BY 4.0.

Male			
<i>Reference</i>	Ob+MU-	Ob-MU+	Ob+MU+
<i>Ob-MU-</i>	1.10 (0.87-1.39) p=0.203	5.96 (4.70-7.55) p<0.001	6.99 (5.72-8.54) p<0.001
<i>Ob+MU-</i>	-	-	6.01 (4.98-7.26) p<0.001
<i>Ob-MU+</i>	-	-	1.25 (1.05-1.49) p=0.036
Female			
<i>Reference</i>	Ob+MU-	Ob-MU+	Ob+MU+
<i>Ob-MU-</i>	0.87 (0.64-1.18) p=0.326	5.10 (3.34-7.80) p<0.001	3.56 (2.66-4.77) p<0.001
<i>Ob+MU-</i>	-	-	5.61 (4.18-7.52) p<0.001
<i>Ob-MU+</i>	-	-	1.16 (0.80-1.68) p=0.315
Table 3.5: Metabolic perturbation confers a greater risk of ventricular arrhythmias than obesity in males and females. The odds ratios (OR) for total ventricular arrhythmias referenced to obesity phenotypes, stratified by sex and			

adjusted for age, ethnicity and Townsend deprivation index, are shown. Metabolic ill-health alone confers greater risks of VA than obesity alone. For male, obesity adds to the risk of VA in the context of metabolic ill-health. Ob, obese; MU, metabolically unhealthy; +, presence; -, absence; 95% confidence intervals in parentheses. P-values for comparisons with reference groups are provided alongside OR.

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3.4. Discussion

The analyses presented herein investigated the associations between adiposity and metabolic ill-health with QTc interval, as well as the burden of ventricular arrhythmias amongst participants of the UK Biobank stratified by weight and metabolic health.

The results can be summarised as follows: (1) QTc interval prolongs with increasing weight even after adjusting for sociodemographic, lifestyle factors and co-morbidity; (2) adiposity-related QTc prolongation is not modulated by genetic variations associated with ventricular repolarisation; (3) QTc interval is prolonged by metabolic obesity as well as metabolic perturbation, their respective effects are additive when they co-exists; and (4) metabolic ill-health confers higher risks of ventricular arrhythmias than obesity. These results support the evidence for obesity-related ventricular electrophysiological remodelling independent of concurrent cardiometabolic ill-health, and uniquely show that it is not offset by any protective effects of individuals' genetic constitution. Despite the association with abnormal ventricular repolarisation, the risks of corresponding arrhythmias is greater in metabolic-ill health than obesity.

3.4.1. Increasing adiposity prolongs QTc interval

Analyses presented herein show that increasing adiposity prolongs QTc interval independent of sociodemographic status, comorbidity and related genetics.

Importantly, this association held across several anthropometric indices. Waist circumference and waist:hip ratio are more sensitive to abdominal visceral adiposity which has been associated with more adverse health outcomes (472, 474) and increased arrhythmic risk (475) compared to subcutaneous fat. Indeed, abdominal obesity quantified by waist circumference is an independent risk factor for incident and recurrent atrial arrhythmias (476, 477). A meta-analysis of 29 prospective studies comprising over 2.5 million patients evaluating the association between different adiposity measures and arrhythmic risks showed that relative risk for AF was 1.28 (95% CI:1.20-1.38) per 5-unit increment in BMI, 1.18 (95% CI:1.12-1.25) per 10cm increase in waist girth, 1.32 (95% CI:1.16-1.51) per 10 cm increase in hip girth, 1.09 (95% CI:1.02-1.16) per 0.1-unit increase in waist:hip ratio and 1.09 (95% CI:1.02-1.16) per 5kg increase in fat mass (478). Similarly, relative risks for sudden cardiac death increased with increasing adiposity quantified by BMI (1.16 [95% CI:1.05-1.28] per 5 unit increment) and waist:hip ratio (1.82 [95% CI 1.61-2.07] per 0.1-unit increment), although not waist girth (1.03 [95% CI 0.93-1.15] per 10cm increment) (115). The mortality risk associated with central adiposity has been corroborated in a more recent systematic and meta-analysis (479). In the context of these studies, the results of this analyses suggest that adverse remodelling extends to ventricular as well as atrial electrophysiology and offers an electrocardiographic explanation for the higher risk of sudden cardiac death in obesity.

The findings of this study showing that obesity is associated with QTc prolongation contrasts with a similar one by Guo et al (459). In their study comprising over 11,000 participants from a rural region of China, Guo et al showed that QTc prolongation became more prevalent with increasing components of metabolic dysfunction irrespective of concurrent obesity, although obesity alone was not associated with longer QTc compared to non-obese metabolically healthy individuals (459). There are a number of differences between the present study and the one by Guo et al that may explain the observed differences. First, Guo et al's study sampled individuals from a specific region of China, whereas analyses presented herein used data from almost twice as many individuals from across different regions of the UK. Second, the numbers of participants in each of the obesity phenotypes were more unequally distributed in the study by Guo et al (56.8% Ob-MU-, 25.5% Ob-MU+, 4.4% Ob+MU- and 13.4% OB+MU+). Third, given the sex-dependent characteristics of ventricular repolarisation, the present study included sex-stratified analyses of the effects of increasing adiposity on QTc prolongation, rather than pooled analyses of the study cohort as presented by Guo et al. Most importantly, however, resting QTc interval was 10ms longer at baseline in the (reference) OB-MS- group ($426.8 \pm 21.5\text{ms}$ vs $416.5 \pm 25.0\text{ms}$) in the study by Guo et al, although QTc was comparable in the OB+MS- groups ($425.4 \pm 24.0\text{ms}$ vs $422.9 \pm 27.5\text{ms}$). QTc interval was also longer at baseline in OB-MS+ ($436.3 \pm 24.3\text{ms}$ vs $420.6 \pm 26.7\text{ms}$) and OB+MS+ ($436.6 \pm 25.9\text{ms}$ vs $427.2 \pm 27.0\text{ms}$) groups compared to the present study. Notably, although BMI amongst obesity phenotypes were similar between respective studies (Guo et al vs present study: Ob-MU- 23.0 ± 2.5 vs $23.8 \pm 2.4\text{kg/m}^2$; Ob+MU- 30.3 ± 3.5 vs $29.2 \pm 4.1\text{kg/m}^2$; Ob-MU+ 25.0 ± 2.1 vs $24.6 \pm 2.4\text{kg/m}^2$; OB+MU+ 30.4 ± 2.3 vs $30.6 \pm 4.5\text{kg/m}^2$), the study by Guo et al had a female preponderance across obesity

phenotypes and were younger, compared to a more equal sex-split and an older cohort in the present study. Detailed information on pharmacotherapy was not available in the present study as well as the study by Guo et al. Thus, the disparity between the results of this study showing obesity prolongs QTc, and the conclusion by Guo et al that QTc interval prolongs with metabolic perturbation but not obesity, may be explained by the differences in baseline QTc in the reference (Ob-MU-) group as well as other baseline characteristics.

3.4.2. Mechanisms underpinning adiposity-related QTc prolongation

The mechanisms underpinning adiposity-related electrophysiological remodelling reviewed in detail in Chapter 6 and have been reviewed by Patel et al (1) and others (422, 480, 481, 482). Briefly, adipose tissue is the largest endocrine organ that secretes pro-inflammatory and arrhythmogenic adipokines, which modulate ionic channels and prolong ventricular action potential duration (APD). This is particularly true in obesity in which adipocytes hypertrophy and adipose depots outgrow circulatory perfusion and resulting in hypoxia. Hypoxic adipocytes secrete pro-inflammatory cytokines that contribute to low-grade chronic inflammation in obesity. The consequence for myocardial tissue is particularly stark, given its intimate relationship with its visceral fat depot. For example, IL-6 increases L-type calcium (I_{CaL}) (483) and suppresses rapid delayed rectifier K^+ (I_{Kr}) currents (484). IL-1 β reduces the transient outward K^+ current (I_{to}) (485) and increases I_{CaL} also causing APD prolongation(194). TNF- α suppresses delayed rectifier K^+ currents (486) and reduces $K_{v4.2}$ and $K_{v4.3}$ protein and K_v channel-interacting protein-2 mRNA expression (487). More specifically, the pericardial fat secretome has been associated with reduced I_{Kr} and increased I_{CaL} currents in metabolic syndrome

resulting in APD prolongation (488). Serum levels of the adipokine fatty-acid binding protein has also been suggested as a biochemical proxy for prolonged QTc interval and reduced ejection fraction in patients with stable angina (489). Similarly, plasma adiponectin may modulate ventricular repolarisation and thereby QTc interval (490). Given that APD prolongation manifests as QTc prolongation on 12-lead ECG, these results are compatible with adipokine-mediated modulation of ionic channels.

3.4.3. Adiposity and adverse clinical cardiovascular outcomes

The experimental evidence for the deleterious effects of adipose volume is supported in clinical studies. For example, amongst 42,598 UK Biobank participants that have undergone cardiac MRI, pericardial adipose volume has been linked to adverse cardiovascular phenotypes. In analyses that resemble the approach adopted in the present study, Ardissino et al first used a convolutional neural network-based tool to automatically quantify pericardial fat in 4-chamber cine images (491) and then, using linear regression, evaluated any independent associations with cardiac structure and function by adjusting for confounders, including adiposity quantified by anthropometric indices used in this study (WHR, body impedance etc) (492). They showed that pericardial fat volume amongst the UK Biobank cohort is associated with greater LV thickness and mass and lower LV global function index, stroke volume and ejection fraction. Importantly, these associations were only partly explained by concurrent hypertension and dyslipidaemia suggesting pericardial fat exerts direct adverse effects on cardiac structure and function (492).

Although QTc prolongation described herein is in the context of increasing abdominal and whole-body adiposity rather than pericardial or epicardial fat volume,

it nonetheless suggests that the adverse effects of abdominal visceral adiposity extends to ventricular electrophysiological remodelling. This is consistent with results from a meta-analysis of 63 observational studies comprising over 350,000 individuals in whom abdominal and overall adiposity conferred higher risks of AF (OR 1.32 95%CI 1.25–1.41, OR 1.11 95%CI 1.08–1.14 and OR 1.22 95%CI 1.17–1.27 per standard deviation increment in WG, WHR and BMI, respectively) (101). Although the odds for AF were greater with increments in epicardial fat volume than abdominal or overall adiposity (101) and that regions of abnormal electrophysiology co-localise with epicardial fat (99), the fact that excess epicardial fat often occurs in the context of excess abdominal visceral adiposity suggests that this depot may also contribute to adverse electrophysiological remodelling via systemic inflammation induced by adipokines (482). Indeed, aside from the proarrhythmic effects of the inflammatory adipose secretome, chronic obesity confers arrhythmic risks via systemic metabolic complications such as non-alcoholic fatty liver disease (NAFLD) (493, 494). NAFLD has been linked to higher rates of adverse cardiovascular outcomes (495), that includes greater risks of atrial fibrillation (496, 497, 498), QTc prolongation (499, 500) and ventricular arrhythmias (501, 502). Insulin resistance and dysregulated lipid metabolism resulting in release of pro-inflammatory, -fibrotic and -oxidising hepatic mediators may underpin these observations (494, 503, 504). Furthermore, NAFLD favours epicardial fat accumulation resulting in cardiac lipotoxicity, fatty infiltration, fibrosis and yet more secretion of inflammatory mediators, that creates a vicious cycle of increasing metabolic perturbation to cause structural, electrical and autonomic remodelling (152, 505).

The effects of obesity on cardiac electrophysiology extends beyond conventional electrocardiographic indices. For instance, Li et al showed that neural network models applied to resting 12-lead ECGs of UK Biobank participants could be used to determine whether they had BMI greater or less than 25kg/m^2 with accuracies of 73% and 75% in males and females, respectively (321). Furthermore, instances in which NN-predicted over-predicted an individual's BMI, i.e. NN-predicted BMI was greater than calculated BMI, were partly explained by central abdominal visceral adipose tissue volume (VAT) and concurrent metabolic ill-health. In other words, participants with calculated BMI $<25\text{kg/m}^2$ who had concurrent hypertension, diabetes, dyslipidaemia or coronary heart disease were more likely to be identified as overweight or obese. Although the authors acknowledge that neural network-based derivations of obesity status from ECGs are unlikely to replace routine anthropometry, their results are proof-of-concept that these methods may add value to standard indices and that obesity and cardiometabolic perturbations induce subtle electrocardiographic changes.

Interestingly, a recent study using MRI and genetic data from the 40,000 participants of the UK Biobank has yielded important insights into how genetics influences distribution of adipose tissue (506). This is noteworthy given that fat distribution varies considerably for a given BMI, which in turn has clinical implications for the development of cardiometabolic perturbations. In their study, Agrawal et al show that non-conventional indices of the adiposity such as ratio of visceral adipose to total adipose volume had a greater heritability than conventional indices like BMI (506). Furthermore, they identified 39 unique SNPs determining adipose distribution and, unlike SNPs determining BMI by modulating satiety in the central nervous system,

these influenced fat distribution by directly modulating adipocyte function. Moreover, individuals with a genetic predilection for accumulating VAT were at higher risk of cardiometabolic ill-health compared to those with a predilection for accumulating gluteofemoral fat (506).

3.4.4. Genetic modulation of QTc in context of increasing adiposity

GWAS studies suggest that up to 10% of QTc interval variation in a given population can be explained by genetic differences associated with myocardial ionic homeostasis(460). Despite this, the influence of genetic variation on QT prolongation in the context of obesity and metabolic dysfunction has not been investigated. In this study, a PRS was generated based on 66 SNPs available in the UK Biobank that are known to influence QTc (460, 465) as a quantifiable metric of an individual's genetic propensity for longer than normal QTc, and thereby a surrogate for repolarisation reserve. There was no consistent evidence to show that low PRS, as a proxy for high repolarisation reserve, attenuates or protects against any QTc-prolonging effects of increasing adiposity. Furthermore, the interaction of PRS with the relationships between adiposity and QTc was not statistically significant. These results suggest that excess adiposity is a sufficiently potent extrinsic stressor that overwhelms any protective effect of genetics on QTc prolongation, and that modifiable rather than non-modifiable risk factors are more important determinants of QT interval.

3.4.5. Metabolic perturbation prolongs QTc interval

One of the aims of this study was to investigate the effects of metabolic syndrome on QTc interval amongst a large sample of the UK population using their most common and widely used definitions. To do this, the International Diabetes Federation (IDF)

global consensus definition for obesity was used in the context of metabolic syndrome. Thus, obesity was defined as BMI $>30\text{kg/m}^2$ or waist circumference $>94\text{cm}$ for males and $>80\text{cm}$ for females. The rationale for so doing is that these metrics are used to define obesity by the National Institute for Health and Care Excellence (NICE) to inform clinical practice in the UK, and are also consistent with those outlined by the European Group for Study of Insulin Resistance (EGIR) (507). The joint interim statement of the IDF Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation; International Atherosclerosis Society and the International Association for the Study of Obesity also support using the IDF global consensus definition of obesity using BMI and WG (71). Furthermore, given that the UK Biobank cohort in this study comprised a largely Caucasian population, 94cm and 80cm were the ethnicity-based WG thresholds to define obesity in males and females, respectively.

Amongst the four clinical phenotypes, QTc was longest in those who are obese and metabolically unhealthy (Ob+MU+) and shortest in those who have neither (Ob-MU). Metabolically healthy obese (Ob+MU-) and metabolically unhealthy lean (Ob-MU+) individuals have comparable QTc intervals that were longer than Ob-MU- individuals but shorter than observed in Ob+MU+. These observations held true in sex-stratified analyses. PRS did not modulate the relationships between phenotype and QTc. These results are consistent with previous small-scale studies and suggest that obesity and metabolic perturbation, whether alone or in combination, are not electrophysiologically benign. For example, metabolic syndrome has been associated with prolonged ventricular repolarisation as a consequence of reduced voltage-gated K^+ channel current, and greater intracellular Na^+ and Ca^{2+} .

concentrations from increased activity of $\text{Na}^+/\text{Ca}^{2+}$ and Na^+/H^+ exchanger proteins (508).

3.4.6. Metabolic ill-health confers a greater risk of VA than obesity

The final aim of this study was to assess the burden of ventricular arrhythmias amongst the UK Biobank population stratified by their obesity and metabolic status. To do this, all available data in the UK Biobank relating to ICD codes for ventricular arrhythmias were included in the analyses, including patients with ventricular extrasystole scheduled for catheter ablation. Participants with ventricular ectopy would be expected to be younger and relatively healthier than those with a history of ventricular fibrillation that their inclusion in this study would have resulted in a more heterogenous study cohort compared to one in which they were excluded. Their inclusion can be justified, however, given that obesity increases risk of ventricular ectopy particularly in the context of left ventricular hypertrophy (44, 509, 510). Given that a critically timed extrasystole may trigger re-entry leading to ventricular tachycardia/fibrillation, inclusion of catheter ablation of the ventricular wall was justified to ensure the analyses comprehensively assessed ventricular arrhythmic risks in the UK Biobank cohort. The effects of left ventricular hypertrophy or ejection fraction on arrhythmic risk could not be considered in the analyses as an insufficient number of the study participants had undergone either echocardiography or MRI at the instances from which their ECG and other data was collected. Therefore, the left ventricular ejection fraction of many of the present study's cohort at the time of their ECG and anthropometry was unavailable and unknown. Although some of a subset study cohort may have subsequently participated in the MRI studies of the UK Biobank, their scans would have been performed a considerable time after their ECG

and anthropometry recordings. Considering these time lags, including any such data in the analyses may have adversely biased the results.

VA occurred most frequently in the Ob+MU+ phenotype and least frequently in Ob-MU-, suggesting that the four obese phenotypes exist on a spectrum of increasing arrhythmic risk. Referenced to Ob-MU-, the odds ratios of VA in metabolically unhealthy individuals (Ob-MU+) were over five-fold greater in males and females. In both sexes, metabolic dysfunction conferred incremental VA risks in the absence and presence of obesity. Obesity in the context of metabolic dysfunction, i.e. Ob-MU+ vs Ob+MU+, was associated with an incremental VA risk in males but not females. Although both obesity and metabolic perturbation can increase the risk of VA, the latter appears to have a disproportional effect. These results were observed in the whole UK Biobank cohort that had comparable sociodemographic baseline characteristics to the subset in which the effect of adiposity on QTc interval was assessed. Factors aside from abnormal repolarisation, such as aberrant depolarisation, fibrosis and increased incidence of structural heart disease and coronary artery disease, may also contribute to arrhythmic risk in this group. For example, diabetic cardiomyopathy is the consequence of chronic activation of renin-angiotensin-aldosterone and sympathetic nervous systems, oxidative stress, and endothelial dysfunction, which promote cardiac tissue interstitial fibrosis and thereby conduction and repolarisation heterogeneity that increases arrhythmia propensity (511). The greater risk of VA in metabolically unhealthy individuals compared to their healthy obese counterparts may also be attributed to a greater and more unstable plaque burden even in non-culprit coronary lesions resulting in more adverse outcomes (512).

3.4.7. Limitations

There are a number of inherent limitations with retrospective analyses using data from a resource as large as the UK Biobank. This was a cross-sectional study in which middle-aged Caucasian individuals were over-represented and therefore these results may not extrapolate to other ethnic groups or ages. Participation in the UK Biobank was by invitation to individuals living within a predetermined vicinity of any of the 22 assessment centres across the UK and therefore is not true representation of the UK population. It is also possible that those partaking in the UK Biobank had a greater interest in health topics or related research that may translate into their own lifestyle choices. Obesity phenotypes were unequally represented and ECG data was available for only approximately 5% of the UK Biobank cohort. It is possible that the results reported herein may change with a more even distribution amongst these groups. Similarly, there were insufficient VA episodes amongst the subset of participants with recorded ECGs to ascertain any associations between QTc and VA.

The analyses were not adjusted for the effects of medication as this information was not available in sufficient detail in the UK Biobank. Although pharmacological data was collected amongst participants, self-reporting questionnaire did not require them to state or provide the name or class of drug. Instead, participants were required to answer general questions, for example, if they were taking blood pressure lowering medication. Incorporating medication into the analyses was further complicated by the fact a class of drug may have more than one clinical indication, making it difficult to accurately or reliably determine the indication for its prescription. For these reasons, it was not possible to establish which of the participants were prescribed

pharmacological agents, if any, that may cause QTc prolongation. Similarly, electrolyte results were also limited for the subjects included in the study. According to the UK Biobank, only biomarkers established as risk factors for disease, used for clinical diagnostic measures, or characterising phenotypes that are not otherwise well assessed were measured in participants. Although this included liver function tests, C-reactive protein, sex hormone profiles and lipid profiles, there were no detailed results on electrolytes (sodium, potassium, calcium and magnesium). It was therefore not possible to ascertain whether these were within normal range for participants included in the analyses. Participants with asymptomatic VA or with VA-related symptoms that did not result in clinical evaluation would not have been included in the analyses presented herein. Finally, the assessment of the association between obesity and metabolic syndrome and VA may be influenced by the accuracy of ICD coding that was used to determine medical diagnoses from healthcare records.

3.5. Conclusion

Amongst individuals with recorded ECG and anthropometry data in the UK Biobank, adiposity and metabolic perturbation are both associated with increased QTc after adjusting for confounding variables, and their co-existence has an additive effect. Individuals' genetics do not significantly modulate the relationship between adiposity and QTc, suggesting modifiable rather than non-modifiable risk factors are more important determinants of QTc interval. Despite comparable QTc-prolonging effects of adiposity and metabolic ill-health, metabolic ill-health alone is associated with a greater burden of VA than obesity alone amongst 500,000 individuals in the UK population, with individuals with both obesity and metabolic ill-health exhibiting the highest burden of VA.

Chapter 4: Developing a standardised framework for signal pre- and post- processing in electrocardiographic imaging

4.1. Introduction

Electrocardiographic imaging (ECGi) involves the reconstruction of epicardial electrograms using body surface ECG signals and individuals' unique heart and torso geometries, by solving the inverse problem of electrocardiography. This problem is inherently ill-posed meaning that the reliability and accuracy of the output (reconstructed unipolar epicardial electrograms) is highly susceptible to noise and artefact in the input (body surface potentials, BSPs) (406). This is compounded by the fact that solving the inverse problem can yield several solutions. Although regularisation processes can constrain these solutions to those that are electrophysiologically plausible (297, 298, 359), ECGi outputs are nonetheless exquisitely sensitive to pre-processing of BSP and post-processing of unipolar electrograms. Indeed, given that ECGi outputs are dependent on the pre- and post-processing techniques and the absence of ground-truth electroanatomic contact mapping data in the study cohort, it was imperative to optimise the methods by which activation and repolarisation parameters could be computed to accurately represent data represented by BSPs before their application in the main study (Chapter 5). Thus, the aim of this chapter is to develop a standardised approach for pre- and post-processing of BSPs and unipolar electrograms, respectively, to generate reproducible and robust ECGi outputs.

4.1.1 Aims

With respect to pre-processing BSP, the primary aims were to:

- 1) Determine a method to select BSP that would yield the most stable averaged signal

- 2) Determine the optimal number of BSP for signal averaging to reconstruct atrial and ventricular epicardial electrograms
- 3) Determine the method by which total activation (AT) and repolarisation (RT) times can be calculated by reducing the effect of outliers

With respect to post-processing unipolar electrograms, the aims were to:

- 1) Develop an automated method to exclude low-amplitude or ambiguous T-waves
- 2) Determine the T-wave upslope gradient threshold that identified low-amplitude T-waves
- 3) Determine the optimal spatial median filter that would preserve spatial repolarisation gradients

4.2. Methods

To achieve the aims outlined above, the optimisation of data process was undertaken in a sample of the study population for two reasons: first, participant recruitment to the clinical study had not been completed during the optimisation phase and, second, optimisation in a small sample was computationally more efficient and avoided introducing bias into the final analyses. Typically, data from between 5 and 10 participants was used, with an equal split between lean and obese participants wherever possible, to avoid bias and ensure that the methods were robust in both groups. The numbers of participants from whom data was used to address each aim is detailed in each of the sections in this chapter. Pre-processing of BSP was performed using Python software (Python Software

Foundation, <https://www.python.org/>) and post-processing of unipolar epicardial electrograms using customised software (MATlab, MathWorks, Natick, USA). The results presented in this chapter were acquired with the technical assistance and expertise of Nikesh Bajaj (research associate, National Heart and Lung Institute).

4.3. Statistical analysis

One-way ANOVA was used to compare data between groups. Comparisons between lean and obese participants was performed using unpaired Student's T-tests for normally distributed data. Free-marginal multi-rate kappa, a chance-adjusted measure of agreement, was used to compare results between two operators working independently (513) using an on-line platform (www.justusrandolph.net/kappa). Data are presented as mean $\pm$ standard deviation, unless otherwise stated. Statistical analyses were performed using Prism 9.3.1 (GraphPad Software, CA, USA). Further relevant details pertaining to methods and statistical analyses are provided within the following sections.

4.4. Results

4.4.1 Pre-processing of BSPs

4.4.1.1. Rationale for averaging BSPs

The aim of the studies in this section was to develop a method of selecting BSPs which, when averaged, would generate the most stable signal that represented the data captured on the body surface. This section provides the rationale for this.

The inverse problem of electrocardiographic is ill-posed and therefore even a small degree of noise on BSPs may be amplified in the computed epicardial electrograms. Noise and artefact may arise from poor skin contact or movement during data acquisition. Furthermore, the optical cable connecting the AD-box and analogue input box to the USB2 receiver was extremely sensitive to ambient lighting that may have resulted in artefact in the recorded signals. Although the IIR, 50Hz and Savitzky-Golay filters would negate some of these effects, it was possible that low amplitude oscillations may not be smoothened by these pre-processing techniques. Previous ECGi studies have therefore averaged BSPs to improve signal quality in addition to application of signal filters (300). However, the selection of BSPs for averaging also has important downstream implications in the ECGi methodology. Although the R-wave is commonly used to align BSPs because it is the most easily discerned and largest amplitude feature within a single cardiac cycle, it is also the least likely to be obscured by artefact. Even though averaging consecutive BSPs aligned by their respective R-waves enables ventricular activation to be studied reliably, this method can result in misalignment of P- and T-waves, particularly in instances of marked sinus arrhythmia and large RR variability, resulting in bifid P- or T-waves. This would inevitably introduce errors in the computation of epicardial electrograms and thereby atrial activation and ventricular repolarisation, respectively. The following sections present the results of a method that reproducibly identified consecutive resting BSPs for signal averaging that minimised P- and T-wave variability.

4.4.1.2. BSPs from the minute-segment with the least RR variability minimises P- and T-wave variability

To determine the selection of BSPs for signal averaging, first, the 10-minute resting BSP data from 10 (5 obese and 5 lean) individuals were divided into one-minute segments and the RR intervals between consecutive BSPs in each minute segment were calculated. RR variability was quantified by the standard deviation (SD) of the RR intervals in each minute-segment. In sinus rhythm and the absence of ectopic beats, the RR variability is likely to be minimal, and either stable or periodic in nature. By contrast, artefact is often non-periodical and characterised by oscillations that are misidentified as the R-wave of the QRS complex resulting in differences in RR interval and therefore RR variability. Thus, in the absence of a conventional method for objectively identifying artefact, an increased RR variability was considered a surrogate indicator of segments of BSPs that may contain noise. In other words, RR variability was considered an indicator of BSP stability in this study.

To determine the effects of RR variability on P- and T-wave alignment before signal filtering on BSPs, 60 consecutive BSPs in the minute-segments with the lowest RR variability and two other randomly selected minute-segments were chosen. The potentials in these three minute-segments were aligned by their respective R-waves to observe the corresponding variability in P- and T-wave alignment.

The results of this are shown in a representative example in Figure 4.1. The alignment of P- and T-waves is most uniform in the minute-segment with the lowest RR variability (column A). Artefact and greater RR variability in the other two minute-

segments results in greater misalignment of the P- and T-waves, which is reflected by variations in the corresponding BSP (column B).

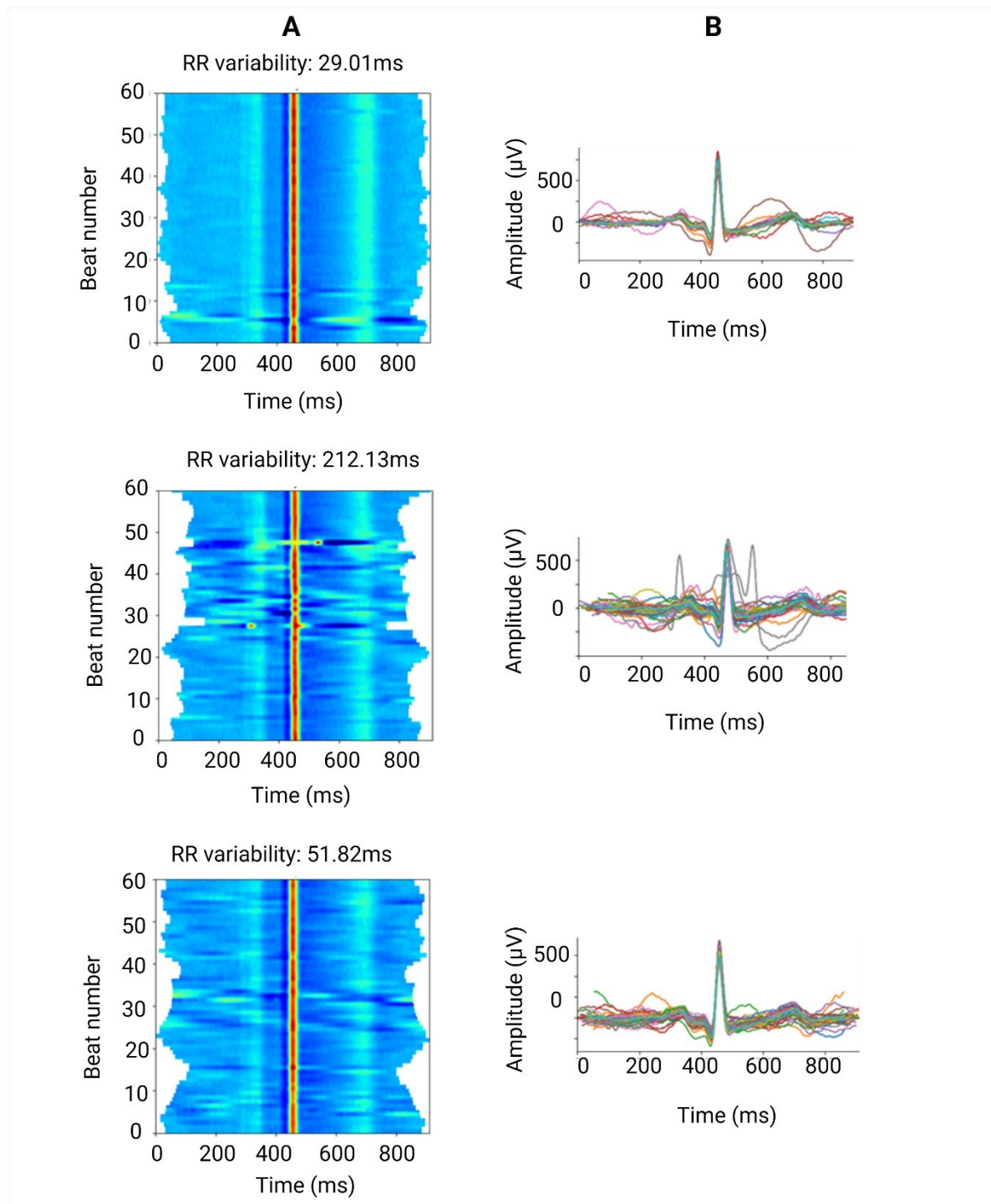


Figure 4.1: Minute-segment with least RR variability demonstrates greatest P- and T-wave alignment. The first 60 BSP from the minute-segment with the least

RR variability (**A**, top) shows more consistent alignment of the P- and T-waves, compared to two other minute-segments with greater RR-variability (**A**, middle and bottom). The differences in alignment of the P- and T-waves between minute-segments are explained by the artefact and temporal variations observed in the minute-intervals with greater RR variability (**B**, top vs middle and bottom).

Figure 4.1 shows that the minute-intervals with greater RR variability contain more artefactual signals. Given that these may adversely affect signal averaging, the method described above was repeated by after removal of artefactual potentials, which were defined as those with a power of more than 1.5 times greater than the interquartile range above the third quartile taken over the entire 10-minute recording. The results of this are shown in Figure 4.2. Even after removal of artefact, the least variation in P- and T-wave alignment is observed in the minute-segment with the least RR variability compared to the other two minute-segments, in which there is more baseline wander and low-amplitude oscillations, resulting P- and T-waves being relatively obscured.

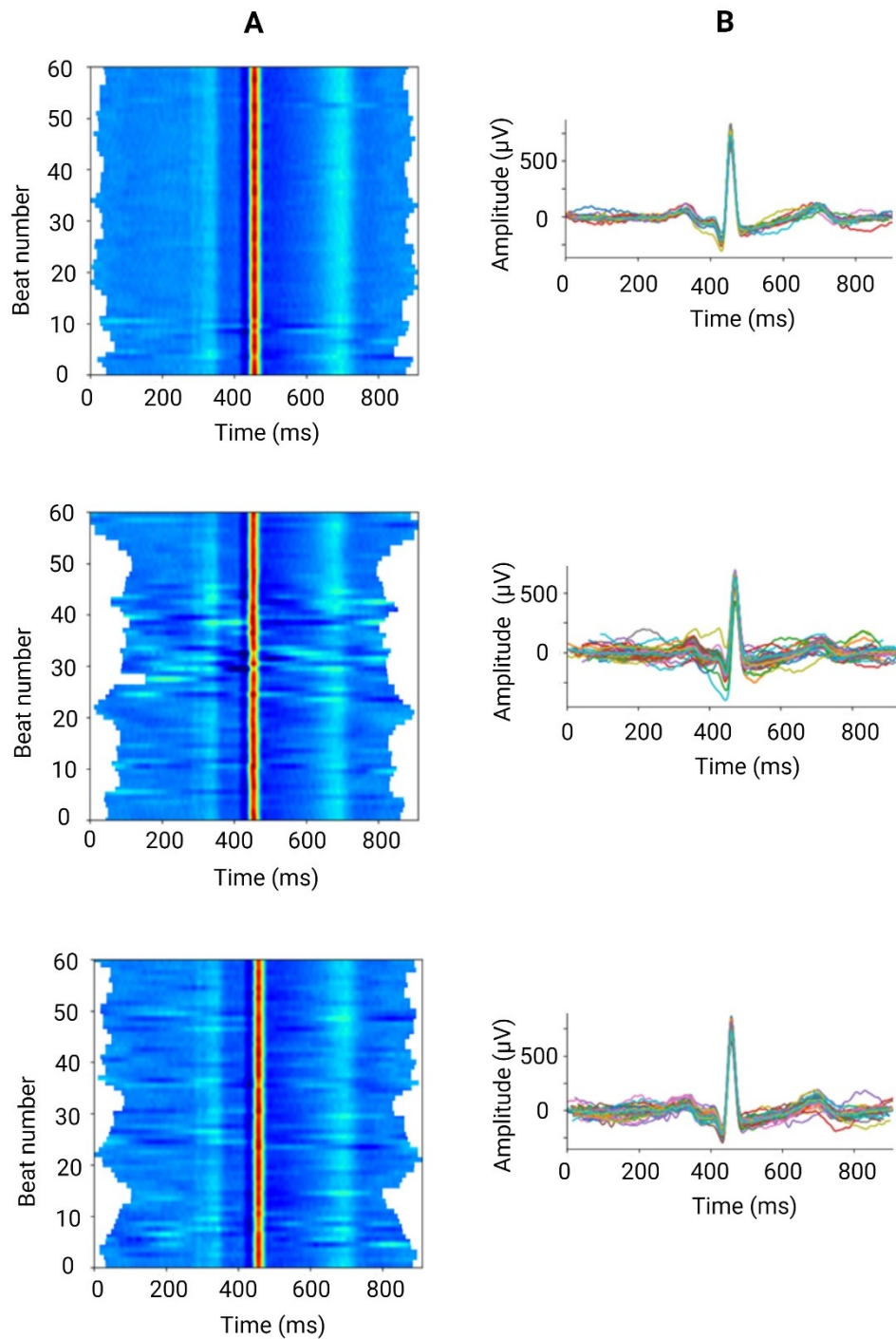


Figure 4.2: Alignment of temporal features of BSP after exclusion of artefact.

(A) P- and T-wave alignment is more consistent in the minute segment with the least RR variability even after removal of potentials defined as noise (top vs middle and bottom). **(B)** Potentials from the minute-interval with the least RR-variability were

more stable enabling P- and T-waves to be more easily discerned (top) compared to two other minute-segments in which baseline wander and noise persisted even after filtering (middle and bottom).

Having determined that P- and T-wave variation was lowest amongst consecutive BSPs in the minute-interval with the least RR variability, the next aim was to compare the averaged BSPs between the minute-segments. Based on the results presented in Sections 4.4.2 and 4.4.3, the hypothesis was that the P- and T-waves of the signal averaged BSP from the minute interval with the least RR variability would most closely resemble the P- and T-waves of their constituent BSPs.

Figure 4.3 shows the effect of averaging randomly selected 10 consecutive BSP in each of the minute-segments shown in Figures 4.1 and 4.2. Two observations can be made from these results. First, the topologies of averaged BSP are visibly different between the three minute-segments. Second, and more importantly, there is less variation in the mean amplitude of the averaged body surface potential from the minute-segment with the least RR variability.

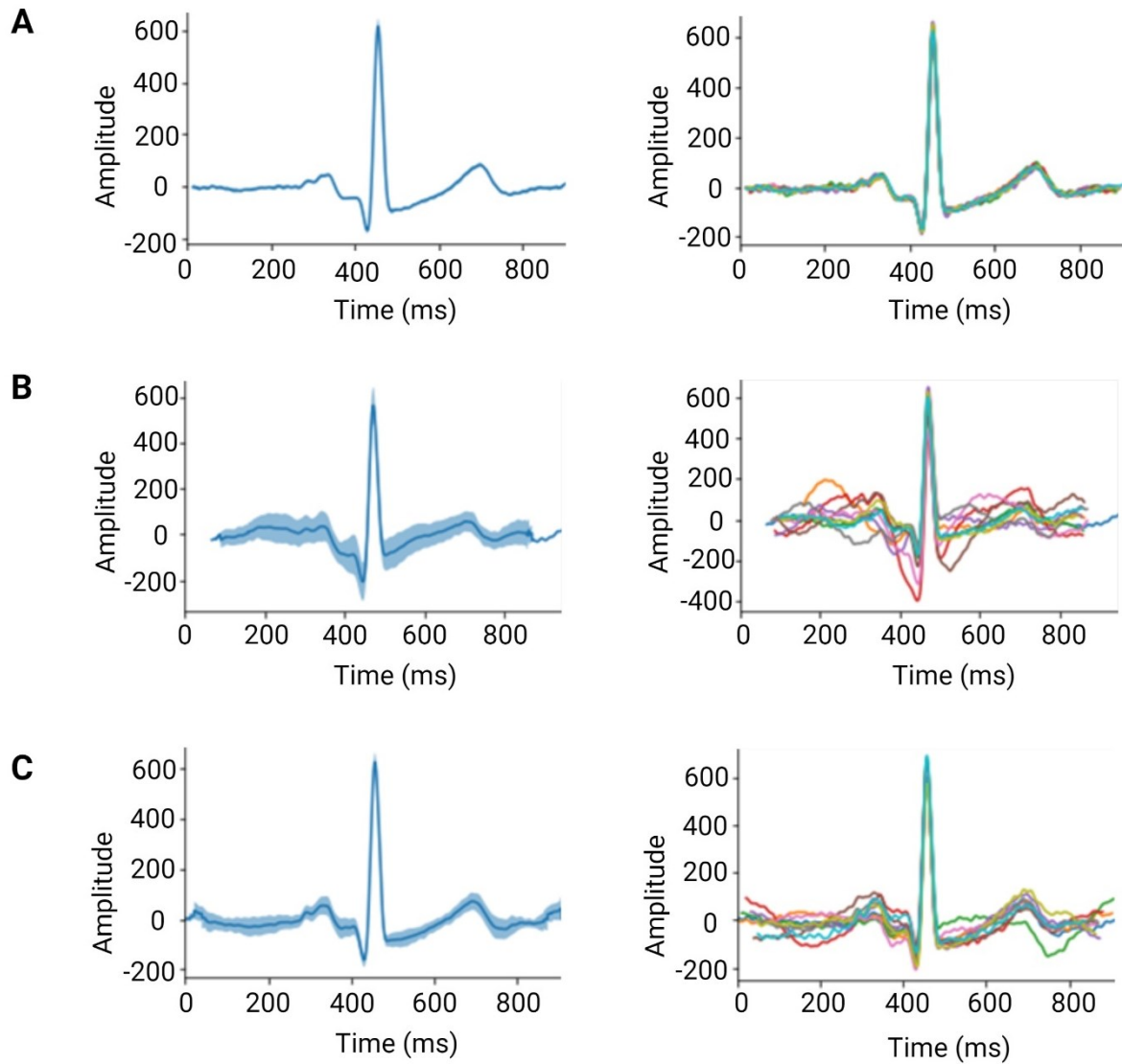


Figure 4.3: Averaging BSPs from the minute interval with the least RR variability generates P- and T-wave topologies that resemble those of their constituent BSPs. Average potentials are shown on the left with shaded areas denoting amplitude variation, quantified by the standard deviation of 10 consecutive potentials. The 10 consecutive potentials used to generate the averaged potential are shown on the right. (A): The minute-segment with the least RR variability has the least amplitude variability and the averaged potential closely resembles each of the 10 potentials from which it was generated. By contrast, there is greater amplitude variability of the averaged potential calculated using 10

consecutive potentials from two other randomly selected minute-segments (B and C). Amplitude in μV .

The implication of these observations is that averaging consecutive BSP recorded at different timepoints at rest yields different waveforms. Thus, the choice of the minute-segment from which signal BSP are used to solve the inverse solution is likely to influence computation of atrial AT and ventricular RT. Selection of potentials from minute-intervals with greater RR variability is likely to generate an averaged potential that bears less resemblance to the BSPs compared to utilisation of BSPs from the minute-segment with the least RR variability. Computing unipolar electrograms from an averaged potential with the former would generate outputs that inaccurately represent activation and repolarisation times and patterns. By contrast, these results show suggest that signal averaging BSPs from the least RR minute-interval is more likely to generate activation and repolarisation patterns that accurately reflect recorded BSP, and also minimising the effect of artefact that would adversely affect solving the inverse problem. Together with results in Section 4.4.2, these results show that averaging BSP from the minute interval with the least RR variability is a robust method to ensure that the signal averaged P- and T-wave topologies most closely resemble those of their constituent BSPs.

4.4.1.3. Averaged BSPs from the minute-segment with the least RR variability have the least T-wave amplitude variation

The qualitative results from the previous section show that averaging BSPs from the minute-segment from the least RR variability improves the quality of input of the ECGi method by minimising the effect of artefact, and also generates an averaged potential that closely resembles its constituent BSPs. These findings were quantified by comparing the mean T-wave amplitude variation of 10 BSPs sampled in the three one-minute segments shown in Figure 4.3 from 5 obese and 5 lean individuals. The T-wave amplitude variation was quantified by standard deviation of the 10 BSPs in each individual and comparisons were made using one-way ANOVA.

Figure 4.4 shows that the T-wave amplitude variation of the 10 BSPs sampled from the minute-segment with the least RR variability was significantly less than that observed in the other two minute-segments that had greater RR variability (least RR variability segment: $12.2 \pm 7.3 \mu\text{V}$; segment 1: $32.4 \pm 12.6 \mu\text{V}$; segment 2: $26.1 \pm 5.3 \mu\text{V}$, $p < 0.0001$). These corroborate the qualitative results of the previous sections, and show that averaging BSPs from the minute-segment with the least RR variability generates the most stable averaged potential that is most akin to the recorded BSPs.

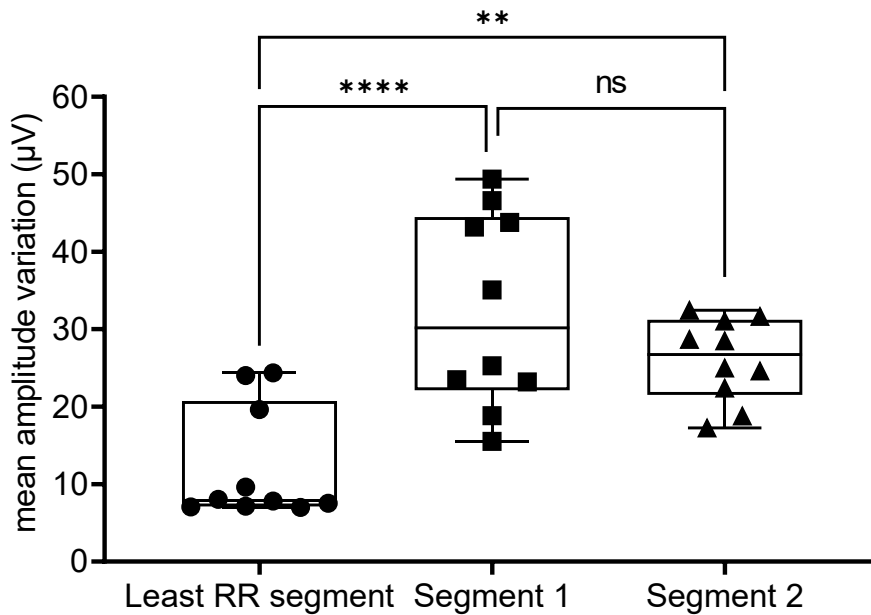


Figure 4.4: T-wave amplitude variation is lowest amongst averaged BSPs sampled from the minute-segment with the least RR variability. Graph shows the mean amplitude of 10 BSPs sampled from three different minute-segments from 10 study participants (5 obese and 5 non-obese).

In summary, Figures 4.3 and 4.4 suggest that BSPs from the minute-segments with the greatest RR variability are also subject to the greatest variability in amplitude that may include artefact. On the other hand, averaging BSP from the minute-segment with the least RR variability generates a waveform that most closely resembles its constituent BSPs, that also has the least amplitude variability, and therefore is most likely to generate activation and repolarisation patterns that accurately correspond to the potentials recorded on the torso. Based on these results, BSPs from the minute segment with the least RR variability were selected for signal averaging in the main study, and were used in the results presented in the following sections of this Chapter. Although the implication of this was that BSPs would be averaged from

different minute-segments in the study population, this was unlikely to introduce bias in the final analysis as the BSP were recorded under otherwise identical resting conditions in an upright position with the same equipment in each participant.

4.4.1.4. Removing poor quality BSPs defined by power

Although the results in the previous sections suggest that the minute-segment with the least RR variability was the least likely to be contaminated with artefact, it was possible that BSPs remained influenced by some noise. Therefore, before the optimal number of BSPs for signal averaging could be determined, it was necessary to eliminate any such signals that may adversely influence this process. To do this, any BSP in the minute-segment with the least RR variability with power 1.5 times greater than the interquartile range above the third quartile were removed. This is represented in Figure 4.5.

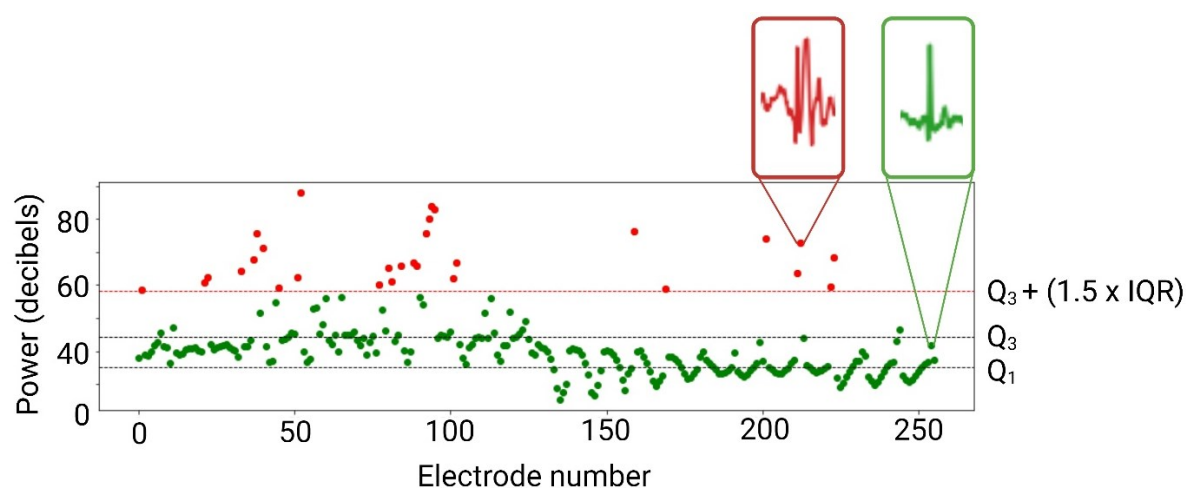


Figure 4.5: Filtering of BSPs by power. An example of the power of BSP (y-axis) recorded using 256 body surface electrodes (x-axis) over a single beat. Potentials with power of more than 1.5 times greater than the interquartile range ($Q_3 - Q_1$) above the third quartile (red dashed line) were defined as artefact, denoted as red

dots, and excluded from computation of the averaged potentials. Examples of BSPs above and below the power threshold are shown in red and green, respectively, in the inset boxes. The interquartile range for BSPs based on their power is shown by black dashed lines.

4.4.1.5. Determining the number of BSPs for signal averaging to generate stable total activation and repolarisation times

Having determined that the BSP in the minute-segment with the least RR variability demonstrate the least variability around their averaged potential, the next step was to determine the optimal number of BSPs for signal averaging, and in tandem, to determine the method by which total activation and repolarisation times should be calculated.

As shown in the previous sections, averaging of BSPs improves signal quality by eliminating artefact, thereby facilitating accurate reconstruction of isochronal maps, and has been utilised in previous studies to study activation and repolarisation patterns (38, 300). On the other hand, averaging an increasing number of BSP beyond a threshold may become time-consuming and require increasing computational power without commensurate improvements in signal quality. For this reason, it was necessary to establish the threshold beyond which averaging increasing BSPs yielded no significant changes in AT and RT. In this regard, total AT denotes the time taken for the entire substrate to depolarise and total RT for the same to repolarise and recover from refractoriness to be activated again. Total ventricular activation and RT are usually calculated as their respective ranges, i.e. the difference between the earliest and latest activating and repolarising times,

respectively. Given that the computation of local AT and RT often yields outlier values that are not likely to be electrophysiologically plausible, calculating total activation and repolarisation based on the earliest and latest times, i.e. the mathematical range, would yield total activation and RT that may be inaccurate. In address this, previous ECGi studies have utilised the smallest range encompassing 95% of local activation and RT to negate the influence of outliers and approximate total activation and RT, respectively (38, 514, 515). Alternatively, the central 95% range that excludes 2.5% of the earliest and latest local activation and RT may also be robust to outliers than the mathematical range. The relative robustness of calculating total activation and repolarisation with these three methods is described in the following sections.

4.4.1.6. Averaging 10 BSPs yields stable total ventricular activation and repolarisation times

This section describes the how the number of BSPs from the minute-segment with least RR variability required for signal averaging was determined to generate total ventricular activation and repolarisation times.

First, BSPs were averaged over a range of 1 to 74 BSPs to ensure that all BSPs within the minute-segment with the least RR variability were utilised. The averaged potentials were used to compute total ventricular AT and RT to identify the threshold after which averaging an increasing number of BSP would not result in any significant changes in these parameters. The effect of BSP averaging was tested using three definitions of total AT and RT: mathematical range, central 95% range or

the smallest range encompassing 95% of local activation and RT, respectively. This was achieved using BSPs from the minute-segment with the least RR variability from 5 randomly selected obese participants.

The results of signal averaging over increasing number of BSPs and the quantification of total ventricular AT and RT is shown in Figure 4.6. It can be observed that irrespective of the calculation method, total AT remains stable after averaging 5 BSP. By contrast, total RT are more sensitive to outliers demonstrate significantly more variability even with BSP averaging. This is particularly stark when total RT are defined by the mathematical and central 95% ranges. However, the smallest range encompassing 95% of local RT is more robust to effects of outliers, such that total ventricular RT remains stable after averaging 10 BSP.

These results suggest that implementation of the smallest 95% range of local AT and RT requires the QRS complexes and T-waves from 10 BSP to be averaged to yield the most stable solution for determining total ventricular AT and RT, respectively. This result is also consistent with previous ECGi studies that have utilised signal averaging to improve the quality of their signals prior to solving the inverse problem of electrocardiography (38, 515).

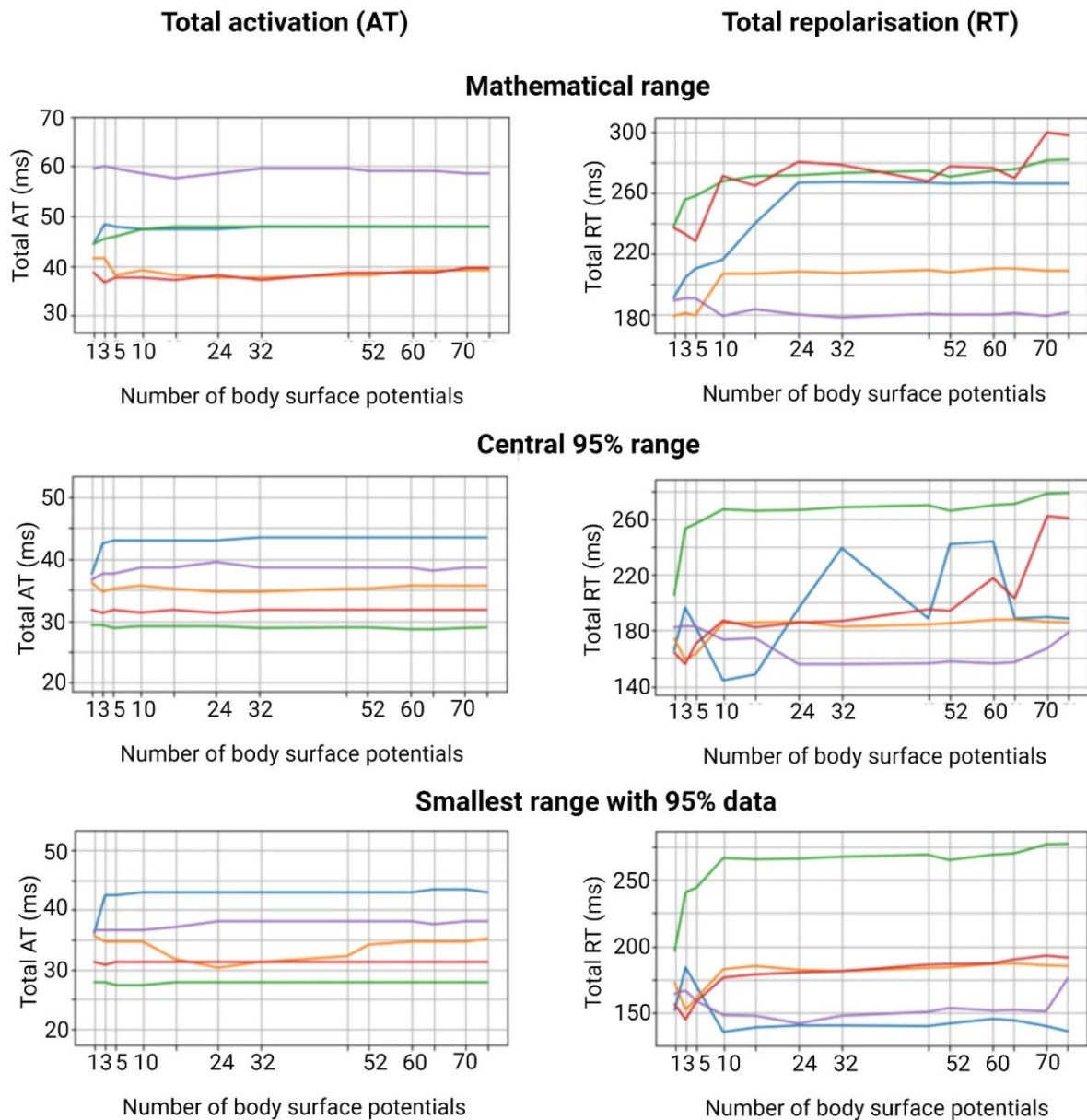


Figure 4.6: Averaging 10 BSPs generates stable ventricular total activation and repolarisation times defined as the smallest range encompassing 95% data. A range of BSP in the minute-segment with least RR variability were used to compute an averaged body surface potential. The total AT and RT corresponding to each of these, defined by three definitions (mathematical range, central 95% range, and smallest range of 95% of data). Total AT was stable after averaging over 10 consecutive potentials (QRS complexes), irrespective of its definition.

Total RT was most stable after 10-beat averaging (of T-waves) when defined as the smallest range encompassing 95% of local RT.

4.4.1.7. Averaging 50 BSPs yields stable atrial activation times

The process described in the previous section was applied for atrial data to determine the threshold for averaging BSPs resulting in stable atrial total AT. Given that the P-wave which denotes atrial activation is a low-amplitude feature, BSPs were averaged starting with 1, 10 and 50 potentials and subsequently in increments of 50 up to 300, and total activation was determined using the three different methods as described above (mathematical range, central 95% range or the smallest range encompassing 95% of local AT).

The results of BSP averaging to determine total atrial AT are shown in Figure 4.7. Similar to ventricular data, atrial data was subject to outliers resulting in inaccurately prolonged total AT when defined by the mathematical range, even in the absence of known atrial conduction disease in any of the participants. By contrast, the central 95% and smallest 95% ranges removed the effects of outliers yielding total AT in the electrophysiologically normal range. Furthermore, total AT defined by the smallest range encompassing 95% of local AT was consistent after averaging 50 BSP. By contrast, total AT defined by the other two methods were more variable necessitating a greater number of potentials (>150) to be averaged to provide a stable solution. Thus, these results suggest that averaging 50 consecutive P-waves to determine total atrial AT defined as the smallest range encompassing 95% of local AT yields the most stable solution.

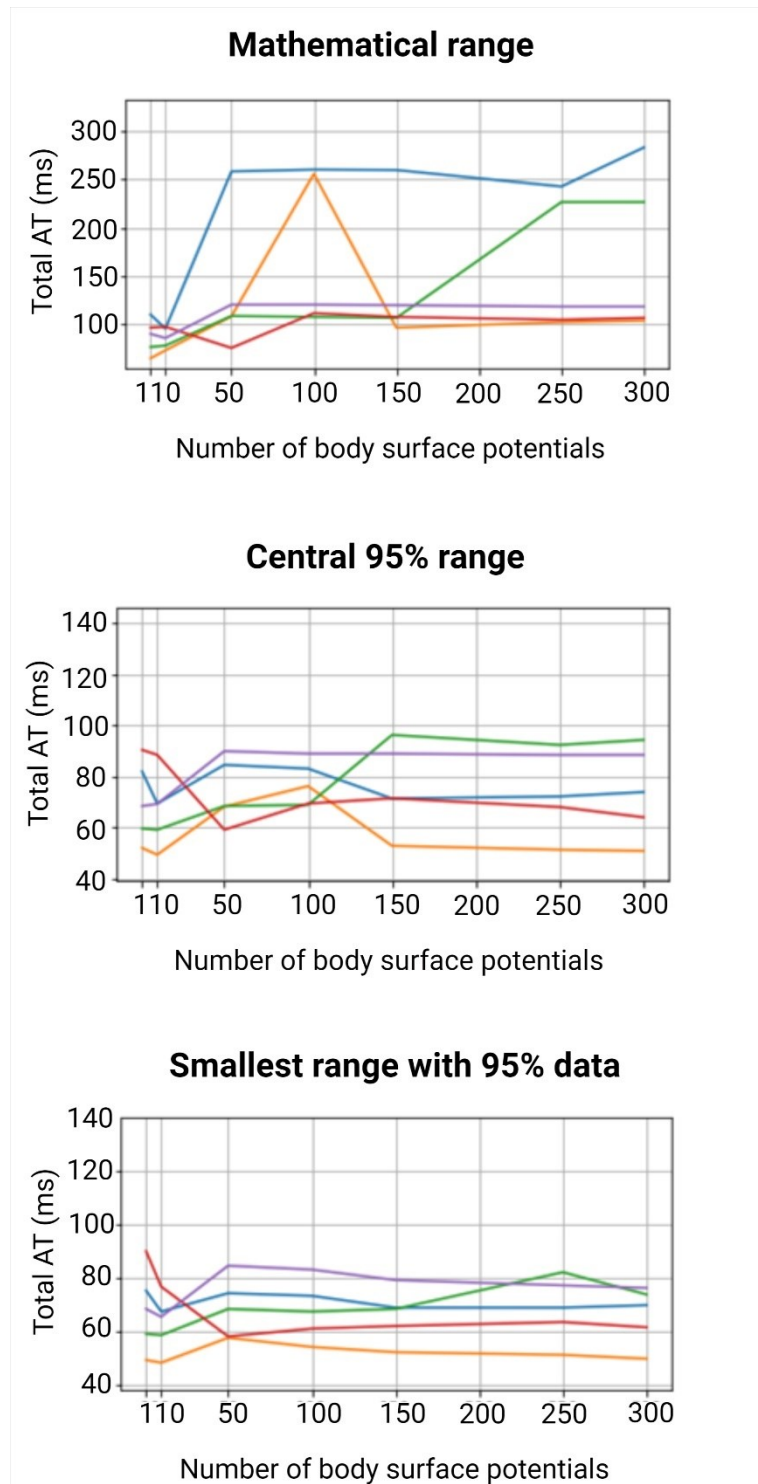


Figure 4.7: Averaging 50 BSPs generates stable atrial total activation times defined as the smallest range encompassing 95% data. A range of BSP in the minute-segment with least RR variability were used to compute an averaged body surface potential. Total AT corresponding to each of these, defined by three

definitions (mathematical range, central 95% range, and smallest range of 95% of data). The most stable solution was provided by averaging 50 consecutive potentials (P-waves) when total AT was defined by using the smallest range encompassing 95% of local AT values.

Informed by these results, the QRS complexes of 10 consecutive BSPs and P-waves of 50 consecutive BSPs from the minute-segment with the least RR variability were averaged to serve as inputs to ECGi used in Chapter 5. In both instances, total AT and RT were calculated as the smallest range encompassing 95% of local AT and RT.

4.4.2. Post-processing of unipolar electrograms

4.4.2.1. Determining ventricular repolarisation times from unipolar electrograms

Local RT has been shown to closely approximate to the steepest upslope of T-wave in epicardial electrograms, irrespective of whether it is upright or inverted (38, 516). The accurate automated annotation of the steepest upslope necessitates the T-wave to be easily discernible and not obscured by oscillations in the isoelectric baseline as a result of artefact. Otherwise, steeper deflections occurring after the QRS complex but outside the T-wave may be incorrectly labelled as the local RT. Similarly, low amplitude T-waves also create ambiguity for the automated labelling of local RT, such that even small oscillations from the isoelectric baseline may result in inaccurate RT annotation. Manual annotation or verification of the automated RT annotation for each of >2000 epicardial electrograms computed from solving the inverse problem would represent a significant practical undertaking. For this reason,

accurate automated RT annotation for robust analysis of the clinical study, particularly in the absence of contact ground-truth data, was imperative. To reconcile these considerations, a two-step process was designed and tested to ensure that only RT labelled on the steepest upslope of T-waves were included in analyses. The first of these was to ensure that only the steepest upslope of the largest amplitude deflection would be annotated as the local RT, and the second to exclude low amplitude T-waves in which automated local RT annotation would likely be inaccurate. The results of these methods are described in the following sections.

4.4.2.2. Maximum derivative-integral method accurately annotates ventricular RT

Two automated methods of annotating local RT were tested: one which identified local RT as the steepest upslope within the window of interest, and another that identified RT as the steepest upslope overlying the absolute maximum integral. For descriptive purposes, the latter will be referred to as the maximum derivative-integral, or MDI, method herein.

The results of these two methods on local RT is shown in a representative example in Figure 4.8. The top panel shows the repolarisation window of an unipolar electrogram with two upslopes and the bottom panel plots the corresponding signal's first derivative. The steepest points of the two upslopes are denoted by green and red crosses and the upslope integrals are shaded in blue and orange. From the bottom panel, it can be deduced that the maximum derivative of the second upslope, denoted by the red cross, is greater than that of the first (green cross). In this

instance, defining local RT as the steepest upslope by the first method yields an inaccurate result (red cross) given that this portion of the unipolar electrogram is unlikely to represent the T-wave based on its topology in the top panel. On the other hand, defining local RT as the point of the maximum derivative that overlies the greatest positive integral (shaded blue) using the MDI method, gives a result that is more plausible (green cross), as this portion of the signal seen in the top panel is more likely to represent the T-wave.

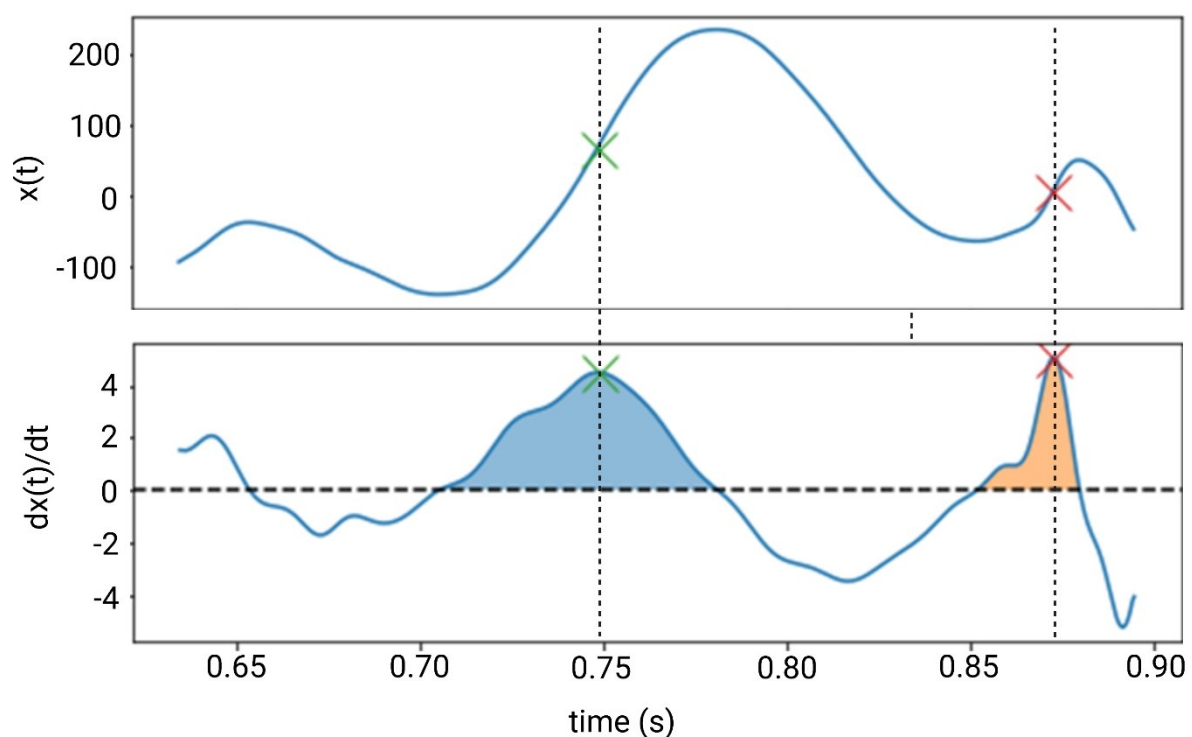


Figure 4.8: Maximum derivative-integral method identifies ventricular repolarisation time on the steepest upslope of the T-wave. The top panel shows a window of interest containing the repolarisation waveform from an unipolar electrogram. The green and red crosses denote the steepest point of the two upslopes in the window of interest. The bottom panel shows the first derivative of the electrogram ($dx(t)/dt$). The MDI method determines the point more likely to

represent the local RT by identifying the steepest upslope with the greater integral of its first derivative (green cross and region shaded blue, rather than red cross and region shaded orange).

Based on these findings, the MDI method was selected for automated annotation of local RT in unipolar electrograms. Any local RT as the maximum derivative that were not within the absolute greatest positive integral were excluded from analysis.

4.4.2.3. Excluding low amplitude signals based on T-wave upslope gradient

Although the MDI method described in the previous section ensured that the steepest upslopes of the greatest amplitude deflections were annotated as the local RT, it was not sufficiently robust for the exclusion of low amplitude (flat) T-waves in which annotation of repolarisation by automated methods would likely be inaccurate. Explicitly, it was possible that the MDI method annotated the steepest upslope of the deflections that did not constitute a T-wave, particularly when the T-wave was ambiguous or of such low amplitude as to be indiscernible.

Therefore, to identify the T-wave upslope gradient threshold below which electrograms could be consistently and reliably excluded on the basis that automated annotation of RT was likely to be inaccurate, 2000 unipolar electrograms from a single randomly selected study participant were reviewed individually by two independent operators experienced at reviewing unipolar electrograms (Kiran Patel and Nikesh Bajaj). Electrograms with visually discernible T-waves were ascribed a score of 1; all other electrograms were ascribed a score of 0. Thereafter, all

electrograms were sorted in ascending order of the maximum upslope gradient (dV/dt , where voltage was recorded in microvolts and time in milliseconds) within their respective T-wave windows and the agreement between the scores ascribed to the electrograms by the two operators were compared using free-marginal kappa statistics (513, 517).

The results of this comparison, with kappa statistics and their 95% confidence intervals, are presented in Figure 4.9. Low-amplitude T-waves in which RT cannot be accurately annotated were consistently ascribed a score of zero resulting in 100% agreement between the two operators for electrograms with upslope gradients <1.2 . The disagreement between operators as gradients increased between 1.2 and 2 was explained by ambiguity in identifying any deviations from isoelectric baseline that may or may not have constituted T-waves. Agreement between operators increased and stabilised as gradients increased above 2.0 and T-waves became more discernible, although artefact in some electrograms occasionally resulted in discordant scores resulting in less than 100% agreement.

These results suggested that utilising a T-wave upslope gradient threshold of 1.2 would exclude low-amplitude and flat T-waves and consequently their corresponding RT that would otherwise introduce inaccuracies in the study analyses. It was likely that this threshold would vary between individuals and be influenced by the quality of the BSP and consequently the reconstructed epicardial electrograms. For instance, noisy electrograms may necessitate a threshold lower than 1.2 to exclude low-amplitude T-waves. On the other hand, a threshold of 1.2 may exclude T-waves in

which RT could have been accurately annotated in less noisy electrograms. Nevertheless, it was unlikely that any inter-patient variability in this regard would significantly affect subsequent analyses, given that over 2000 electrograms were computed for each participant which afforded sufficient redundancy within datasets to preserve inter-group differences even if inaccurate RT may have been included or accurate data excluded inadvertently. Nevertheless, the next section outlines a subsequent post-processing method that specifically aimed to minimise any such inaccuracies.

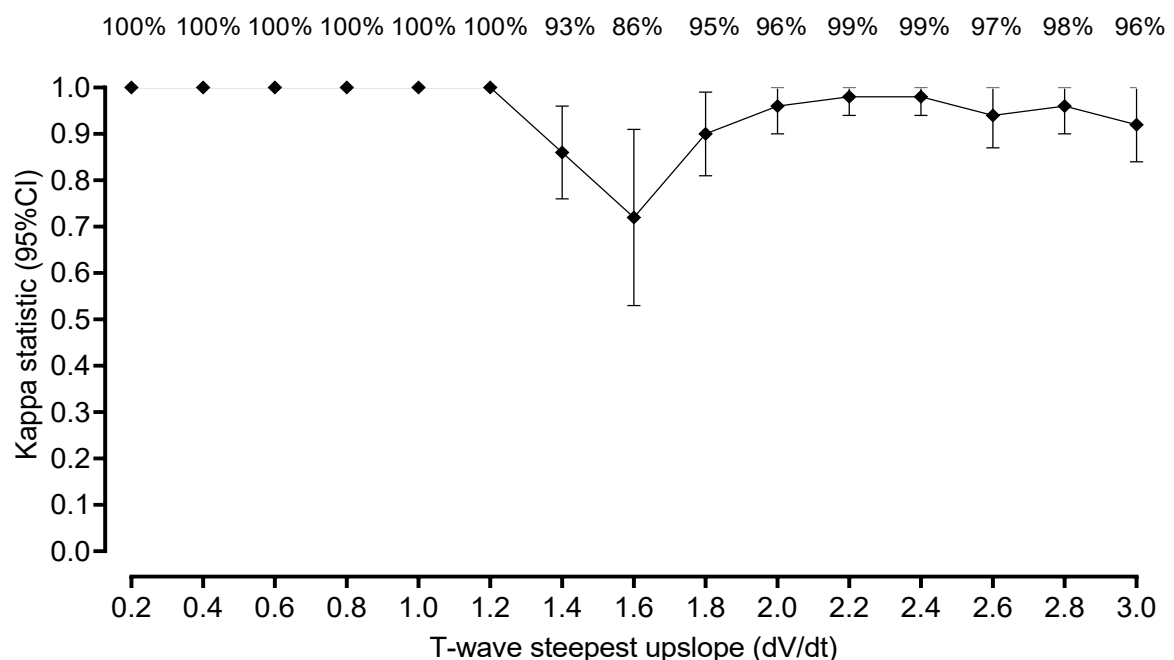
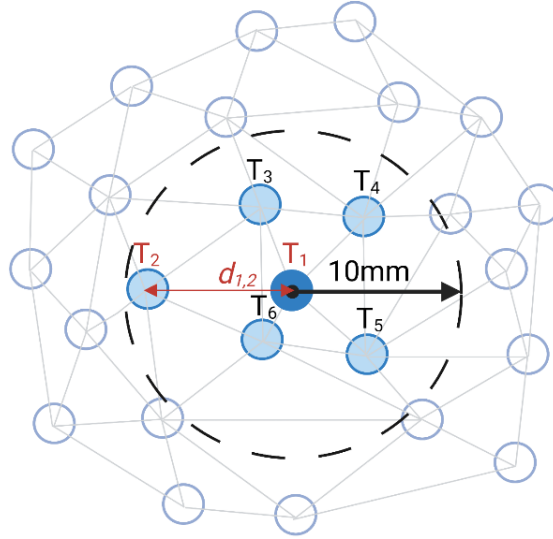


Figure 4.9: Upslope gradient of less than 1.2 consistently identifies low amplitude T-waves. 2000 electrograms from a single case were reviewed and categorised based on the whether the T-wave was discernible by two operators independently. The agreement between the two operators for each T-wave based on its steepest upslope gradient (x-axis) was quantified using free-marginal kappa statistics (y-axis) and shown as percentages. Flat T-waves were relatively unambiguous and were therefore unanimously identified resulting in 100%

agreement. Agreement between operators decreased as T-wave gradient increased owing to differences in opinion whether a deflection from isoelectric baseline constituted a T-wave. Agreement between operators increased thereafter as T-waves become more obvious with increasing gradient of upslopes.

4.4.2.4. Determining the optimal spatial median filter radius

Differences between neighbouring regions of early and late repolarisation creates a spatial gradient, which results in a substrate that can facilitate re-entry in way that has been eloquently described by Cluitmans et al (38). A critically timed premature beat arising near an early repolarising region will activate it but will be blocked in tissue that has a longer RT and is therefore refractory. During the time the early repolarising region becomes activated, the late repolarising region would have recovered its excitability, enabling the wavefront to activate it. As the late repolarising region activates, the early repolarising region would have recovered from its refractoriness allowing it to activate again and thereby re-starting the circuit (38). Quantifying repolarisation heterogeneity that may facilitate re-entry is therefore of electrophysiological importance. RTG was calculated as the maximum difference in RT between an index electrogram and its neighbouring electrograms within a 10mm radius divided by their corresponding distance (38) (Figure 4.10).



$$\text{gradient} = \frac{\Delta T_{\max}}{d} = \frac{|T_2 - T_1|}{d_{1,2}}$$

Figure 4.10. Calculation of spatial gradients of activation and repolarisation.

Nodes on the epicardial mesh are represented by open circles. Spatial activation and repolarisation time gradients (ATG and RTG, respectively, in mm/ms) were calculated by dividing the maximum difference in AT and RT ($\Delta T_{\max}$) between an index node (T_1 , filled dark blue circle) and any other node within a 10mm radius (T_2 , filled light blue circles) by their anatomical distance (d , mm). This was repeated for all nodes in turn.

The quantification of RTG is influenced by two important factors: first, the annotation of RT, and second the spatial range in which RT are considered. Although methods to identify inaccurately labelled RT and low-amplitude T-waves have been described earlier in this chapter, T-waves distorted by artefact would also result in inaccurate RT annotation. This is supported by Figure 4.9 in which <100% inter-operator agreement in non-flat T-waves ($>dV/dt$ 1.8) was the consequence of ambiguous topologies resulting from noise in the BSPs. To address this, previous studies have

utilised a spatial median filter to minimise the effect of noise or mis-annotation within a predefined region (38). It does so by ascribing the median of all neighbouring local RT within a predetermined radius to the index electrogram and is underpinned by the rationale that in health and normal circumstances the differences in RT between adjacent and neighbouring epicardial electrograms are expected to be minimal (Figure 4.11).

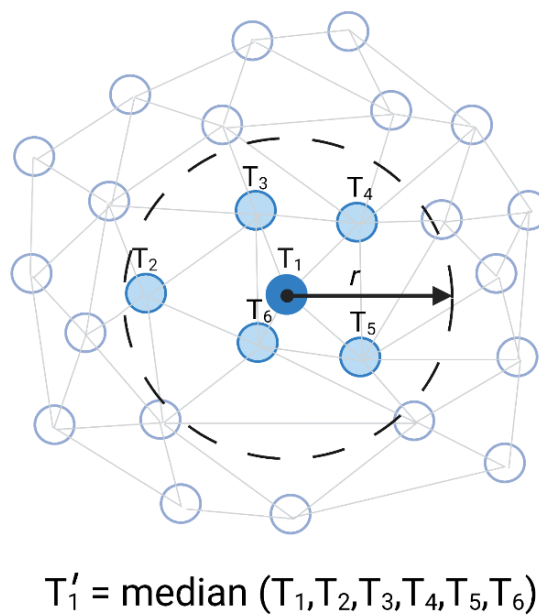


Figure 4.11: Spatial median filter. A form of spatial binning minimised the effect of outlier or inaccurately labelled AT and RT in analyses by ascribing the median RT of all nodes (filled light blue circles) within a radius, r , to the index node (filled dark blue circle).

4.4.2.5. Increasing spatial median filter radius causes over-smoothing of repolarisation isochronal maps

The effect of increasing spatial median filter radius on ventricular repolarisation RT annotation and isochronal maps was tested using median filters ranging from 0mm

to 25mm in increments of 5mm. An illustrative example from an obese participant is shown in Figure 4.12. This demonstrates that repolarisation maps become increasingly homogenous with increasing median filter radius. Importantly, the 5mm setting smoothens islands of early repolarisation (coloured red) observed in the 0mm isochronal map that are the consequence of artefact and inaccurate annotation of RT. It also results in RT annotation to appropriately shift toward the steepest part of the T-wave upslope based on its neighbouring electrograms in instances where RT has been misannotated.

Increasing the median filter radius above 5mm, however, results in correctly annotated early repolarising regions to become less prominent because of over-smoothing. This effect is exaggerated as the median filter increases from 15mm to 25mm such that regions coloured red, indicating early repolarisation, become yellow which is suggestive of later repolarisation. These results show that increasing the radius of the median filter introduces inaccuracies of RT annotation and over-smoothing beyond a threshold of 5mm, and identify this value as the optimal setting for correcting misannotated RT.

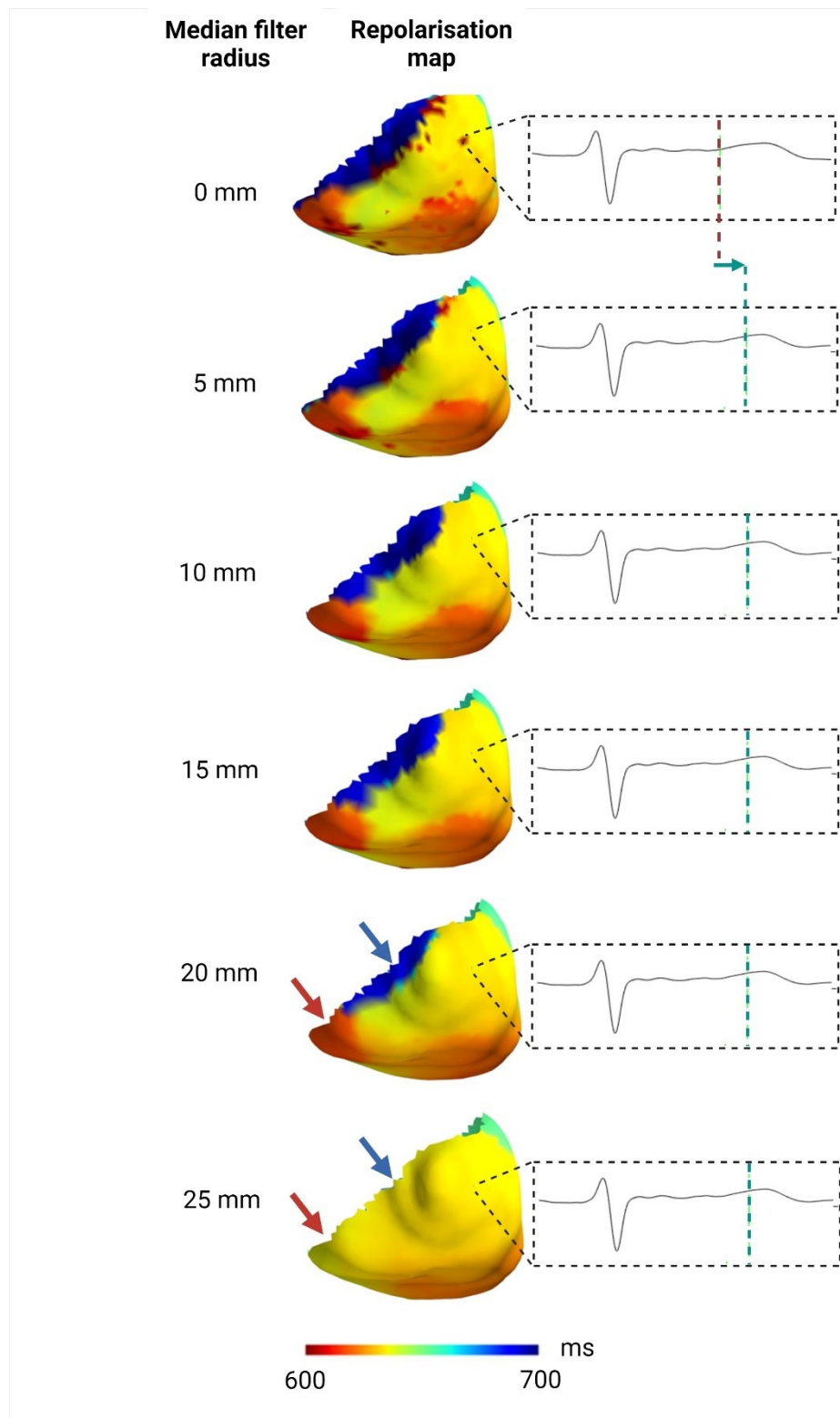


Figure 4.12: Increasing spatial median filter results in over-smoothing of repolarisation isochronal maps. An representative example from an obese participant shows increasing median filter from 0 to 25mm in increments of 5mm

results in progressive smoothing of repolarisation patterns. In the top repolarisation map (0 ms, i.e. no median filter), mis-labelling of the steepest upstroke of the T-wave in an electrogram results in a region being incorrectly identified as an area of early local repolarisation (red dashed line). Application of 5ms median filter shifts this to the right (green dashed line) resulting in more accurate labelling of the steepest upslope of the T-wave in the same electrogram. Although increasing median filter radius beyond 5mm does not significantly change local RT in this electrogram, it nevertheless results in over-smoothing of regions of early and late repolarisation (red and blue bold arrows, respectively). Median filter was applied to RT only and therefore the activation complex, and therefore local AT, remain unchanged.

4.4.2.6. 5mm spatial median filter preserves spatial repolarisation gradients

As demonstrated in Figure 4.12, increasing spatial median filter causes progressive smoothing of ventricular repolarisation isochronal maps that would mask steep RTG that are of electrophysiological interest in characterising a proarrhythmic substrate. Given that the degree of smoothing is related to the radius of the median filter, this section presents results to determine the optimal median filter radius to correct mis-labelled RT while simultaneously preserving RTG.

The effect increasing median spatial filters ranging from a radius of 0 to 25mm on ventricular RTG were tested in increments of 5mm in 10 obese and 10 age- and sex-matched non-obese individuals. The results of this are shown in Figure 4.13.

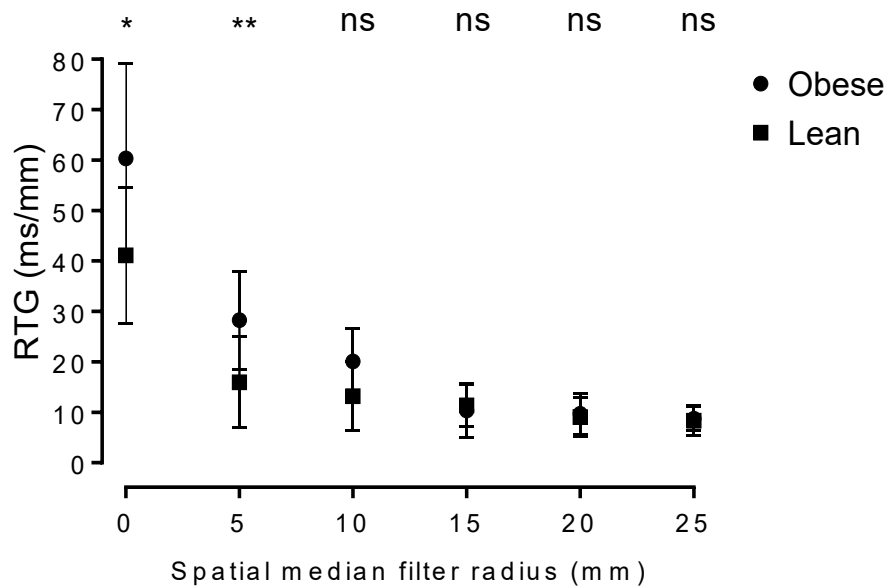


Figure 4.13: Increasing spatial median filter obscures differences in

repolarisation gradients between obese and non-obese groups. RTG

decreases in lean (n=5) and obese (n=5) individuals as the radius of the spatial median filter increases, resulting in the progressively smaller differences between the two groups.

There was a significant difference between obese vs lean groups at median filters of 0mm (60 ± 19 vs 41 ± 13 ms/mm, $p=0.04$) and 5mm (28 ± 10 vs 16 ± 9 ms/mm, $p=0.0089$).

There were no differences at other median filter radii: 10mm: 20 ± 7 vs 13 ± 7 ms/mm, $p=0.62$; 15mm: 10 ± 5 vs 11 ± 4 ms/mm, $p=0.59$; 20mm: 10 ± 4 vs 9 ± 4 ms/mm, $p=0.59$; 25mm: 9 ± 2 vs 8 ± 3 ms, $p=0.85$.

A number of observations are noteworthy from these results. First, RTG decreases in obese and lean groups as the median filter radius increases which, as demonstrated in **Figure 4.12**, is a predictable consequence of increased

smoothing that minimises the effects of noise at the expense of eliminating differences between groups that may be of clinical interest. Second, this effect of smoothing is reflected by the convergence of RTG between groups as well as the narrowing of standard deviation bars as the median filter radius increases. Third, although obese patients have steeper ventricular RTG than their lean counterparts, these differences are only significant at median filter settings of 0 (i.e. no median filter) and 5mm. Thus, increasing median filter setting above 5mm in a sample of the study population results in inter-group RTG homogeneity that may mask differences of electrophysiological importance. On the other hand, not utilising a median filter may result in mis-annotated RT remaining uncorrected, as shown in Figure 4.12, and thereby introduce error in the calculation of repolarisation parameters.

These results support the implementation of 5mm radius spatial median filter to study RTG differences between the cohorts in the main study. This is consistent with a recent ECGi study demonstrating that RTG are preserved below a 15mm-filter in models of idiopathic VF (38). Although in their study Cluitmans et al opted for a 15mm-filter, they also showed there were no statistical differences in RTG between 15mm and 10mm filters (38). Results presented here support implementation of a more modest filter of 5mm which may be explained by differences in experimental and technical methodologies as well as respective study populations.

4.4.2.7. Manual review of repolarisation post-spatial median filtering

As shown in Figure 4.11, the median filter adjusts local RT based on its neighbouring RT. This method assumes that the neighbouring RT are correctly annotated as the

steepest upslope of the T-wave. In this instance, RT of the index electrogram will be appropriately corrected as shown in Figure 4.12 and Figure 4.14. Conversely, if neighbouring RT are incorrectly annotated away from the steepest point of the upslope, this may result in inaccurate and inappropriate corrections of RT of the index electrogram even in instances when the original annotation was correct.

To ensure that the corrections by the median filter were appropriate, all electrograms recording >5ms difference between median filtered and annotated RT were manually reviewed individually in tandem with the corresponding repolarisation isochronal map. Any median filter corrections resulting in RT deviating away from the steepest upslope of the T-wave as a consequence of neighbouring inaccuracies were rejected (Figure 4.14). In this way, only corrections resulting in RT being annotated closer to the steepest T-wave upstroke were accepted. A threshold of 5ms was chosen as a trade-off between the practical challenge of manually verifying RT annotation in up to 2000 electrograms for each repolarisation isochronal map and any differences between automated vs median filter RT annotations that would be of electrophysiological consequence. This verification process also ensured that the post-processing method was robust to any remaining mis-annotated RT or ambiguous T-waves.

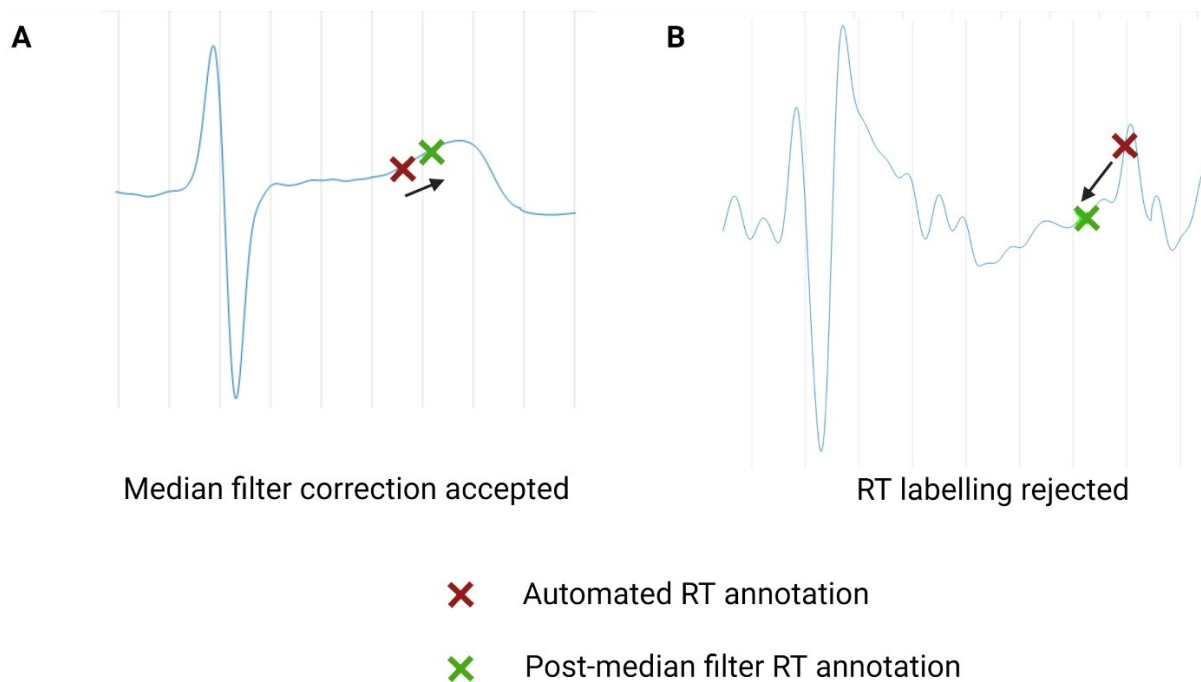


Figure 4.14: Manual review of automated local repolarisation time

annotation. Local RT was defined as the steepest upslope of the T-wave (516) and detected by automated methods. Each electrograms recording >5ms difference between automated and post-median filter RT was reviewed manually. Automated detection of RT denoted by red crosses and post-5mm median filter RT by green crosses in two unipolar electrograms. Arrows show the direction of change of RT after 5mm median filter. **(A):** Automated annotation incorrectly ascribes local RT on a flatter segment of the T-wave. Based on the RT values of electrograms within a 5mm radius, the median filter results in RT annotation of this electrogram to shift toward the steepest upslope of the T-wave. In this case, the median filter correction of RT was accepted given that the steepest upslope corresponds to local RT (516). **(B):** Although median filtering results also a change in RT annotation, the T-wave is ambiguous. In this case, local RT annotation by pre- and post-median filter is rejected and this datapoint excluded from analyses.

4.4.2.8. Determining the number of electrograms available for ECGi after post-processing

Given that optimisation of post-processing methods described hitherto focussed on ventricular repolarisation, the result of their implementation on total RT was tested in 10 obese and non-obese individuals ($n=5$ each). Figure 4.15 shows the number of electrograms remaining after sequential exclusion with each post-processing method following computation from BSP by solving the inverse problem of electrocardiography. In total, 2002 unipolar epicardial electrograms were computed in each of the 10 cases. First, 559 ± 95 electrograms computed over the valve plane were excluded. Next, a further 60 ± 39 electrograms were excluded by the MDI method, followed by 127 ± 41 electrograms based on upslope $dV/dt < 1.2$. Finally, 35 ± 9 electrograms were excluded during a review of repolarisation isochronal maps after implementation of the spatial median filter. Thus, 1220 ± 102 electrograms ($55.9 \pm 6.5\%$ of the total) remained from which total RT were calculated. This result is favourable compared to a previous study that used the same ECGi system (ActiveTwo, BioSemi) to compute approximately 1000 electrograms prior to exclusion of those contained within valvular planes to investigate electrophysiological abnormalities in another population (300). An illustrative example of these processes is shown in Figure 4.16.

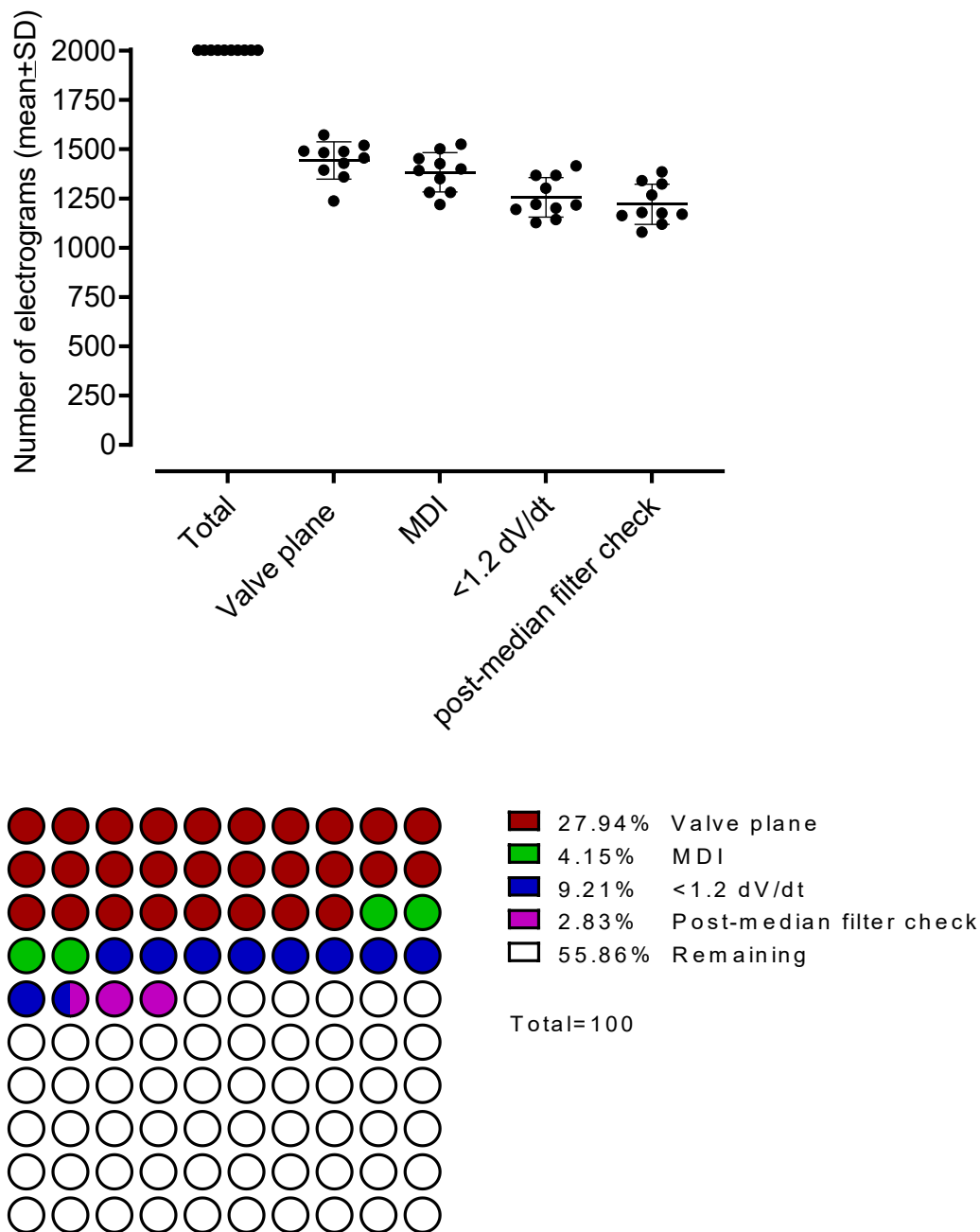


Figure 4.15: The number of unipolar electrograms remaining at each stage of post-processing. All ventricular meshes comprised 2002 nodes, and therefore a corresponding number of electrograms. Sequential post-processing methods described in the main text resulted in progressive exclusion of electrograms resulting in an average of 1220 ± 102 electrograms ($n=10$, 5 obese, 5 lean), from which RT parameters were calculated. The number of electrograms excluded by

the post-processing methods expressed as a percentage of the whole (2002 electrograms) is depicted below the graph.

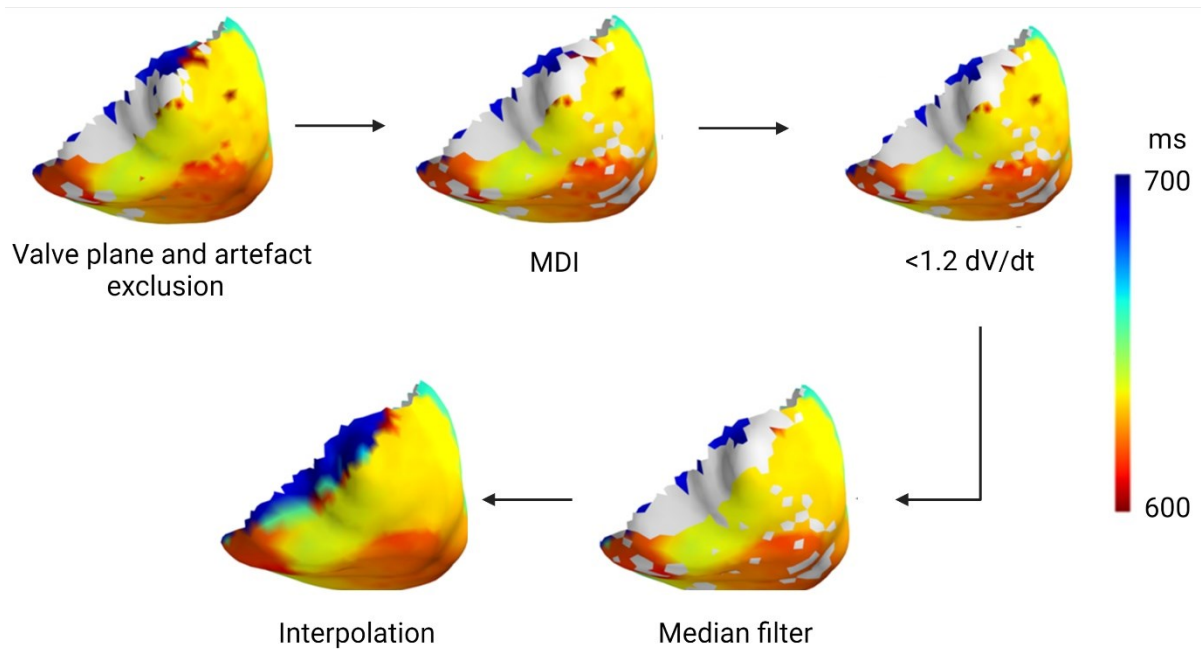


Figure 4.16: Effect of post-processing of electrograms on ventricular repolarisation. Illustrative example from an obese patient demonstrating the effects of post-processing quantified in Figure 4.15. Grey regions denote excluded local RT. Although repolarisation maps may be interpolated following application of median filter, RT data after median filtering, not interpolated data, were used for the analyses presented in Chapter 5.

4.5. Discussion

The main finding from this study is that pre-processing of BSPs and post-processing of unipolar electrograms determines the accuracy, robustness and reproducibility of ECGi outputs. The results of optimising pre- and post-processing methods for ECGi described in this chapter may be summarised as follows: 1) BSP from sections of data with the least RR variability are the most stable and therefore least likely to introduce error in the computation of unipolar electrograms; 2) following removal of artefact based on power, averaging 10 consecutive BSP (QRS complexes) yields stable ventricular activation and RT (50 consecutive P-waves for atrial AT); 3) defining total AT and RT as the smallest range encompassing 95% of their respective local times is robust to outliers; 4) the automated MDI method reliably annotates the RT on the steepest upslope of oscillations from isoelectric baseline that are most likely to constitute the T-wave; 5) T-wave upslope $dV/dt < 1.2$ identifies low-amplitude T-waves in which automated annotation of repolarisation is -likely to be inaccurate; 6) a spatial median filter of 5mm corrects incorrectly annotated local RT and excludes artefact while simultaneously preserving ventricular RTG.

4.5.1. Annotation of repolarisation time is more prone to error than activation time

The inherently ill-posed nature of the inverse problem of electrocardiography necessitated the development of standardised and robust BSP pre-processing and unipolar electrogram post-processing methods to minimise introducing further error in the computation of reproducibly accurate AT and RT. Annotation of local RT is more sensitive to error than local AT mainly because of the differences in the topologies of waveforms from which they are derived. The QRS complex is usually a

discreet, high amplitude waveform in which the steepest downslope can be more reliably determined by automated methods. By contrast, the relative low amplitude and highly variable waveform of the T-wave in the unipolar electrogram renders automated local RT annotation subject to error. This is compounded by artefact in the BSPs that introduces error in ECGi outputs. For this reason, post-processing results presented in this chapter focussed on ensuring accurate annotation of RT.

4.5.2. BSPs sampled from the minute-segment with least RR variability provide stable inputs for ECGi

In the absence of a conventional method for sampling BSPs from a body surface mapping for ECGi, the results presented herein show that selecting BSPs from minute-segment with the least RR variability are the relatively homogenous and results in better alignment of P- and T-waves. A consistent topology of P- and T-wave ensures that the averaged BSP bears resemblance to the constituent BSPs. Inconsistent topologies of P- and T-waves between consecutive BSPs would result in warping of the averaged BSP, resulting in computation of atria activation and ventricular repolarisation times and patterns that do not faithfully recapitulate electrophysiological data captured by BSPs. Although any differences arising from this may be subtle, the fact the inverse problem is ill-posed necessitates pre-processing rigour and is first qualitative description of this method.

Averaged BSPs from the minute segment with the least RR variability also showed the least variation in amplitude. Although amplitude variation does not introduce error in solving the inverse problem, this result suggests that BSPs from the minute

segment with the least RR variability may also contain less artefactual signal.

Artefact interrupts the recognition of an otherwise regular RR interval. Thus, an increased RR variability may be considered a surrogate measure of the degree of noise. Although this method in itself is not sufficiently robust to excluding artefactual BSPs, it nonetheless ensured that the BSPs used for signal averaging were sufficiently alike.

4.5.3. Averaging BSPs beyond a threshold does not change total activation and repolarisation times

BSP averaging serves to minimise artefact. However, averaging over an increasing number of BSP becomes increasingly computationally inefficient without incremental gains in signal quality. Results in this chapter demonstrate that averaging more than 10 QRS-T complexes and 50 P waves from the BSPs to did not improve the stability of the ECGi output, defined by total ventricular activation and repolarisation and total atrial activation. These results are in-keeping with the methods adopted by others (300) and suggest that they may be consistent amongst different cohorts.

4.5.4. The smallest range encompassing 95% local data define total activation and repolarisation times

Total activation and repolarisation times may be calculated in a number of ways (38, 514, 515). To mitigate the effect of any outliers on these parameters, three methods of calculating total activation and repolarisation were tested to determine the one that yielded stable outputs. Mathematical range, calculated as the difference between the earliest and latest AT and RT, are the most vulnerable to artefact. Utilising the central 95% range eliminates outliers that are consistently in the extremes of the

mathematical range. By contrast, the smallest range encompassing 95% of data is not restrained to the central 95% of data and considers the distribution of data as well as the number of local AT or RT utilised. The results herein show that the smallest range encompassing 95% of local activation and repolarisation consistently yields robust and electrophysiologically sound solutions for atrial and ventricular data.

4.5.5. Accurate annotation of local RT is achieved with automated and manual post-processing

Accurate computation of total ventricular RT and RTG necessitates all local RT to be reliably and accurately annotated. The results of this chapter demonstrate that this may be achieved with a combination of automated and manual post-processing steps. First, the automated MDI method ensures that the steepest upslope the deflection with the greatest area under the curve is labelled as the local RT in an unipolar electrogram. Second, an upslope gradient of the T-wave of less than 1.2, with amplitude in microvolts and time in milliseconds, identified low amplitude T-waves in which automated or manual annotation of local RT could not be determined or verified in the absence of ground-truth data. Third, a median spatial filter with a radius of 5mm served to correct inaccurately labelled local RT by automated methods, whilst preserving RTG. Fourth, a manual check of electrograms with more than 5ms difference between local RT pre- and post- median filter provided the opportunity to verify that any corrections of RT by the median filter were appropriate. As shown in Figure 4.15, these sequential steps excluded approximately 800 local RT values, resulting in approximately 1200 data points being used in the analyses presented in Chapter 5, and is consistent with other studies (300). The post-

processing methods tested herein also expand on those implemented by others in other cohorts and computational models (33, 38, 313). For example, the comparison with the MDI method showed that determining the steepest upslope is insufficient to identify the local RT, and that a manual review of the RT after spatial median filtering ensures that the local RT have not been inappropriately modified. Methods described herein therefore constitute a systematic approach to RT post-processing that is robust to noisy reconstructions of electrograms and may be adapted as a standardised approach in other ECGi studies.

4.5.6. Rationale for non-signal processing ECGi methods

Optimisation of the methods outlined in this chapter was necessary prior to their implementation in the main study as ECGi outputs are highly dependent on the choice of technical and post-processing methods, that often explains the variability between studies(38, 290). A number of ECGi methods adopted in the main study that were not optimised with preliminary data were chosen based on their validation in previous studies. This included implementation of the Boundary Element Method based on Green's theorem to generate heart-torso geometries and using Delaunay's triangulation for meshing, segmentation of two atria and ventricles as single rather than double entities, application of zero-order Tikhonov regularisation to constrain the solutions to the inverse problem of electrocardiography and utilisation of spatiotemporal and Wyatt methods to determine local activation and RT (38, 292, 310, 404, 407, 418, 516, 518). The rationale for these choices are summarised in the following sections.

4.5.6.1. Discretisation of heart-torso geometries

Solving Laplace's equation in a source-free volume between the torso and epicardial surface enables the computation of epicardial electrograms from BSP. Irrespective of the models used to represent the cardiac source (potential-based, bi-domain model or uniform dipole layer), solving the inverse problem of electrocardiography necessitates discretisation of the heart and torso surfaces into smaller individual and non-overlapping elements, using a process called meshing(407). Delaunay's triangulation was implemented to discretise the epicardium and torso into triangular elements, such that no node was encompassed inside the circumcircle of any triangle. This is represented in Figures 4.10 and 4.11 and minimised the surface element aspect ratio and thereby the error between the anatomical and discretised epicardial and body torso surfaces. This method was chosen because it conforms to any anatomical boundaries (Ruppert, 1995). Atrial and ventricular meshes comprising 16,000 nodes was reduced to 2,000 nodes and smoothened to create a realistic representation of the epicardial surface. Solving the inverse problem enabled the computation of an electrogram for each of epicardial node.

Amira software (ThermoFisher Scientific) was used to identify the anatomical positions of body surface electrodes on cross-sections acquired during cardiac MRI. Each electrode was manually labelled in sequence of the electrodes placed on the torso. For cardiac geometry, the atrial and ventricular perimeters were manually traced on each of the 94 coronal sections in the end-diastolic phase. The apex and annuli of the tricuspid and mitral valves demarcated the ventricles. The same annuli and the pulmonary outflow tracts demarcated the atria. Generation of the heart-torso geometries using MRI data enabled computation of the transfer matrix, which defines

the electrical and spatial relationship between epicardial and torso surfaces in volume conductor (the thorax). Thus, the matrix is the mathematical function by which the epicardial potentials can be determined by recorded BSP. Given that previous studies reported that the introduction of torso inhomogeneities introduced complexities in solving the inverse problem of electrocardiography without significantly improving accuracy of electrogram reconstruction (382, 406), the torso was considered as a homogenous volume conductor in the present study. Furthermore, determining the specific conductive and electrical properties of each structure and medium within the torso of each participant would not have been practically possible. Consequently, the conductivity of homogenous torso was fixed at 0.239/metre, for blood 0.685/metre and the heart 0.1/metre.

The transfer matrix can be determined using the Boundary Element Method (BEM) or the infinite element method. BEM has been widely implemented in ECGi and is applicable when Green's theorem hold true, namely that electrical sources reside inside a closed volume (519). As the name suggests, only the boundaries ("mesh outline") of the structures of interest (i.e. the epicardial and torso surfaces) are discretised. This reduces the complexity and dimensions of the matrix and thereby computational time for subsequent processing. It is suited for solving linear problems (body surface ECG has a 1:1 relationship with epicardial electrograms) in a relatively stable or steady state medium (the torso volume conductor) and does so by solving integral equations. By contrast, the infinite element method discretises the entire structures of interest (epicardium and torso surfaces) to create a mesh of the entire domain and generates a matrix by solving differential equations. It is better suited for non-linear problems in a dynamic medium. Even though the heart and torso are

dynamic structures, the BEM was chosen because it has been implemented in ECGi development and validation studies (366) and requires less computational time. The entire body surface geometry was used to compute the transfer matrix even if the ECG signal recorded by an electrode in an anatomical region was discarded owing to noise, as described earlier in this chapter. This is because solving the inverse problem requires a complete mesh boundary. For the same reason, the valve plane was included in the atrial and ventricular epicardial meshes despite being electrically inert. Atrial and ventricular meshes created with Amira (ThermoFisher Scientific, UK) were imported into ParaView (v5.9.0-RCS) software as .surf files to manually delineate the valve planes and exclude electrograms computed over them.

4.5.6.2. Single vs double volume atria and ventricles

As described in the previous section, 3D epicardial meshes were generated separately for atria and ventricles. The majority of ECGi studies studying atria or ventricles consider each of these as a single volume unit, such that a single mesh encompasses both atria or both ventricles. The effect of separating the atria into two volumes using BEM method prior to solving the inverse problem has been investigated in one study in the context of AF (518). In their study, Gonzalez-Ascasco et al showed that dominant-frequency metrics relating to the study of AF were not significantly influenced by meshing atria as individual entities compared to combining both atria into a single mesh (518). For this reason, and to more accurately reflect cardiac anatomy, atria and ventricles were combined in their respective meshes.

4.5.6.3. Regularisation

The derivation of epicardial potentials from BSP requires inverting the transfer matrix. However, given that the transfer matrix is not a full-rank (square) matrix, epicardial potentials can only be approximated by using the least squares solution to compute the inverse of the transfer matrix. Solving the inverse problem in this way does not yield a unique solution because the input (BSP) is outnumbered by the output (epicardial electrograms). Furthermore, small oscillations in the ECG signal are amplified by the inverted transfer matrix resulting in large oscillations in reconstructed epicardial electrograms. For these reasons, the inverse problem has been described as ill-posed. Regularisation tempers this by applying constraints on the reconstruction. Although several regularisation methods have been described, Tikhonov regularisation was chosen in this study because it is commonly used in the context of ECGi (299). Specifically, zero-order Tikhonov regularisation was chosen as it limits the amplifying effect of the inverse matrix to minimise the magnitude of any errors imposed on the solution (epicardial electrogram). Other options included first-order Tikhonov regularisation, which constrains the solution by considering neighbouring epicardial signals and thereby constrains local epicardial heterogeneity, or second-order Tikhonov regularisation, which constrains the solution by minimising the rate of change in activation and repolarisation on the epicardial surface. Given that this study aimed to study activation and RT, patterns and gradients, first- and second-order Tikhonov regularisation were not utilised for regularisation, as the former would smoothen the solution and thereby obscure local heterogeneities and latter may alter the times and patterns of activation and repolarisation. Solving the inverse solution has been described mathematically in Chapter 2.

Application of regularisation requires the optimal regularisation parameter (λ) to be determined first. As described above, epicardial electrograms are estimated using the recorded body surface ECG and inverting the transfer matrix. In the first instance in the absence of knowing which regularisation parameter to implement, the L-curve method was used to constrain the solution (358). The relationship between the regularisation parameter and error in the reconstructed signal is described as a L-curve, such that increasing the regularisation parameter beyond the maximal deflection does not result in further reductions in error. Thus, the regularisation parameter at the maximal deflection was implemented to estimate epicardial potentials from BSP. Thereafter, the body surface ECG was reconstructed using the transfer matrix to solve the forward problem of electrocardiography. This iterative process, by which the recorded and reconstructed body surface ECG were compared, identified the regularisation parameter that minimised the difference between the two at each time sample, i.e. the optimal λ . Given that ECG signals are a series of voltage against time that were recorded at a sampling frequency of 2048Hz, in theory 2048 regularisation parameters may be calculated for every second of record ECG. In reality, the optimal regularisation parameters were only computed for each time sample in the windows of the interest, i.e. P-wave for atrial ECGi and QRS complex and T-wave for ventricular ECGi, for computational efficiency. In this way, the optimal regularisation parameter was found for at each time sample in these windows of interest. The median of the optimal regularisation parameters in each of window of interest was then calculated and applied across all electrodes in their respective windows. In this way, separate regularisation

parameters were implemented to compute epicardial atrial activation, ventricular activation and ventricular repolarisation.

4.5.6.4. Wyatt vs alternative method for determining local RT

Two methods have been proposed by which local RT may be determined from unipolar electrograms. The Wyatt method (418) considers the steepest upslope (maximum dV/dt) of the T-wave, whether upright or inverted, as the local RT given that it is determined by phase 3 of the local action potential. RT determined by this method have been shown to correlate with the effective refractory period (419, 520, 521) as well as the maximum downslope on transmembrane action potentials (522). By contrast, the alternative method defines local RT as the steepest upslope of inverted T-waves and the steepest downslope of upright T-waves, and was proposed following discrepancies observed between the activation-recovery interval determined by the Wyatt method and 90% action potential duration (523). Thus, the method by which RT is defined has important repercussions for calculating other parameters, such as RTG. Specifically, regions with inverted T-waves adjacent to those recording upright T-waves would result in steep RTG by the Wyatt method but not with the alternative method. Unsurprisingly, therefore, a previous study using ECGi to investigate repolarisation abnormalities in survivors of cardiac arrest and an inherited predisposition to sudden cardiac death demonstrated discordant results between the Wyatt and alternative methods (301). Although the alternative method approximates to MAP90 (523), the Wyatt method is based on the theoretical basis of coinciding with the ion currents determining local RT. Recent studies have shown that that the upslope of the T-wave in epicardial unipolar electrograms is an accurate measure of local refractoriness ($r=0.95$) irrespective of T-wave morphology in intact

human hearts (38, 516). For this reason, the Wyatt method has been favoured over the alternative method as a more accurate representation of local RT in a number of studies (38, 300, 417, 516, 524, 525). On this basis and given that this study was performed in the absence of ground-truth epicardial data with which to validate the Wyatt and alternative methods, calculations of RT in this chapter and for the primary analyses in the main study were performed using the Wyatt method.

4.5.6.5. Spatiotemporal method for defining local activation and repolarisation

Temporal-only methods for determining activation and repolarisation are usually robust to identify activation and RT when their respective waveforms are discrete. However, the steepest downslope of the activation complex and upstroke of repolarisation complex may not be accurately annotated by computational algorithms in fractionated electrograms, or when quality of electrogram-signal is suboptimal or obscured by artefact. Combining this temporal-only approach with a spatial component has been proposed as a more robust method for activation and repolarisation annotation (292, 404). At the time of activation, the first-order derivative of activation (spatial gradient) of the point of anatomical interest is expected to be at its greatest, and its second-order derivative (Laplacian) would be expected to change signs (526). Combining the first- and second-order spatial gradients with the temporal derivatives from the electrograms, the so-called spatiotemporal approach, has been shown to accurately estimate activation and RT by identifying sharp changes in time and space simultaneously (404). As such, this method considers local activation and RT in the context of their respective neighbouring times. The spatiotemporal approach has been validated in *in silico* and animal models, in which it has been shown to be superior than temporal-only

approach for correctly identifying activation in fractionated and ECGi-derived electrograms. It also offers better resolution for discriminating pacing locations (38, 292). For these reasons, a spatiotemporal approach as outlined by Cluitmans et al (Cluitmans et 2021) was implemented in this study to annotate activation and RT and was accessed by a Github code repository at <https://github.com/jcollfont/activationtimes>. Automated activation and RT determined by the spatiotemporal approach were subjected to the post-processing methods described in this chapter.

4.6. Conclusion

ECGi optimisation and its translation into the clinical realm is challenging for two main reasons: it is practically challenging and cumbersome, and technically requires electrophysiological and computational expertise. This chapter has attempted to address the latter to ensure the robust computation of epicardial electrograms and calculation of activation and repolarisation parameters. In so doing, it has presented the results of various methodological options for pre-and post-processing data unique to the obese and lean individuals recruited to this ECGi study. This included identifying BSP for signal averaging, defining electrophysiological parameters, ensuring correct annotation of activation and RT and excluding the effects of outlier data. This chapter has also provided the rationale for the methodological choices that were not optimised by either referencing studies that have or explaining the scientific basis for their implementation. These optimisation and exploratory processes were particularly important given that there was no ground-truth data for validation of ECGi in this cohort given the absence of clinical indications for invasive electrophysiological studies in either the obese or lean groups. Thus, the

optimisation of pre- and post-processing methods described in this chapter will have contributed to the robustness of the analyses presented in the next chapter.

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Chapter 5: Bariatric surgery reverses electrophysiological remodelling in morbidly obese patients

5.1. Background

5.1.1. Obesity increases arrhythmic risks

Approximately half the world's population is either overweight or obese (527). This global epidemic is associated with numerous healthcare problems, including diabetes, hypertension, sleep disorders as well as cardiovascular disease. The magnitude of this problem has been summarised in a scientific statement from the American Heart Association (528), which also identifies obesity as a risk factor for atrial and ventricular arrhythmias. For example, amongst 5 population-based cohort studies comprising almost 80,000 adults across the Western hemisphere, the relative risk of AF amongst obese individuals was 1.49 (95% CI 1.36-1.64) compared to non-obese counterparts and increased further with increasing BMI (529). Similarly, in another meta-analysis of 63 observational studies comprising over 350,000 individuals, every standard deviation increments in WG, WHR and BMI was associated with 32%, 11% and 22% greater risks of AF, respectively (101). Similarly, every 5-unit increment in BMI confers approximately 13% increase in post-ablation AF and 10% increase in post-operative AF (100). A population-based prospective study of almost 50,000 Danish individual followed-up for approximately 6 years, the hazard ratio for incident AF per unit increment in BMI was 1.08 (95% CI 1.05-1.11) for males and 1.06 (95% CI 1.03-1.09) for females. These increased to 2.35 (95% CI 1.70-3.25) and 1.99 (95% CI 1.31-3.02) when comparing the risks of AF in obese to lean males and females, respectively (530).

Although relatively uncommon compared to atrial arrhythmias, obesity also confers higher risks of ventricular arrhythmias and sudden cardiac death (45, 116, 249) (531). For example, after 26 years follow-up, the Framingham Heart Study showed

obese males and females had 2.6- and 5.8-fold increased risks of sudden cardiac death (SCD), respectively, compared to lean individuals (532). The increased tendency to arrhythmias in obesity is thought to be partly mediated by increased volumes of epicardial adipose tissue (EAT), which has been shown to cause adverse electrophysiological remodelling of adjacent myocardium by fatty infiltration and paracrine mechanisms (1, 533).

5.1.2. Weight loss reduces arrhythmic burden

Given that obesity increases risk of cardiac arrhythmias, and atrial arrhythmias are more common than ventricular arrhythmias, much of the evidence for weight loss reducing arrhythmia burden is derived from studies of obese patients with pre-existing AF. By contrast, the effect of weight loss on ventricular electrophysiology is relatively sparse. For example, amongst overweight or obese individuals with paroxysmal AF, enrolment into a weight management programme is associated with significant improvements in symptoms and arrhythmic burden quantified by the Atrial Fibrillation Severity Scale compared to general lifestyle advice (252). Similarly, arrhythmic-free survival post-AF ablation amongst overweight or obese patients with metabolic ill-health, smoking history or alcohol excess was greater in those that also received aggressive risk factor management according to American Heart Association/American College of Cardiology guidelines compared to standard care (253). In a similar subsequent observational cohort study comprising 355 obese patients with AF, there was a dose-response relationship between weight loss and arrhythmia-free survival (254). Specifically, >10% weight loss was associated with a six-fold greater probability of arrhythmia-free survival compared to patients with 3-9% or <3% weight loss. Interestingly, the degree of weight loss corresponded with

participation in weight management clinic suggesting a positive reinforcement effect of losing weight. Arrhythmia-free survival was also greater in those who maintained weight loss in excess of 5% of their total body weight compared to those who achieved either more modest weight reduction or experienced fluctuations in weight (254). Similarly in another Australian cohort study, of those patients experiencing 3-9% weight loss, 33% progressed from paroxysmal to persistent AF and 25% reversed from persistent to paroxysmal or no AF. By comparison, of those recording >10% weight loss, only 3% progressed from paroxysmal to persistent AF and almost 90% reversed from persistent to paroxysmal or no AF (255). The dose-dependent relationship between weight loss and AF-freedom in overweight and obese individuals has also been corroborated in an European population (256).

On the other hand, although bariatric surgery is a cost-effective intervention for moderately to severely obese people compared with non-surgical interventions (239), there is conflicting evidence for whether it mitigates incident AF risks (278, 281). For instance, one meta-analysis comprising 39 prospective and retrospective cohort studies demonstrated bariatric surgery reduced all-cause mortality (pooled HR 0.55; 95%CI: 0.49-0.62, $p < 0.001$ vs. controls), cardiovascular mortality (HR 0.59, 95%CI: 0.47-0.73, $p < 0.001$), as well as incidence of heart failure (HR 0.50, 95%CI: 0.38-0.66, $p < 0.001$), myocardial infarction (HR 0.58, 95%CI: 0.43-0.76, $p < 0.001$) and stroke (HR 0.64, 95%CI: 0.53-0.77, $p < 0.001$), but not in incident AF (HR 0.82, 95%CI: 0.64-1.06, $p = 0.12$) (240). Similarly, amongst a national primary and secondary healthcare database of over 11 million patients in the UK, although rate of AF resolution among patients undergoing bariatric surgery was greater compared to age-, sex- and BMI-matched controls, there was a downward but not

significant trend for AF incidence (adjusted hazard ratio 0.72, 95% CI: 0.492–1.054; $p=0.091$) (278). Another retrospective study of over 1000 obese ($\text{BMI}>35\text{kg/m}^2$) patients in the USA undergoing gastric bypass ($n=308$) or medical management ($n=701$) showed that although surgery was associated with lower all-cause mortality, stroke, heart failure hospitalisation and myocardial infarction, there was no difference in AF incidence (279). Höskuldsdóttir et al showed that although risk for hospitalisation for pre-existing AF was 41% lower after bariatric surgery, they did not specifically evaluate incident AF risk, AF severity or burden post-surgery (280). In fact, others have shown that AF requiring emergency care may persist 2 years after surgical weight loss (534). By contrast, in a Swedish prospective multicentre cohort (SOS) study with median 19-year follow-up and comprising over 4000 obese patients of which half underwent bariatric surgery, AF risk was 29% lower in the surgery group versus the control group (HR 0.71; 95%CI: 0.60-0.83, $p<0.001$), although these effects only became apparent after several years follow-up and not all patients were randomly allocated to bariatric surgery that may have introduced bias (283). Another single centre retrospective cohort study showed AF incidence was lower in obese patients that had undergone bariatric surgery compared to those that were medically managed (0.8% vs 2.9%, $p=0.0001$) (284).

5.1.3. Adverse electrophysiological remodelling in obesity

The studies discussed hitherto suggest obesity confers a higher risk of incident atrial arrhythmias and that weight loss partly mitigates arrhythmic burden or recurrence in patients with pre-existing atrial arrhythmias. With respect to the latter, the proarrhythmic substrate in obesity patients with AF can be invasively characterised. Indeed, this has enabled others to show that conduction abnormalities co-localise

with EAT in obese patients undergoing catheter ablation (99). On the other hand, the proarrhythmic substrate in obese patients *without* a history of arrhythmias has not been studied in-depth. Currently, therefore, it is unclear if and how weight loss reverses any subclinical electrophysiological remodelling in arrhythmia-free obese patients. Mechanistic insights into the proarrhythmic remodelling in these patients would be helpful to explain the inconclusive evidence for the effects of weight loss on incident AF risks discussed above, and moreover, inform and guide primary preventative strategies. Furthermore, in comparison to atrial arrhythmias, there is a paucity of evidence for adiposity-related adverse ventricular electrophysiological remodelling and, importantly, its reversibility with weight reduction. Given that ventricular arrhythmias are more common in obese patients (45) and are likely to be life-threatening or poorly tolerated, establishing the reversibility of a proarrhythmic ventricular substrate represents an important clinical endeavour that could also inform primary prevention.

The hypothesis that weight reduction induces reverse electrical remodelling even in patients without a history of arrhythmias is plausible given that metabolic complications of obesity are mitigated by bariatric surgery. Indeed, there is increasing support for the BMI threshold for bariatric surgery to be reduced to enable a greater number of relatively younger obese patients with more modest BMI to become eligible for surgical weight loss intervention before the onset of irreversible end-organ damage due to obesity (535, 536, 537, 538). Given that P wave and QTc interval abnormalities reverse with weight loss in arrhythmia-free obese individuals (105, 539), it is possible that bariatric surgery early in natural history of obesity may

mitigate incident arrhythmic risk before adverse electrophysiological remodelling becomes irreversible.

5.1.4. Non-invasive characterisation of proarrhythmic substrates

One of the practical reasons why the proarrhythmic substrate and any reverse remodelling in arrhythmia-free obese patients has not been characterised to date is the absence of the clinical indication for invasive electrophysiological studies. However, this can be addressed by an alternative method by which activation and repolarisation parameters are reconstructed non-invasively. Using data from high-density body surface mapping and cross-sectional imaging to acquire individuals' unique heart-torso geometries, electrocardiographic imaging (ECGi) solves the inverse problem of electrocardiography to computer epicardial electrograms by which activation and repolarisation patterns can be studied non-invasively (309). The principles, development, validation and clinical applications of this mapping technique are described in detail in Chapter 2 and have also been reviewed by several others (289, 290, 309, 316, 317, 350). Amongst these, it is noteworthy that ECGi has been used to study arrhythmic substrates in other at-risk cohorts that may not have a clinical indication for invasive electrophysiological studies. This includes cardiac amyloidosis (300), Brugada syndrome (540), structural heart disease (417) and arrhythmogenic right ventricular cardiomyopathy (408). However, it has also been used to map haemodynamically stable and unstable ventricular arrhythmias (311) and study atrial fibrillation (306, 310) and is therefore a versatile modality that can be used to study cardiac electrophysiology as well as guide treatment (317).

5.1.5. Aims and hypotheses

Using ECGi, this study sought to address the limitations of current understanding of the proarrhythmic substrate in arrhythmia-free obese patients compared to non-obese individuals, and the extent to which any observed subclinical electrophysiological remodelling is reversed following bariatric surgery. The pre-specified hypotheses were:

- 1) Total atrial activation time is prolonged and dispersion of atrial activation is greater in obese compared to lean individuals, and reverse post-bariatric surgery
- 2) Total ventricular repolarisation time is prolonged and dispersion of ventricular repolarisation is greater in obese compared to lean individuals, and reverse post-bariatric surgery
- 3) Spatial gradients of atrial activation and ventricular repolarisation are steeper in obese compared to lean individuals, and reverse post-bariatric surgery
- 4) Bariatric surgery is associated with structural and metabolic adaptations in obesity, including regression of epicardial adipose tissue (EAT)
- 5) Activation or repolarisation abnormalities in obesity that are not observed in the resting state become manifest post-exercise.

5.2. Methods

5.2.1. Study population

Upon application for ethical approval, a favourable opinion for this study was received from the Health Research Authority London (Surrey) Research Ethics Committee on 25 June 2019 (IRAS project ID 255939, protocol number 19HH4965, reference 19/LO/0762, sponsor Imperial College London). This was a single centre

prospective clinical study conducted at Hammersmith hospital campus, Imperial College London, between November 2019 and February 2022. The bariatric service at Imperial College Healthcare NHS Trust is amongst one of the highest volume centres in the UK and performs approximately 500 bariatric surgeries per year. Prospective surgical candidates were considered eligible for the study if they were aged 18 to 75 years, were morbidly obese ($\text{BMI} > 40 \text{ kg/m}^2$), had no history of atrial or ventricular arrhythmias, and were awaiting their first laparoscopic sleeve gastrectomy or gastric bypass surgery for weight management in line with NICE guidelines (541). Exclusion criteria included pregnancy, breastfeeding, previous bariatric surgery and history of cardiac arrhythmias. Patients undergoing bariatric surgery with the intention to induce remission of diabetes were not recruited. Recruitment took place at the pre-operative dietitian clinic led by the clinical bariatric services at Imperial College Healthcare NHS Trust at St. Mary's hospital. Recruitment took place virtually during the coronavirus pandemic. In both settings, the study was introduced to obese patients attending the dietetic clinic by a healthcare professional from the bariatric team, and referred to the research team if they expressed an interest in the present study. Interested participants were provided with verbal and written information about the study by the research team and afforded as much time as required to consider it and ask any questions. Non-obese (lean) individuals with a normal BMI ($18\text{-}25 \text{ kg/m}^2$) were recruited by advertisement on Hammersmith hospital campus, Imperial College London. All participants provided written informed consent. This study conformed to the Declaration of Helsinki.

Given that no previous studies had implemented ECGi before and after bariatric surgery, results from another study (113), in which patients had QTc intervals measured before and after bariatric surgery using 12-lead ECG, were used to estimate power calculations. Assuming a conservative effect size (change in QTc interval following bariatric surgery) of 12ms (standard deviation 10ms), delta of zero milliseconds in the non-obese (control) group (standard deviation 10ms), and a 1:1 randomisation ratio, 16 patients would be needed in each group to provide 90% power to detect an effect at the two-tailed 5% significance level. Allowing for loss to follow-up, the original aim was to recruit 20 participants in each group (obese and non-obese), however, this was not possible owing to unforeseen circumstances presented by the coronavirus pandemic.

5.1.2. Electrocardiographic imaging protocol

The methods used in this study are described fully in Chapter 2, and summarised herein for context. Consenting obese patients underwent ECGi before bariatric surgery according to their convenience and MRI availability in the Robert Steiner MRI unit, Hammersmith Hospital. Obese patients were recalled for repeat ECGi at least 6 months after their surgery. Lean participants had ECGi once only. The ECGi protocol was identical in all instances, which comprised the manual application of 256 numbered electrodes using the ActiveTwo (BioSemi, Netherlands) system across participants' torsos to record body surface potentials for 10 minutes at rest at a sampling frequency of 2048Hz. Participants then undertook incremental exercise on an Cardiostrong recumbent exercise bicycle BC50 at 25 Watts per minute until 85% age-predicted heart rate ($220 - \text{age}$) was achieved or were unable to continue exercise, whichever was reached first. Body surface potentials were recorded in the

final minute of exercise and the following nine minutes into the post-exercise recovery period. Each electrode was then replaced with magnetic resonance imaging (MRI)-safe markers in identical anatomical locations prior to non-contrast cardiothoracic MRI (CMR) on a Siemens MAGNETOM Aera 1.5T scanner (Erlangen, Germany).

The CMR protocol included a respiratory-navigated and electrocardiogram-gated *Half-Fourier Acquisition Single-shot Turbo spin Echo* (HASTE) sequence in the transverse plane. 94 slices were acquired at end-diastole throughout the lung fields ensuring visualisation of all electrode markers. In addition, cardiac cine imaging was acquired in two-, three- and four-chamber views as well as left ventricle short-axis orientation using a balance steady-state free precession (bSSFP) sequence. The ECG method and study protocol are shown in **Figures 5.1 and 5.2 respectively**.

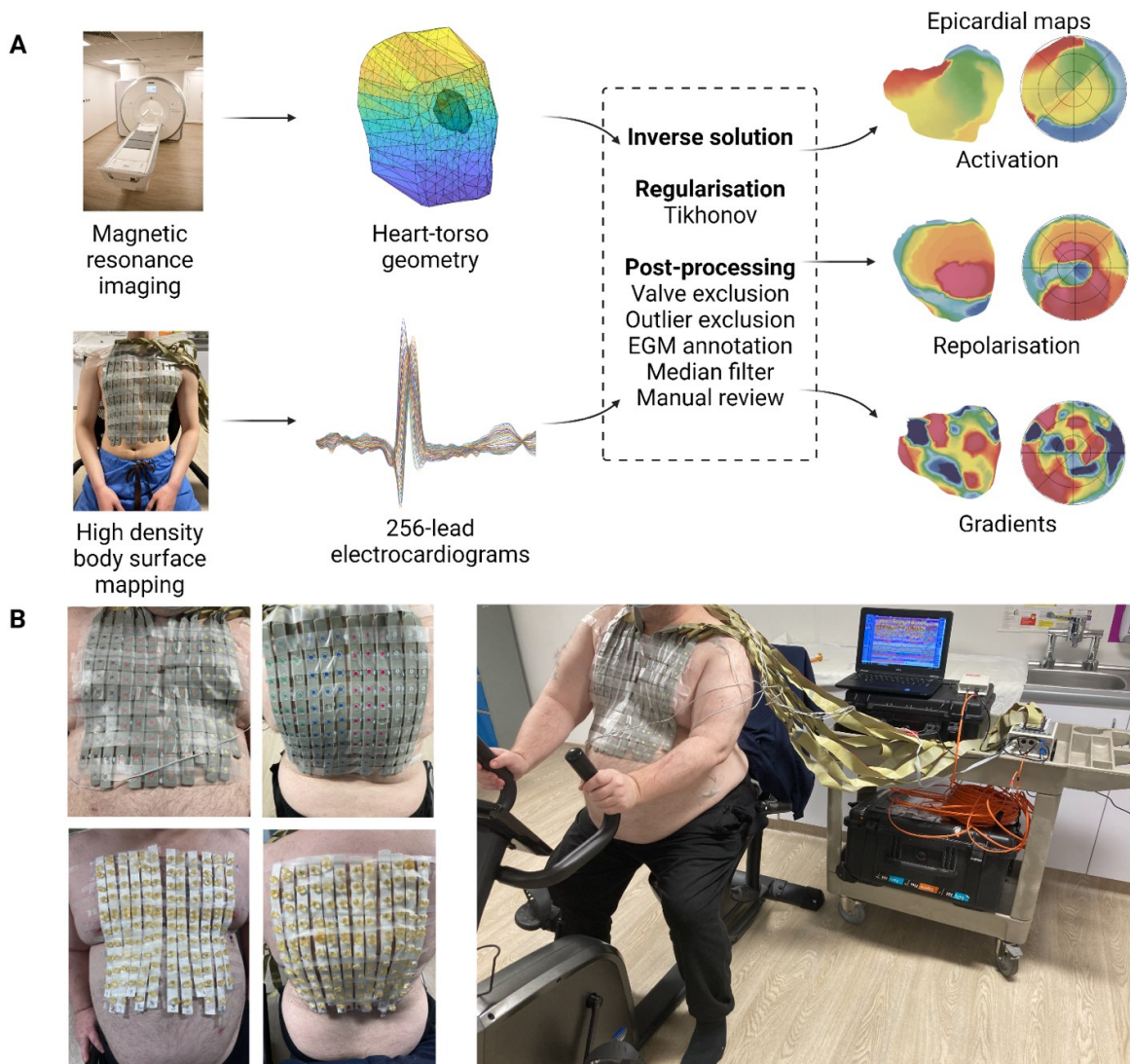


Figure 5.1: Electrocardiographic imaging. (A) High-density body surface mapping using 256 electrodes and MRI-derived heart-torso geometries were used as inputs to solve the inverse problem of electrocardiography to compute epicardial activation and repolarisation patterns. **(B)** Images showing strips of electrodes and their markers in situ, as well as ECGi in progress in an obese participant (photograph taken and included with permission from participants).

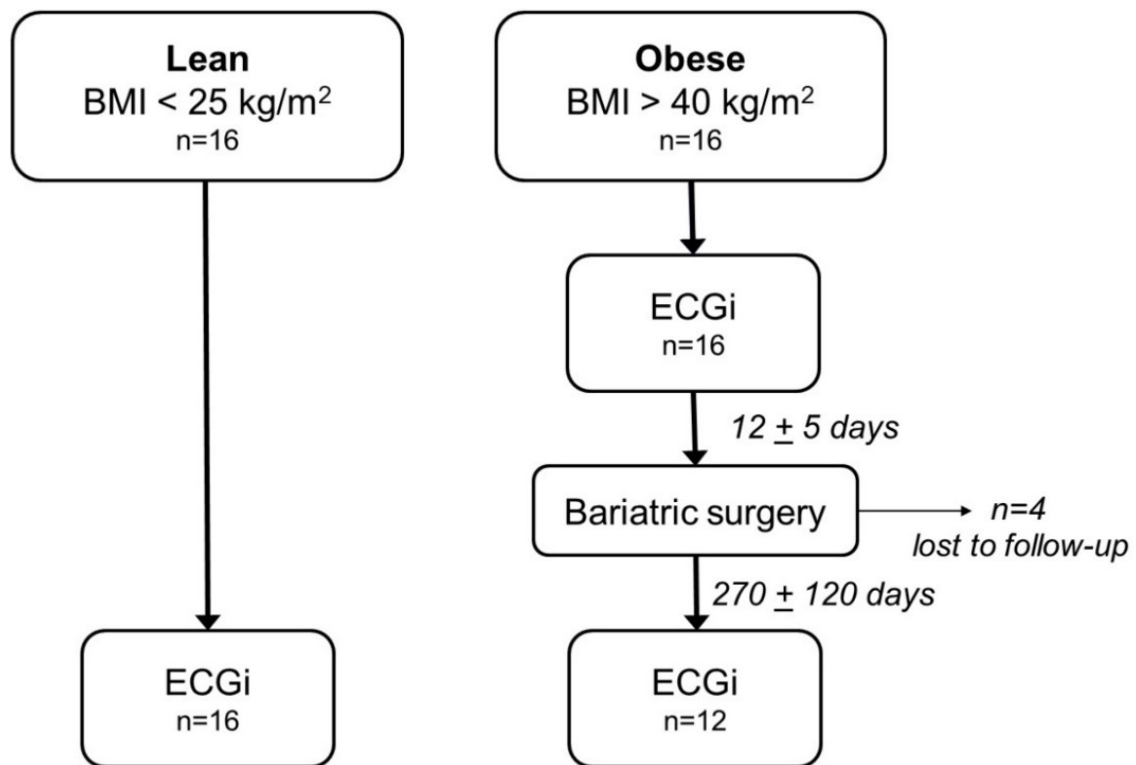


Figure 5.2: Study protocol. Sixteen lean and morbidly obese participants were recruited to the study. Lean participants underwent ECGi once and obese participants had ECGi before and after bariatric surgery. The ECGi protocol was identical in all instances. Four participants did not return for ECGi post-surgery and were lost to follow-up. Results were compared between lean and obese groups (unpaired analyses), as well as before and after surgery (paired analyses) in the obese cohort.

Practical assistance with body surface mapping and performing MRI was provided by Ben Statton (superintendent radiographer), Alaine Berry (radiographer) and Hannah Bergman (radiographer) from the Medical Research Council London Institute of Medical Sciences, Robert Steiner MRI unit, Hammersmith Hospital. Maria Brezitski (research nurse), Ross Davidson, Kimberley Nyamakope, Nihara Herath

(intercalating medical students at Imperial College London), Stelutsa Savvidou (Master of Research student at Imperial College London), and Danya Agha-Jaffar all provided practical assistance with application of body surface electrodes.

5.2.3. 12-lead ECG derivation

Body surface electrodes that spatially corresponded to conventional placement of precordial leads (V1-6) were used to derive their corresponding ECG signals. A neutral electrode was placed on the right side of the abdomen, and electrodes placed on the right arm, left arm and left side of the abdomen were used to calculate bipolar limb leads I, II and III. Augmented unipolar leads aVL, aVR and aVF were calculated from bipolar leads. Wilson's central terminus (reference) was computed by averaging signals from the limb leads. In this way, 12-lead ECG was recorded from all participants to calculate QRS duration, QTc interval, QTc dispersion (difference between maximum and minimum QTc interval), P-wave duration and P-wave dispersion (difference between maximum and minimum P-wave duration).

5.2.4. MRI analysis

EAT quantification and ventricular chamber volume analysis was performed by Ben Statton using methods described previously (99). Image analysis was performed using commercial software (CVI42, v5.13.5, Circle Cardiovascular Imaging Inc., Canada). EAT was defined as a region of high signal intensity between the myocardium and the visceral pericardium on the short-axis bSSFP MR images. Areas of EAT were manually traced on consecutive end-diastolic short axis slices from the level of the mitral valve down to the ventricular apex, including the apical cap. These EAT measurements were then multiplied by the thickness of the

imaged slice (10mm) and then summed to determine the total EAT volume. Biplane method was used to quantify left and right atrial volumes in four- and two-chamber (4ch and 2ch, respectively) views; LA volume was calculated using the formula $(0.848 \times 4\text{ch area} \times 2\text{ch area}) / ([2\text{ch length} + 4\text{ch length}] / 2)$ and RA volume using $3.08 \times (2\text{C area}) + 3.36 \times (4\text{C area}) - 44.4$.

5.2.5. Signal processing

The ECGi method and the process for solving the inverse problem of electrography are described in Chapter 2. The methods for pre- and post-processing of ECG and epicardial electrogram signals are described in Chapter 4. The optimisation of the ECGi method described in Chapter 4 served to minimise any inaccuracies introduced in electrogram reconstruction by noise and artefact in the body surface potentials, as well as to ensure that activation and repolarisation times were accurately identified in their respective waveforms in the epicardial electrograms. These ensured the robustness of the electrophysiological data presented in this chapter. A brief explanation of the ECGi method is provided herein for context. Computational expertise in signal processing to achieve the methods described herein was provided by Nikesh Bajaj (research associated, National Heart and Lung Institute).

Atria and ventricles were segmented from MR images taken at end-diastole and their respective epicardial meshes, each comprising 2002 nodes, were reconstructed using Amira (ThermoFisher, MA) by Ben Statton. Electrode positions were identified by their corresponding radio-opaque markers and manually annotated on volume-rendered images. MR images provided heart-torso geometries from which a transfer

matrix was computed to reconstruct unipolar epicardial electrograms from body surface potentials by solving the inverse problem of electrocardiography. In this way, the cardiac electrical source was determined from recorded body surface potentials. However, the inverse problem is ill-posed, such that even a small degree of noise recorded on the body surface signals is amplified in the computed epicardial electrograms, and it yields several solutions (different patterns of cardiac electrical activity produce similar body surface potentials) (542). To address this, 10-beat signal averaging of body surface potentials was performed to enhance signal quality using customised software (MATLAB, MathWorks, MA). Ten consecutive beats in the minute-segment with the least RR interval variability were averaged to compute epicardial electrograms in the resting state. The first ten consecutive beats in the second minute after exercise cessation were averaged to do the same for the post-exercise state. These methods are described in Chapter 4. Zero-order Tikhonov regularisation was used to constrain solutions to the inverse problem. An extracellular potential-based model was used to represent the cardiac source. Atrial and ventricular epicardial surfaces were artificially closed across the valves to compute epicardial electrograms. Thus, unipolar epicardial electrograms, representing the change in potential over time at a given anatomical site, and potential maps, showing the potential distribution over the epicardium at a given time point, were computed non-invasively. Given the conical anatomy of ventricles, activation and repolarisation patterns were reconstructed in conventional three-dimensional maps, as well as in a bulls-eye plot enabling a panoramic overview of right and left ventricular electrophysiology (543).

In total, 2002 electrograms were computed over the atrial and ventricular epicardium. A spatiotemporal approach was used to identify activation and repolarisation times (292). This method considers the fact that activation and recovery are linked by electrotonic coupling and their respective temporal characteristics are also influenced by regional, i.e. spatial, flow of current (292). Local activation time (AT) was taken as time of the steepest downslope ($-dV/dt_{\min}$) of the activation complex in atrial and ventricular electrograms, and ventricular repolarisation times (RT) as the maximum upslope ($dV/dt_{\max}$) of the T-wave according to the Wyatt method (418). All electrograms underwent automated analysis with manual verification to ensure AT and RT were correctly identified. AT and RT in electrograms reconstructed over the valve plane were excluded from analyses. Given that total AT and RT can be skewed by outliers caused by noise or temporal mis-annotation, we defined total AT and RT as the smallest range encompassing 95% of all local AT and RT, respectively (33, 515). Global dispersion of ATs (ATD) for atria and ventricles, and RTs (RTd) for ventricles, were quantified as their respective standard deviations. Local spatial gradients of activation (ATG) for atria and ventricles, and repolarisation (RTG) for ventricles, were measured as the maximum difference between electrograms within 10mm radius divided by their corresponding distance (38). Activation-recovery intervals (AR_{IC}), a surrogate measure of local action potential duration (419), was calculated as the difference between local AT and RT and corrected for heart rate using Bazett's formula.

5.2.6. Autonomic function quantification

Resting body surface potentials were recorded under identical steady-state physiological conditions between groups and were used to compare heart rate

variability (HRV) as a measure of autonomic function between non-obese and obese groups, as well as before and after surgery (544). HRV was quantified using time-domain features relating to the RR interval. Given that sympathetic and parasympathetic tone co-exist in vivo, delineating their individual functions is complex. Indeed, any attempts to do so is likely to be an over-simplification. Nevertheless, SDRR (standard deviation of RR intervals) may be considered a surrogate for overall HRV and is an indication of sympathetic tone (545). Root mean square of successive differences of RR intervals (RMSSD in milliseconds) and the standard deviation of successive differences (SDSD in milliseconds) quantify beat-to-beat variability have been used to assess parasympathetic tone (545). The number successive RR intervals with $>50\text{ms}$ and $>20\text{ms}$ between them are denoted by nni50 and nni20 , respectively. Nni50 and nni20 divided by the total number of RR intervals are denoted by pnni50 and pnni20 , respectively. These metrics, therefore, also quantify beat-to-beat variability. For resting data, HRV metrics were calculated over the first 5 minutes of body surface potential recordings. In the post-exercise phase, HRV metrics were calculated over the second minute in the post-exercise period.

5.2.7. Statistical analyses

Pre-processing of body surface potentials and post-processing of reconstructed electrograms were performed blinded as much as practically possible. Analyses comprised of three comparisons: lean vs pre-surgery (PreSurg) obese ($n=16$, unpaired analysis), lean vs post-surgery (PostSurg) obese ($n=12$, unpaired analysis), and pre- vs post-surgery obese ($n=12$, paired analysis). This accounted for the 4 obese participants that did not return for their post-operative ECGi (Figure 5.2).

Electrophysiological data was compared between groups at rest and at 2 minutes post-exercise into recovery phase (301). These are presented separately as indicated in the each of the results sections.

Data were tested for normality using the Shapiro-Wilk test. All normally distributed continuous variables are presented as mean $\pm$ standard deviation (SD). Non-normally distributed parameters are presented as median and interquartile range (IQR). For normally distributed data, unpaired analyses were performed using unpaired two-tailed Student's T-test, and paired analyses were performed using paired two-tailed Student's T-test. For non-normally distributed data, unpaired data were analysed with Mann-Whitney U-test, and paired data with Wilcoxon matched-pairs signed rank test. Chi-squared test was used to compare categorical variables. Spearman's rank test was used to test the strength and significance of any correlations between variables. Two-away ANOVA was used to investigate whether exercise unmasked any activation and repolarisation abnormalities. $P < 0.05$ was considered significant and all statistical analyses were performed using Prism 9.3.1 (GraphPad Software, California, USA). Ns, non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

5.3. Results

5.3.1. Participant characteristics

Sixteen obese patients (13 female, age 43 ± 12 years) underwent ECGi pre-bariatric surgery (PreSurg), and due to participant dropout, twelve of these also had ECGi post-surgery (PostSurg). One patient was excluded from the study as they were unable to undergo CMR and their data was not included in analyses presented in

this thesis. The mean time difference between ECGi recordings in these patients was 270 ± 120 days. Sixteen age- and sex-matched lean individuals (42 ± 11 years) underwent ECGi once. Baseline characteristics for lean versus PreSurg individuals are shown in Table 5.1. All participants were in sinus rhythm during ECGi and had no history of atrial or ventricular arrhythmias, ischaemic heart disease or heart failure. No participant was taking QT-prolonging or anti-arrhythmic medication.

5.3.2. Bariatric surgery induces left ventricular reverse remodelling and improves glycaemic control

BMI was greater in PreSurg obese vs lean (46.7 ± 5.5 vs $22.8 \pm 2.6 \text{ kg/m}^2$, $p < 0.0001$). Bariatric surgery led to a mean weight reduction of $28.6 \pm 11.0 \text{ kg}$ (ΔBMI $10.1 \pm 4.1 \text{ kg/m}^2$; $\Delta 21.7 \pm 8.3\%$ total body weight), though patients remained clinically obese defined by BMI following bariatric surgery (PreSurg vs PostSurg: 46.7 ± 5.5 vs $36.8 \pm 6.5 \text{ kg/m}^2$, $p < 0.0001$). LV mass/height^{2.7} was greater in PreSurg obese vs lean ($33 \text{ g/m}^{2.7}$ IQR 30-36 $\text{g/m}^{2.7}$ vs $22 \text{ g/m}^{2.7}$ IQR 20-25 $\text{g/m}^{2.7}$, $p < 0.0001$) and decreased following bariatric surgery (PostSurg $25 \text{ g/m}^{2.7}$ IQR 23-28 $\text{g/m}^{2.7}$, $p < 0.0005$). Left ventricular ejection fraction (LVEF) was greater in PreSurg obese vs lean ($67 \pm 4\%$ vs $63 \pm 6\%$, $p = 0.037$), but not different between PostSurg obese vs lean. Indexed to height^{2.7}, left ventricular end-diastolic (LVEDV) was greater in PreSurg obese vs lean ($35 \pm 5 \text{ ml}$ vs $30 \pm 5 \text{ ml}$, $p = 0.0058$), while no difference in LV end-systolic (LVESV) volume PreSurg obese vs lean (12 ± 3 vs $11 \pm 3 \text{ ml}$, $p = 0.54$). LVEDV and LVESV did not change pre- vs post-surgery (LVEDV 35 ± 5 vs $35 \pm 5 \text{ ml}$, $p = 0.83$; LVESV 12 ± 3 vs $13 \pm 3 \text{ ml}$, $p = 0.09$). HbA1c decreased following bariatric surgery (11 PreSurg vs 10 PostSurg, unpaired analysis): 38.4 ± 8.6 vs $47.5 \pm 15.3 \text{ mmol/mol}$, $p = 0.018$. These results are presented in Figure 5.3.

	Lean (n=16)	Pre-surgery obese (n=16)	P
Demographics			
Age	42 ± 11	43 ± 12	0.77
Female	13	13	-
Body mass			
Weight (kg)	65.4 ± 9.8	132.7 ± 16.5	<0.0001
BMI (kg/m ²)	22.8 ± 2.6	46.7 ± 5.5	<0.0001
Surgery			
Sleeve gastrectomy	-	9	-
Roux-en-Y gastric bypass	-	7	-
Co-morbidities			
Obstructive sleep apnoea	0	5	0.043
Hypertension	0	7	0.0068
Type 2 diabetes	0	5	0.043
Dyslipidaemia	0	3	0.23
Table 5.1: Baseline characteristics of lean and pre-surgical obese participants. Co-morbidities were more prevalent in obese patients.			

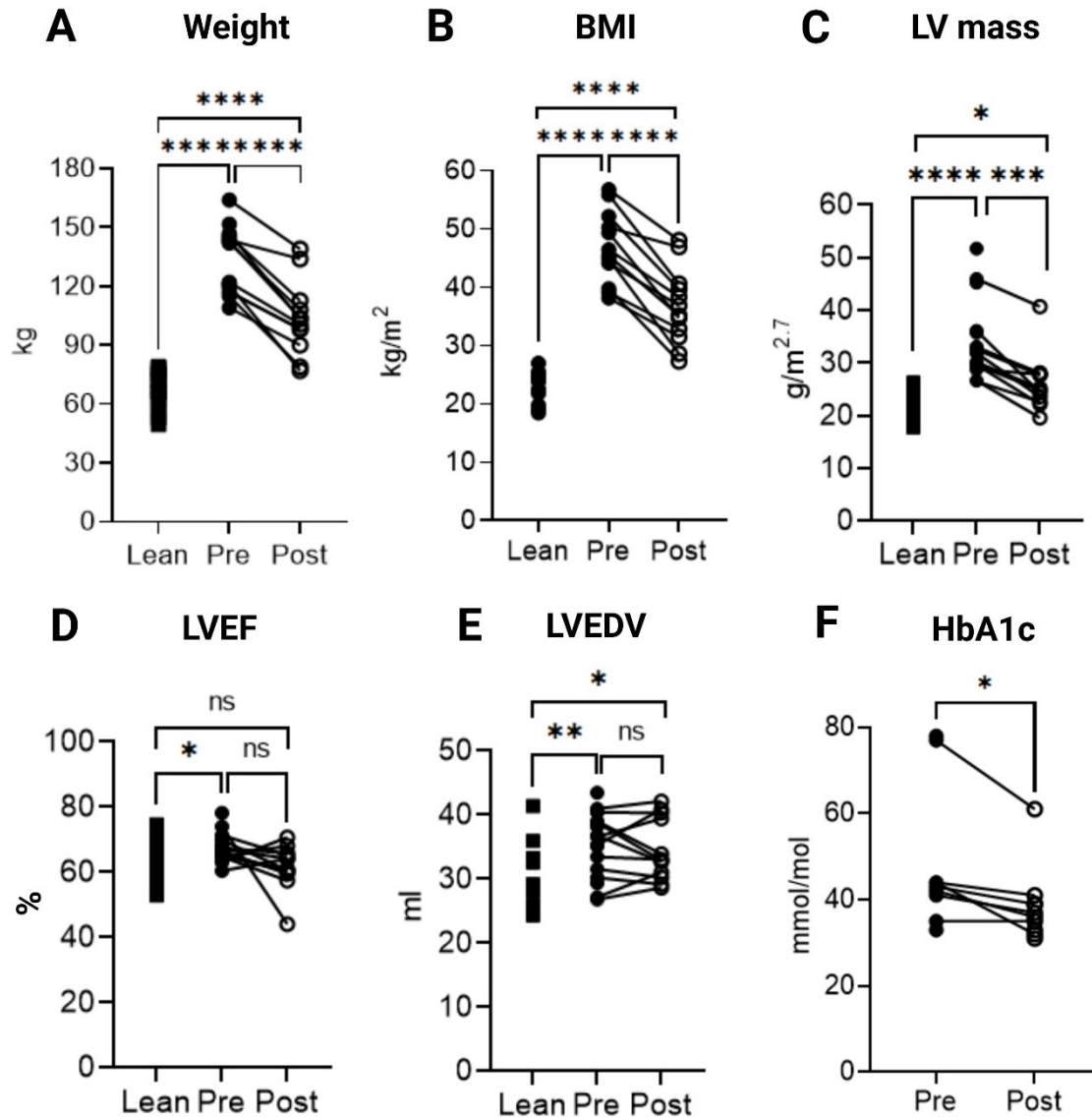


Figure 5.3: Weight loss with bariatric surgery induces left ventricular reverse remodelling and improves glycaemic control. Weight (A) and BMI (B) were greater in pre-surgical obese vs lean and decreased post-bariatric surgery. (C) LV mass index to height raised to power 2.7 was greater in obese patients and reduced following bariatric surgery. Left ventricular ejection fraction (LVEF) (D) and left ventricular end-diastolic (LVEDV). (E) were greater in pre-surgical obese vs lean and did not change significantly post-surgery. (F) HbA1c decreased post-bariatric surgery in obese patients. Pre, pre-surgical obese; Post, post-surgical obese.

5.3.3. Epicardial adipose tissue volume decreases post-bariatric surgery

EAT volume was greater in PreSurg obese vs lean: 68.7cm^3 (IQR: $49.9\text{--}98.6\text{cm}^3$) vs 24.7cm^3 (IQR: $17.4\text{--}37.3\text{cm}^3$), $p < 0.0001$. EAT volume decreased post-surgery (64.5cm^3 IQR $39.3\text{--}78.0\text{cm}^3$, $p = 0.0010$), though it remained greater in PostSurg vs lean ($p = 0.0003$) (Figure 5.4). EAT volume was positively associated with BMI in lean and PreSurg ($r = 0.71$, $p < 0.0001$).

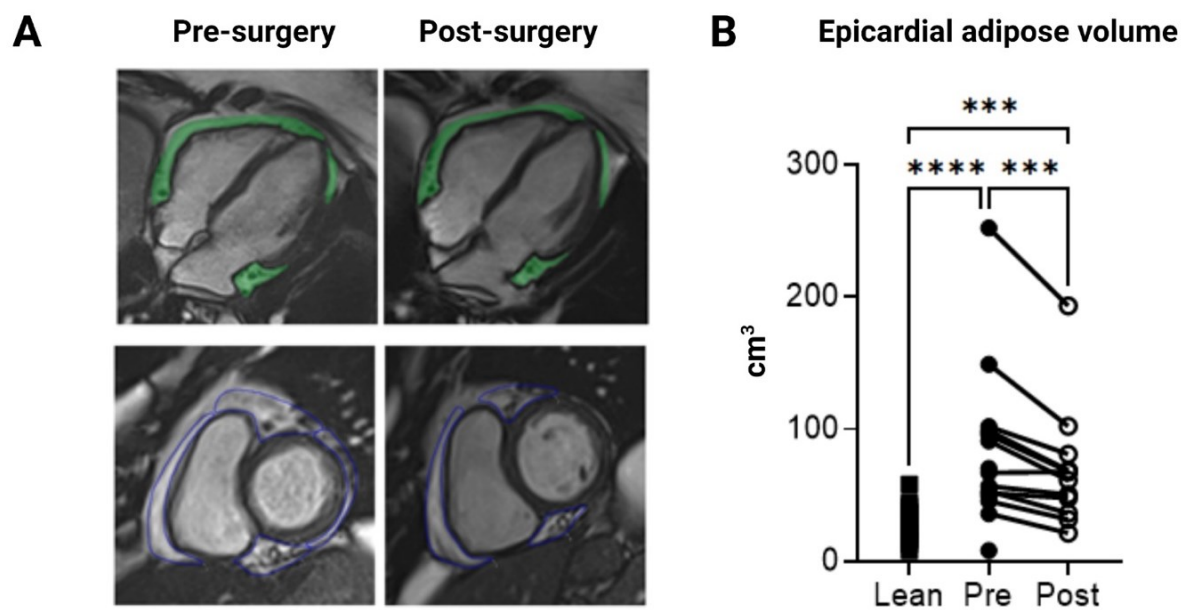


Figure 5.4: Epicardial adipose tissue volume regresses post-bariatric surgery. (A) Cross-sectional images showing EAT pre- and post-surgery. (B) EAT was greater in pre-surgical obese patients vs lean and decreased post-surgery. Pre, pre-surgical obese; Post, post-surgical obese.

5.3.4. Atrial conduction is slower in obese patients and persists post-bariatric surgery

Rest:

Total atrial AT was prolonged in PreSurg obese vs lean at rest (62 ± 15 vs 46 ± 12 ms, $p=0.0028$) and was unchanged following bariatric surgery (PreSurg vs PostSurg: rest 62 ± 15 vs 67 ± 15 ms, $p=0.86$). Atrial AT remained prolonged in PostSurg vs lean ($p=0.0005$). ATD was also greater in PreSurg obese vs lean (19 ± 6 vs 13 ± 4 ms, $p=0.0039$) and did not change post-surgery (PreSurg 20 ± 6 ms, $p=0.82$). ATD was greater in PostSurg vs lean ($p=0.0023$). Maximum and mean atrial ATG were steeper in PreSurg obese vs lean (maximum 102 ± 37 vs 52 ± 21 ms/mm, $p<0.0001$; mean 26 ± 11 vs 14 ± 8 ms/mm, $p=0.0007$). ATG did not change following bariatric surgery (PreSurg vs PostSurg: maximum 102 ± 37 vs 97 ± 38 ms/mm, $p=0.16$; mean 26 ± 11 vs 25 ± 12 ms/mm, $p=0.44$). Consequently, maximum and mean atrial ATG were also steeper PostSurg vs lean ($p=0.0006$ and $p=0.0044$, respectively). Resting atrial ECGi results are shown in Figure 5.5.

Post-exercise:

The differences observed between groups for atrial activation parameters at rest were preserved between groups at 2 minutes into the post-exercise recovery phase. Total atrial AT was prolonged in PreSurg obese vs lean (59.8 ± 14.9 vs 47.4 ± 12.9 ms, $p=0.0181$) and did not change post-surgery (PostSurg 57.8 ± 13.6 ms, $p=0.3344$; lean vs PostSurg $p=0.0502$). The same pattern of differences was observed with ATD (lean 13.2 ± 3.4 ms, PreSurg 17.1 ± 5.1 ms, PostSurg 17.4 ± 4.8 ms; lean vs PreSurg $p=0.0164$, PreSurg vs PostSurg $p=0.9485$, lean vs PostSurg $p=0.0115$), maximum ATG (lean 42.7 ± 16.7 ms/mm, PreSurg 92.8 ± 48.4 ms/mm, PostSurg 81.8

$\pm 45.3\text{ms/mm}$; lean vs PreSurg $p=0.0005$, PreSurg vs PostSurg 0.1787 , lean vs PostSurg $p=0.0037$) and mean ATG (lean $12.8 \pm 4.6\text{ms/mm}$, PreSurg $23.5 \pm 14.4\text{ms/mm}$, PostSurg $24.2 \pm 14.9\text{ms/mm}$; lean vs PreSurg $p=0.0081$, PreSurg vs PostSurg 0.6041 , lean vs PostSurg $p=0.0077$).

There were no statistically significant interactions between time (i.e. rest vs post-exercise) and obesity status, i.e. P (time $\times$ obesity status), for total atrial AT ($p=0.4885$), dispersion of atrial AT ($p=0.3078$), maximum atrial ATG ($p=0.9570$), and mean atrial ATG ($p=0.7310$).

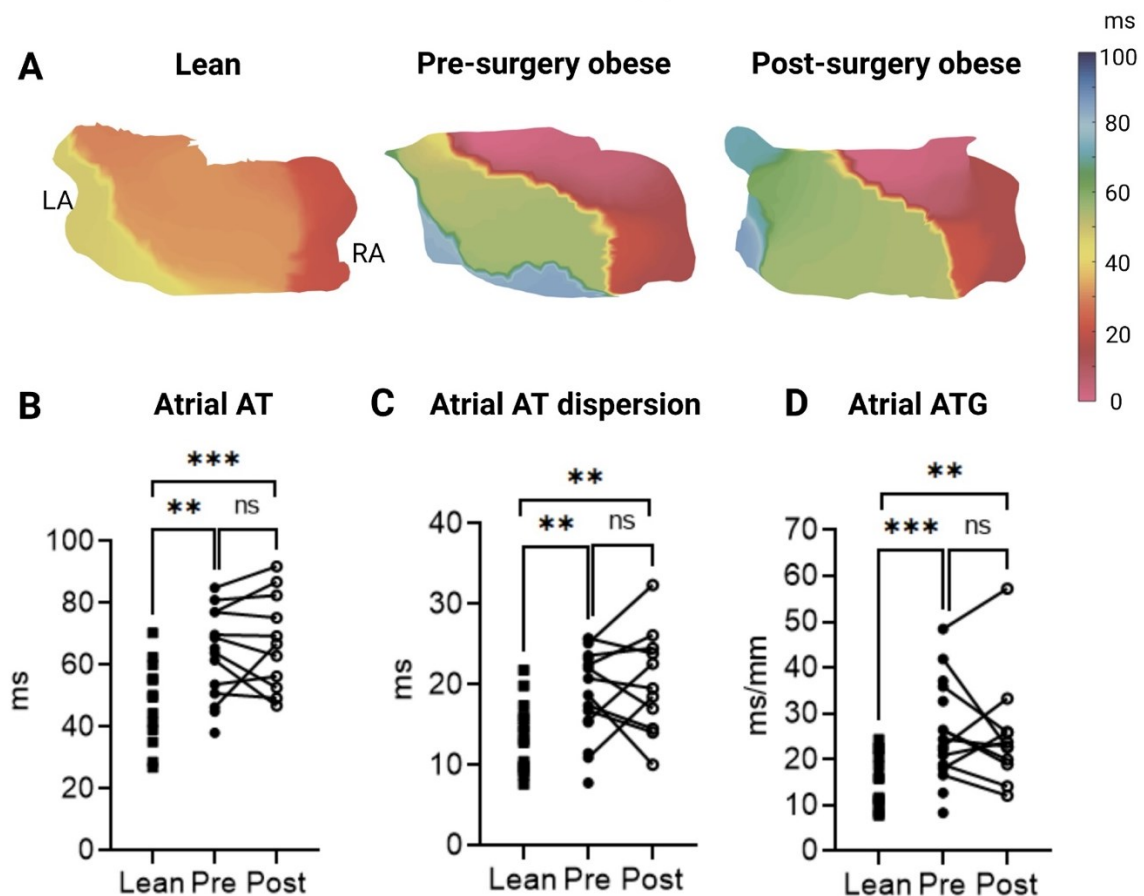


Figure 5.5: Total atrial activation is prolonged in obesity compared to lean individuals and persists post-bariatric surgery. (A) Activation maps showing earliest AT in the RA and latest AT in the LA across all groups. Total atrial AT was slower in pre- and post-surgical obese patients compared to lean individuals, with no difference following bariatric surgery. These results are represented in **(B)**. **(C)** Dispersion of atrial activation was greater in obese patients and persisted after surgical weight loss. Similarly, mean atrial activation gradients (ATG) were greater in pre- and post-surgical obese groups compared to lean individuals **(D)**. Data shown refers to resting state. Pre, pre-surgical obese; Post, post-surgical obese.

5.3.5. Spatial ventricular repolarisation gradients are steeper in obese patients and reverse with bariatric surgery

Rest:

Total ventricular RT was prolonged in PreSurg obese vs lean (105 ± 16 vs 87 ± 25 ms, $p=0.02$). Although total ventricular RT was not altered by bariatric surgery (PreSurg vs PostSurg: 105 ± 16 vs 100 ± 20 ms, $p=0.70$), there was no statistical difference between PostSurg and lean groups ($p=0.15$). There were no differences in global ventricular RTD between groups: lean 25 ± 7 ms, PreSurg 29 ± 5 ms, PostSurg 28 ± 6 ms. Similarly, mean ARlc and its global dispersion were also not significantly different in lean vs PreSurg vs PostSurg (ARlc: 245.7 ± 25.3 ms vs 247.2 ± 18.7 ms vs 247.8 ± 16.2 ms; ARlc dispersion: 28.8 ± 8.1 ms vs 33.8 ± 5.4 ms vs 32.7 ± 6.8 ms). Spatial ventricular RTGs, however, were steeper in PreSurg vs lean (maximum 107 ± 23 vs 62 ± 29 ms/mm, $p<0.0001$; mean 26 ± 11 vs 15 ± 8 ms/mm, $p=0.0024$). Ventricular RTG decreased post-bariatric surgery (maximum 107 ± 23 vs 65 ± 32 ms/mm, $p=0.0014$; mean 26 ± 11 vs 19 ± 8 ms/mm, $p=0.0009$), with no significant differences in ventricular RTG between PostSurg and lean (maximum RTG $p=0.80$; mean RTG $p=0.20$). These results are presented in **Figure 5.6**.

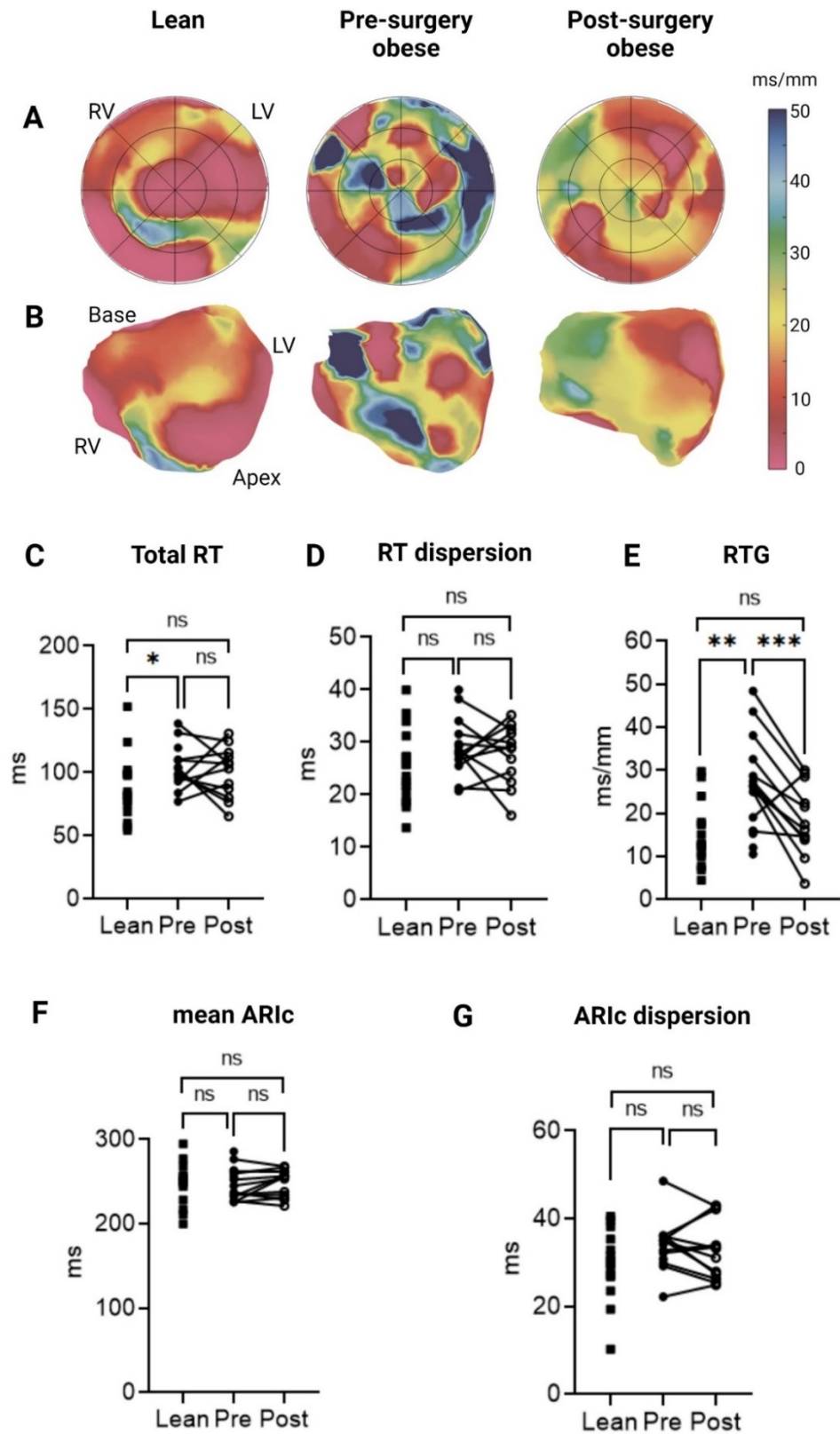


Figure 5.6: Spatial ventricular repolarisation gradients are greater in obesity and reverse post-bariatric surgery. Ventricular RTG maps are shown in

panoramic “bullseye” (543) **(A)** and 3D **(B)** views. The inner and outer rings in the panoramic view **(A)** represent the apex and base, respectively. These maps show that mean RTG were greater in pre-surgical obese vs lean, and were fully reversed with bariatric surgery, with no significant difference between post-surgical obese and lean individuals **(E)**. **(C)** Total ventricular repolarisation times (RT) were prolonged in obesity and there were no significant changes after bariatric surgery. **(D)** RT dispersion was not different between groups. Mean ARlc (F) and ARlc dispersion **(G)** were not significantly different between groups. RV, right ventricle; left ventricle; Pre, pre-surgical obese; Post, post-surgical obese.

Post-exercise:

The differences observed between groups at rest were preserved in the post-exercise phase. Total ventricular RT was prolonged in PreSurg obese vs lean (83.4 ± 18.7 vs 55.6 ± 21.4 , $p=0.0006$) and persisted after surgery (PostSurg 77.9 ± 18.9 ms, PreSurg vs PostSurg $p=0.579$, lean vs PostSurg $p=0.0087$). Ventricular RTD was greater in PreSurg vs lean (22.6 ± 5.9 ms vs 16.2 ± 7.1 ms, $p=0.0092$), and also persisted after surgery (PostSurg 22.8 ± 4.6 ms, PreSurg vs PostSurg $p=0.18$; lean vs PostSurg $p=0.0094$). The same pattern was observed with mean ARlc (lean 221.1 ± 21.9 ms, PreSurg 235.4 ± 19.4 ms; PostSurg 232.9 ± 13.9 ms; lean v PreSurg $p=0.034$, PreSurg vs PostSurg, $p=0.096$, lean vs PostSurg $p=0.0062$) and ARlc dispersion (lean 21.9 ± 9.2 ms, PreSurg 30.8 ± 7.8 ms, PostSurg 29.8 ± 7.4 ms; lean vs PreSurg $p=0.0073$, PreSurg vs PostSurg $p=0.3725$; lean vs PostSurg $p=0.0238$). Maximum RTG were greater in PreSurg vs lean (88.1 ± 32.8 ms/mm vs 36.0 ± 21.3 ms/mm, $p<0.0001$), and reversed post-surgery (PostSurg 46.7 ± 29.1 ms/mm;

PreSurg vs PostSurg $p=0.001$, lean vs PostSurg $p=0.2770$). Mean ventricular RTG showed the same pattern of change (lean $9.2 \pm 5.2\text{ms/mm}$, PreSurg $18.8 \pm 11.2\text{ms/mm}$, PostSurg $12.9 \pm 6.9\text{ms/mm}$; lean vs PreSurg $p=0.0053$, PreSurg vs PostSurg $p=0.0032$, lean vs PostSurg $p=0.1284$).

There were no statistically significant interactions between time (i.e. rest vs post-exercise) and obesity status, i.e. P (time $\times$ obesity status), for total ventricular AT ($p=0.6530$), dispersion of ventricular AT ($p=0.1113$), maximum and mean ventricular ATG ($p=0.6873$ and $p=0.3831$, respectively), total ventricular RT ($p=0.1746$), dispersion of ventricular RT ($p=0.3425$), maximum and mean ventricular RTG ($p=0.5850$ and $p=0.4849$, respectively), ARlc ($p=0.1187$) and ARlc dispersion ($p=0.8402$).

5.3.6. Ventricular activation is comparable between lean and obese individuals and does not change post-bariatric surgery

Rest:

In sinus rhythm, total ventricular ATs were not different lean, PreSurg and PostSurg (31 ± 6 , 34 ± 5 , $35 \pm 9\text{ms}$) groups. Similarly, ventricular AT dispersion was similar between all 3 groups (lean 8 ± 1 , PreSurg 8 ± 2 , PostSurg $9 \pm 2\text{ms}$) (Figure 5.7).

Post-exercise:

All participants remained in sinus rhythm following exercise. Total ventricular AT did was not different between groups at 2 minutes post-exercise: lean $32.6 \pm 6.0\text{ms}$, PreSurg $34.1 \pm 6.8\text{ms}$, PostSurg $35.2 \pm 10.3\text{ms}$; lean vs PreSurg $p=0.5376$, PreSurg vs PostSurg $p=0.4142$, lean vs PostSurg $p=0.4168$. Ventricular AT dispersion was

also similar between groups (lean $8.3 \pm 1.5\text{ms}$, PreSurg $7.9 \pm 1.7\text{ms}$, PostSurg $8.6 \pm 2.3\text{ms}$; lean vs PreSurg $p=0.4949$, PreSurg vs PostSurg, $p=0.2348$, lean vs PostSurg $p=0.6135$).

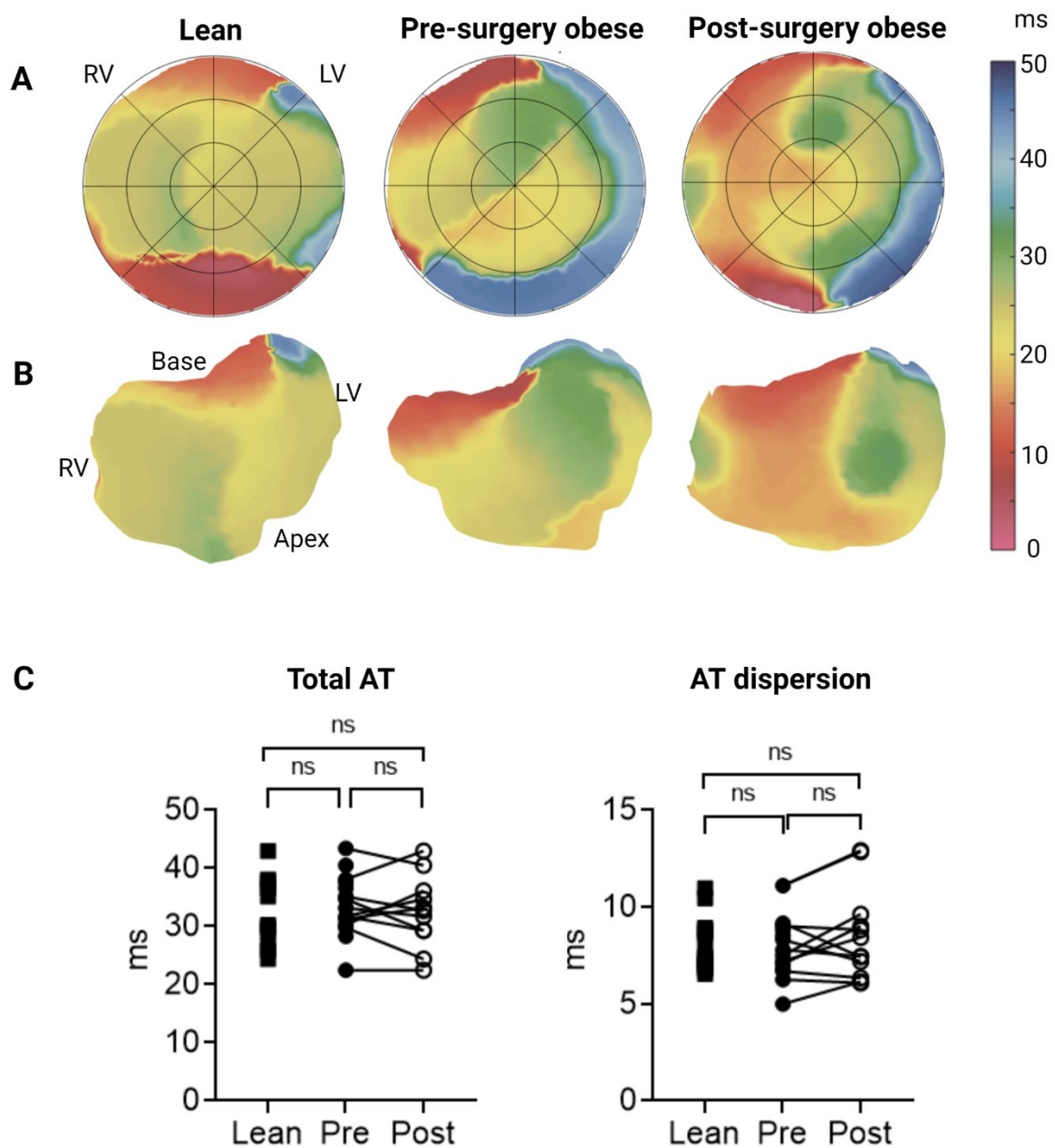


Figure 5.7: Total ventricular activation times and global dispersion of ventricular activation are similar between groups. Patterns of activation preserved between lean and obese groups, including pre- and post-surgery. Earliest activation occurred in the RV base, and the activation wavefront propagated anterolaterally with the latest activation occurring at the LV base. This is seen in the panoramic (A) and 3D (B) views. Comparisons of total AT and its

dispersion are shown in (C). RV, right ventricle; left ventricle; Pre, pre-surgical obese; Post, post-surgical obese.

5.3.7. Atrial activation and ventricular repolarisation gradients correlate with epicardial adipose tissue volumes

At rest, maximum atrial ATG correlated with EAT volume in lean and PreSurg obese individuals ($r=0.36$, $p=0.044$). There was also a positive correlation between maximum and mean ventricular RTG and EAT in lean and PreSurg individuals: $r=0.58$ ($p=0.0005$) and $r=0.54$ ($p=0.0014$), respectively. These results are shown in **Figure 5.8**. Correlations between EAT and ATG and RTG were similar at 2-minutes post-exercise (maximum atrial ATG vs EAT $r=0.3237$, $p=0.0707$; maximum RTG $r=0.5238$, $p=0.0025$; mean RTG $r=0.4141$, $p=0.0206$).

5.3.8. 12-lead ECG parameters are comparable between obese and non-obese individuals

Resting 12-lead ECG parameters were derived from body surface potentials by manual annotation using customised software on MATLAB (MathWorks, MA) written by Nikesh Bajaj. Mean P-wave duration was not different between lean (102 ± 5 ms), PreSurg (100 ± 7 ms) and PostSurg (97 ± 7 ms). Similarly, QRS duration and QTc interval were not different between groups (QRS duration: lean 85 ± 14 ms, PreSurg 84 ± 12 ms, PostSurg 85 ± 7 ms; QTc interval: lean 435 ± 11 ms, PreSurg 441 ± 14 ms, PostSurg 443 ± 10 ms). P-wave and QTc dispersions was calculated as the difference between their respective maximum and minimum values. P-wave dispersion, was greater in PreSurg vs lean (22 ± 7 ms vs 16 ± 8 ms, $p=0.0279$), and

did not change post-bariatric surgery (PostSurg 22 ± 5 ms, $p=0.0720$; lean vs PostSurg, $p=0.0265$). QTc dispersion as similar between groups (lean 45 ± 15 ms, PreSurg 51 ± 14 ms, PostSurg 48 ± 19 ms). These results are shown in Figure 5.9. Ectopy burden was assessed by manually screening resting and post-exercise body surface mapping recordings. One male non-obese participant recorded atrial ectopy at rest but none in the post-exercise phase. No atrial or ventricular ectopy was noted at rest or post-exercise in other lean or obese participants before or after bariatric surgery.

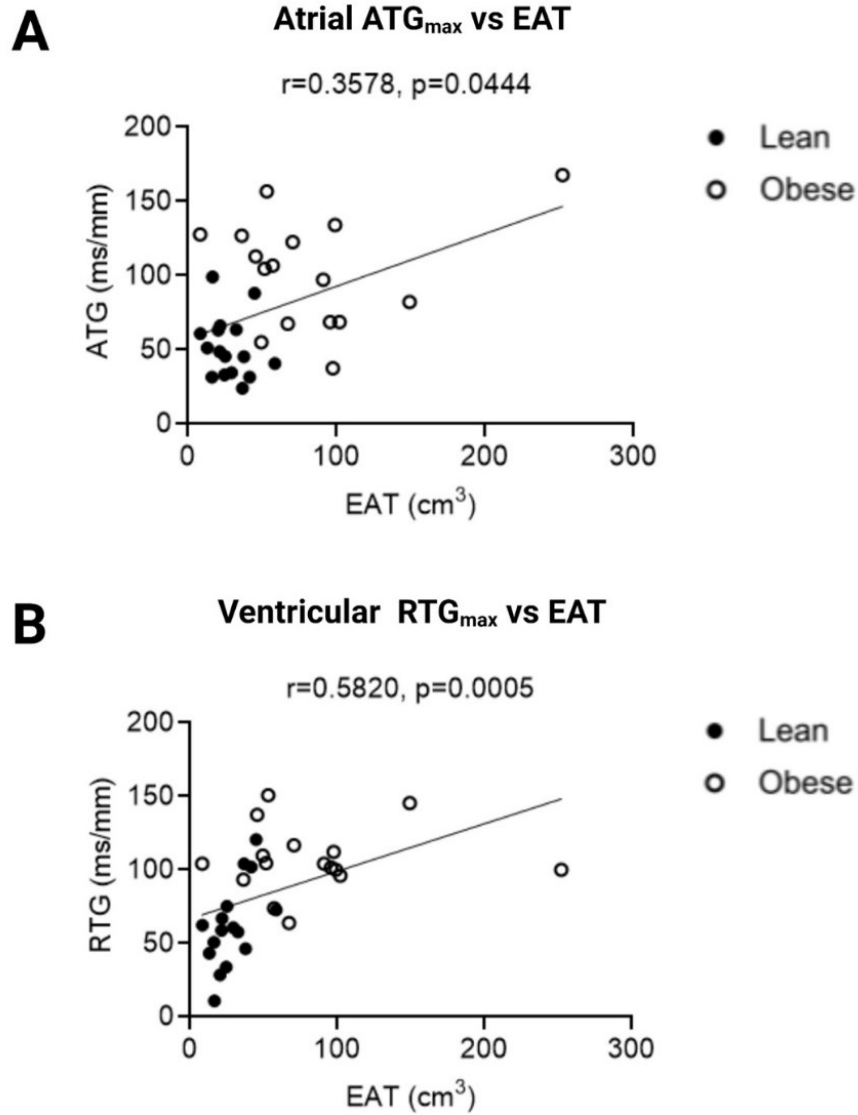


Figure 5.8: Degree of electrophysiological remodelling in obesity correlates with epicardial adipose tissue volume. Maximum atrial activation (ATG) (**A**) and ventricular repolarisation gradients (RTG) (**B**) correlated with epicardial adipose volume. Data shown for resting state.

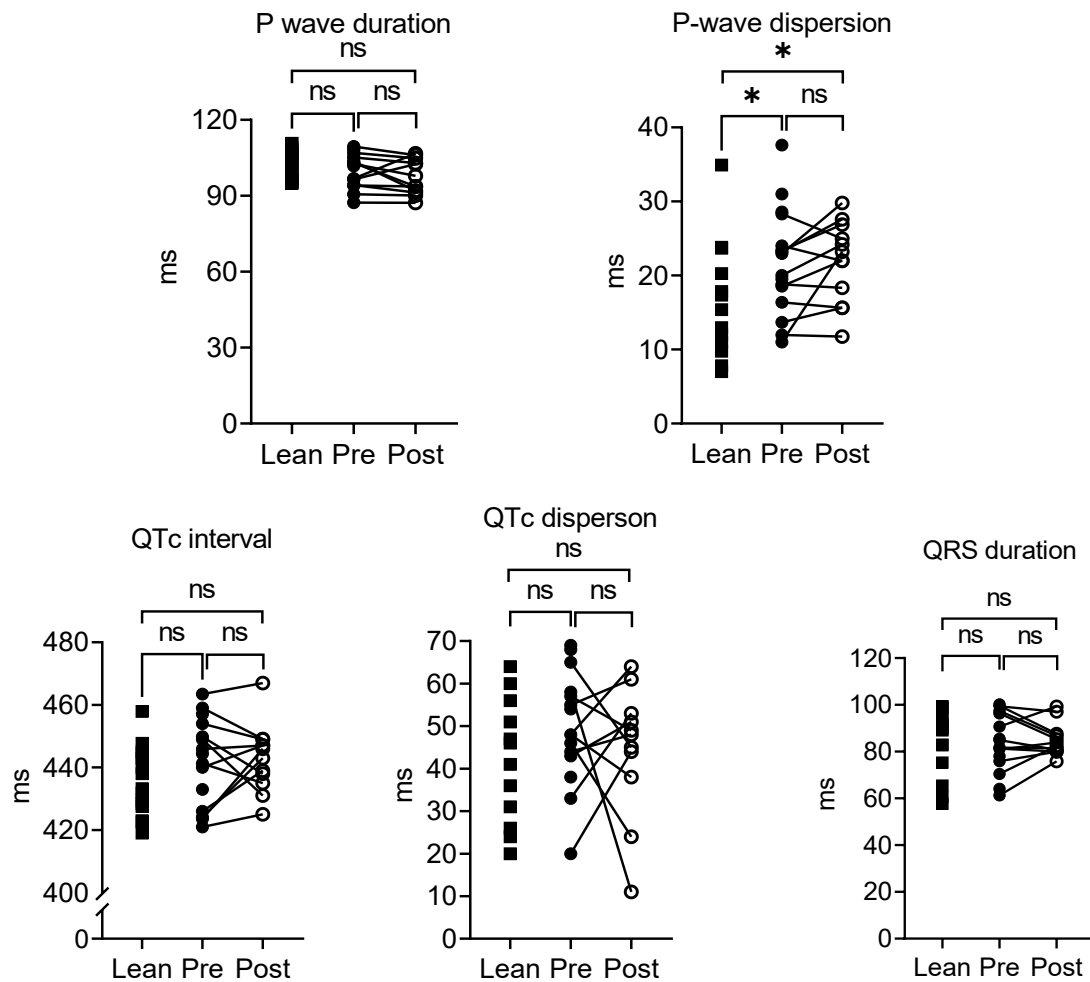


Figure 5.9: Resting 12-lead ECG parameters are comparable in lean and obese individuals. P-wave and QRS durations, QTc interval and its dispersion were not different between groups. P-wave dispersion was greater in PreSurg vs lean but did not change post-bariatric surgery.

5.3.9. Bariatric surgery partially reverses autonomic dysfunction in obesity

Rest:

Mean resting heart rate was greater in PreSurg obese vs lean (86 ± 13 vs 73 ± 11 bpm, $p=0.0091$) and was lower post-surgery (74 ± 12 bpm, $p=0.0007$). There was no difference between lean and PostSurg ($p=0.89$). SDRR was significantly lower in

PreSurg vs lean (25.2 ± 13.5 ms vs 53.8 ± 22.4 ms, $p=0.0002$) and was not significantly different in PostSurg obese (30.0 ± 13.7 ms, $p=0.10$; PreSurg vs lean, $p=0.0035$). SDDSD was also lower in PreSurg obese vs lean (18.7 ± 14.7 ms vs 44.5 ± 20.7 ms, $p=0.0002$), and although it increased post-surgery (25.9 ± 18.0 ms, $p=0.017$), it remained lower than in the lean group ($p=0.0215$). The same differences were observed between groups with RMSSD. Nni50 was greater in lean vs PreSurg obese (13 [IQR 6-18] vs 1 [IQR 0-3], $p=0.0041$), and was not different pre vs post-surgery (PostSurg 2 [IQR 0-6], $p=0.0895$; lean vs PostSurg $p=0.0762$). Correspondingly, ppni50 showed the same pattern of differences between groups (lean 18 [IQR 8-34], PreSurg obese 1 [IQR 0-4], PostSurg obese 3 [IQR 0-7]; lean vs PreSurg obese, $p=0.0015$; PreSurg vs PostSurg $p=0.0914$; lean vs PostSurg obese $p=0.1123$). Nni20 was greater in lean vs PreSurg obese (37 [IQR 27-50] vs 10 [IQR 28], $p=0.0002$), and increased post-surgery (PostSurg 22 [IQR 3-40], $p=0.0077$), but remained lower than in the lean group ($p=0.0326$). Ppni20 was greater in lean vs PreSurg obese (53 [IQR 41-71] vs 19 [IQR 3-35], $p<0.0001$). Compared to PreSurg, ppni20 was greater in PostSurg (36 [IQR 5-62], $p=0.0020$) and there were no differences in ppni20 between lean and PostSurg obese groups ($p=0.0612$). These results are presented in Figure 5.10.

Post-exercise:

Mean heart rate was similar between PreSurg obese vs lean (111 ± 15 bpm vs 111 ± 23 bpm, $p=0.9603$) and was decreased post-surgery (99 ± 18 bpm, $p=0.0002$). There was no difference between lean and PostSurg ($p=0.1601$). SDRR was not significantly different between lean (35.8 ± 20.2 ms), PreSurg (24.8 ± 12.0 ms) and PostSurg (33.2 ± 19.5 ms); lean vs PreSurg, $p=0.0727$; PreSurg vs PostSurg

p=0.3768; lean vs PostSurg, p=0.7382. No statistical differences in SDSD, RMSSD, nni50, pnni50, nni20 and pnni20 were observed between groups.

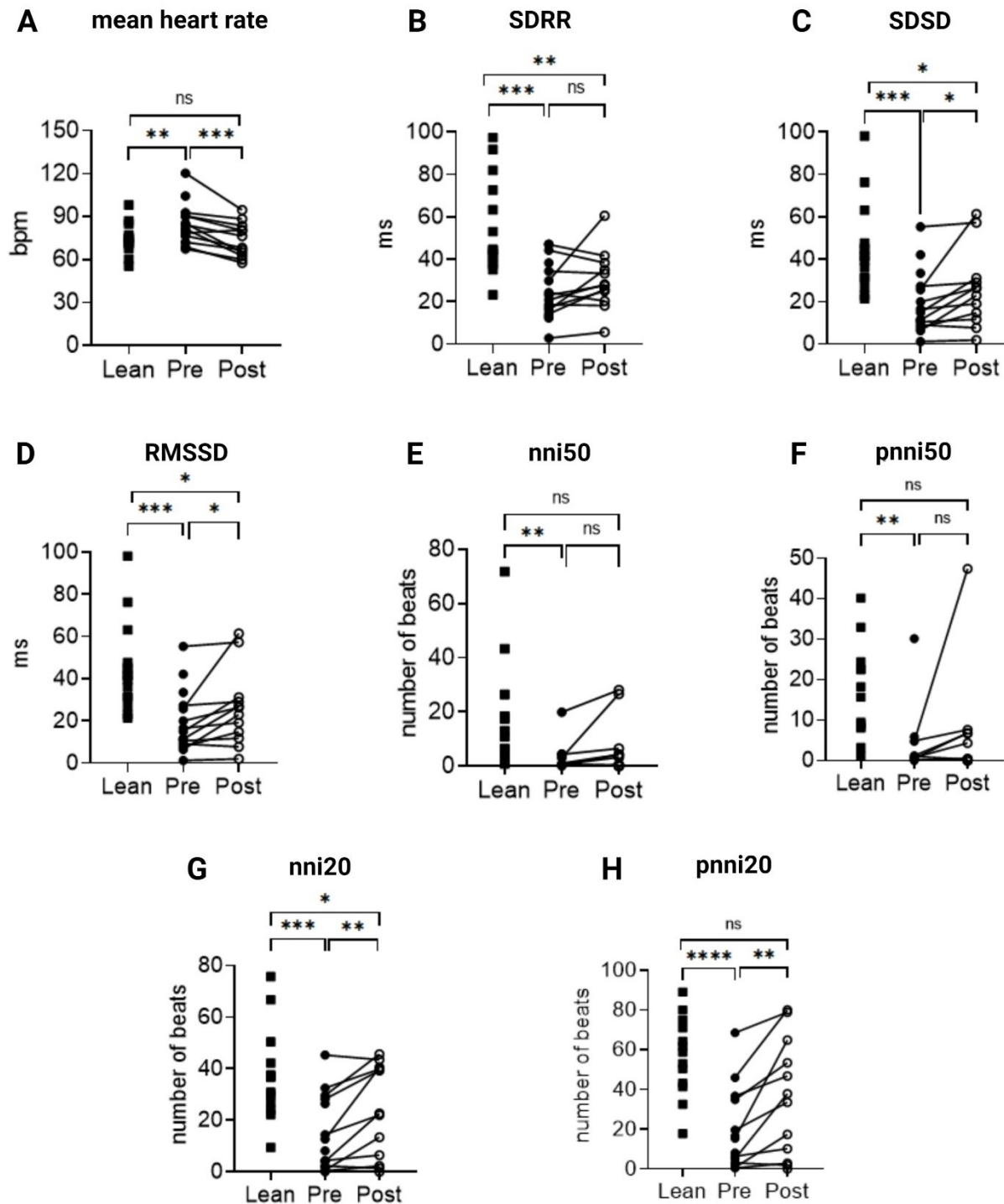


Figure 5.10: Bariatric surgery induces partial improvement in overall and beat-to-beat heart rate variability function in obese patients. (A) Mean resting heart rate was greater in pre-surgical obese patients compared to lean counterparts, and decreased after bariatric surgery. **(B)** Overall heart rate

variability, quantified by SDRR (standard deviation of RR intervals) was lower in pre-surgical obese patients vs lean and did not improve significantly post-bariatric surgery. Beat-to-beat variability quantified by the standard deviation of successive differences (SDSD) **(C)** and root mean square of successive differences of RR intervals (RMSSD) **(D)** were lower in pre-surgical obese patients and improved post-surgery but remained less than the lean group. A similar pattern of differences between groups and post-surgery were observed with the number successive RR intervals with >20ms between them (nni20) **(G)**, as well as nni20 divided by the total number of RR intervals (pnni20) **(H)**. The number successive RR intervals with >50ms between them (nni50), and pnni50, were also lower in the pre-surgical obese group, but did not improve significantly after weight loss **(E-F)**.

5.4. Discussion

This study aimed to characterise the proarrhythmic substrate in arrhythmia-free obese patients and determine whether any subclinical electrophysiological remodelling could be reversed with bariatric surgery. ECGi was used to non-invasively study epicardial activation and repolarisation patterns in the atria and ventricles of obese patients before and after bariatric surgery and compared the results with age- and sex-matched lean individuals. The results can be summarised as follows: (1) total atrial activation is prolonged in obese patients, which persists after bariatric surgery; (2) dispersion and spatial gradients of atrial activation also did not change after surgery; (3) spatial ventricular repolarisation gradients completely reversed after bariatric surgery in obese patients, such that there were no differences between lean and post-operative patients; (4) total ventricular repolarisation is prolonged in obesity and was not statistically different post-surgery;

(5) the electrophysiological adaptations occurred in the context of weight loss and concurrent reductions in LV mass, HbA1c and epicardial fat volume; (6) spatial gradients of atrial activation and ventricular repolarisation correlated with epicardial fat volume in lean and pre-operative obese individuals; (7) exercise did not unmask any electrophysiological differences between obese and lean individuals that were not observed at rest; (8) weight loss is associated with a partial improvement in autonomic function quantified by the indices of HRV. These results suggest that bariatric surgery partially reverses subclinical electrophysiological remodelling in morbidly obese patients and provide mechanistic insights into obesity-related arrhythmic risks that may help to guide primary preventative lifestyle and clinical interventions.

5.4.1. Bariatric surgery causes reverse left ventricular remodelling

Obesity is associated with haemodynamic and physiological adaptations, including LV hypertrophy, raised LVEDP and LVEF (546). Given that LV hypertrophy promotes abnormal ventricular repolarisation in obesity (113, 509, 547) and atrial conduction is slower in regions co-localised with EAT (99), MRI images were used to quantify LV mass and volumes as well as EAT volume before and after bariatric surgery to contextualise electrophysiological adaptations with weight loss. Consistent with previous studies (548, 549, 550, 551), LV mass (indexed to height^{2.7}) was greater in obese versus lean individuals and decreased post-surgery, though only one obese participant had LV mass exceeding the threshold for defining LV hypertrophy (552). LV mass was not indexed to body surface area as this results in inaccuracies owing to the disproportionately large body habitus. There were no significant changes in LVEDV or LVEF after weight reduction in the participants of the present study and

this may be explained by the absence of any significant heart disease at baseline in the current cohort.

Although the benefits of bariatric surgery are particularly marked in patients with insulin resistance (553), only 5 patients in the current cohort had a recorded diagnosis of diabetes of which 2 had a pre-surgical HbA1c >48mmol/mol.

Furthermore, four of 16 obese patients had a pre-surgical HbA1c that was within normal range (<42mmol/mol), 5 were borderline (42-47mmol/mol) and one patient had stopped diabetic medications. Similarly, only one obese participant had ceased using continuous positive airway therapy for OSA and taking anti-hypertensive medication between pre- and post-surgical ECGi. In this context, any electrophysiological remodelling in the obese cohort is unlikely explained by resolution of diabetes, hypertension or OSA.

5.4.2. Atrial activation is prolonged in obesity and persists post-bariatric surgery

Total atrial activation quantifies the time taken for the atria to depolarise and is calculated as the difference between the latest and earliest activating region. Given that all participants in this study were in sinus rhythm, the patterns of activation were similar between lean and obese individuals; activation started in the right atria in a region that closely approximated to the sinus node and ended in the left atria. The total activation times described herein are consistent with the first ECGi study to map atrial activation under physiological conditions (309, 402). At rest, atrial activation is prolonged, dispersion of global activation is increased and local gradients are steeper in obese patients without a history of arrhythmias. Importantly, these

differences persisted after bariatric surgery. Given that the post-operative patients remained clinically obese by their BMI, this result is consistent with studies showing longer P wave durations in obesity (539). The absence of reverse remodelling suggest by these results is also consistent with studies demonstrating persistence of symptomatic AF post-bariatric surgery (534) and that bariatric surgery lowers risk of major cardiovascular events but not incident AF (240, 279, 554).

On the other hand, others have shown that weight loss is associated with greater arrhythmia-free survival (255) and lower AF recurrence post-ablation (555). For instance, in a single centre cohort study, post-ablation AF recurrence was lower in obese patients undergoing bariatric surgery (20%) compared to age- and sex-matched obese counterparts not undergoing surgery (55%), which was similar to that observed in the non-obese group (24.5%) over a mean follow-up of 29 months (277). Similarly, in another retrospective analysis of 239 obese patients, 20% of those that had bariatric surgery prior to ablation had recurrent AF compared to over 60% in those not undergoing surgery, and repeat ablation was also more common in the non-surgical vs surgical group (12% vs 41%) (555). In the same cohort, rates of AF recurrence was lowest (4%) in patients who intensively managed their weight, sleep apnoea, hypertensive and hyperglycaemia, compared to those who achieved better control of none or one (97%), two (85%) or three of (36%) of these risk factors (556). Although these studies suggest weight management alleviates arrhythmic burden, bariatric surgery is not a curative arrhythmic intervention and post-bariatric surgical patients with a history of AF nevertheless remain vulnerable to recurrent arrhythmias. Results of the present study suggest that adverse atrial remodelling persists after bariatric surgery even in obese patients without a history of cardiac

arrhythmias, and provide a mechanistic explanation for why surgical weight loss may not mitigate incident AF risk (220, 240, 278).

In the absence of studies utilising ECGi pre- and post-bariatric surgery, it is acknowledged that the sample size and power calculation for the present study was based on changes in QTc interval from 12-lead ECGs before and after weight reduction (113) and not changes in P-wave duration (539). In the context of these previous studies, and given that the P-wave duration is significantly shorter than the QTc interval, it is possible that the present study may have been insufficiently powered to detect changes in total atrial activation times pre- vs post-bariatric surgery. Thus, it is possible that differences in atrial parameters may have been observed with a greater number of obese participants.

5.4.3. Ventricular repolarisation gradients reverse post-bariatric surgery

In the present study, obese patients had steeper local ventricular RTG, which were fully reversed with bariatric surgery. These results are in-keeping with the pre-specified hypothesis that adverse ventricular electrophysiological remodelling can be reversed with weight loss in patients without prior arrhythmias.

A region of early repolarisation adjacent to a region of relatively late repolarisation creates a steep RTG. This constitutes increased heterogeneity of repolarisation that may confer higher risk of ventricular arrhythmias by two mechanisms. First, an ectopic beat arising in a region of early repolarisation is conducted towards regions of late repolarisation but fails to activate it while it is still refractory. In this instance, the activation wavefront bypasses the refractory tissue and re-enters the region from

which it came thereby creating a re-entrant circuit. Second, repolarisation heterogeneity creates a potential gradient so that the electrotonic current flows from late-repolarising cells to those that repolarised earlier. This has the effect of depolarising the early-repolarising cells, and if the activation threshold is reached, a premature beat is initiated that may act as a trigger for an arrhythmia in a substrate that also has the capability of sustaining re-entry. The initiation and maintenance of arrhythmias in this context have been demonstrated in several studies. For instance, experimental studies in isolated cardiomyocytes and optical mapping in transgenic rabbit hearts with long QT syndrome have shown premature ventricular complexes (PVCs) spontaneously originating from regions of steep RTG can lead to re-entry even in the absence of other proarrhythmic electrophysiological or structural adaptations (557). Furthermore, the vulnerability for re-entry is also increased by enhanced L-type calcium currents that could trigger early afterdepolarisations (EADs) equally in regions of steep and shallow repolarisation gradients (557). Most recently, in Langendorff-perfused rabbit hearts, Alexander et al also showed that PVCs originated from steep voltage gradients at borders of regions with prolonged action potential independent of EADs as a consequence of electrotonic triggering of L-type voltage currents, which was abolished with nifedipine (558). Similarly, another in silico study showed that PVC can be classified according to their site of origin, specifically regions of steep RTG, or initiation by phase 2 EADs (559). Importantly, PVCs originating from regions of steep RTG could cause re-entry even in the absence of EADs and weak L-type calcium currents (559). Correspondingly, steep RTG regions have also been showed suggested to initiate ectopy in other in silico models (560).

Consistent with these experimental findings, clinical ECGi studies have also shown that steep RTG explain vulnerability to ventricular arrhythmias in high-risk cohorts, such as Brugada and long QT syndromes (33, 36, 37). Although the RTGs in the current study were smaller compared to survivors of idiopathic VF observed in a recent ECGi study (38), their presence and reversibility with bariatric surgery supports obesity as a modifiable risk factor for ventricular arrhythmias. It also suggests that obese hearts may not only enable re-entry but also harbour a greater tendency for spontaneous PVCs that may facilitate re-entry compared to leaner hearts. These results are also consistent with studies showing bariatric surgery partly reverses abnormal ventricular repolarisation (105) which may translate into a lower of risk of ventricular arrhythmias and sudden cardiac death (45, 115). Establishing whether rates of incident ventricular arrhythmias were different between lean and obese individuals was beyond the scope of this study, as this would have required prolonged follow-up. Although no ventricular ectopic beats were noted in the 10-minute body surface potential recordings in lean, pre-surgical or post-surgical obese groups, a longer duration of telemetry may have been helpful to assess differences in ectopic burden. However, in the absence of any clinical indication or ethical approval, this was not practically possible in this study.

In this study, local RT was defined as the steepest upstroke of the T-wave in reconstructed epicardial unipolar electrograms (418). This correlates with the local effective refractory period (38, 419, 520, 521) as well as the local RT defined as the maximum downslope on transmembrane action potentials (522). Thus, this definition follows the theoretical basis of coinciding with the ion currents in phase 3 that

determine local RT, irrespective of T-wave morphology, in intact human hearts (516, 524, 525).

5.4.4. Epicardial adipose tissue volume regress post-bariatric surgery

In the present study, there was a significant reduction in EAT volume following bariatric surgery. Furthermore, atrial ATG and ventricular RTG correlated with pre-surgical EAT volume. EAT is the visceral fat depot of the heart and may cover up to 80% of its surface. It receives its blood supply from the coronary circulation and is intimately related to myocardium owing to the absence of fascial separation between the two structures (1). Unfortunately, although epicardial fat and pericardial fat are anatomically different (1, 561), these terms are often used interchangeably and arguably incorrectly (188). In the absence of a consensus agreement on what constitutes epicardial, pericardial and paracardial fat it can be difficult to contextualise and compare results between studies. Given that adipose tissue across different sites around the myocardium demonstrate unique transcriptomic signatures (421), determining what constitutes epicardial and pericardial adipose tissue is a matter that transcends mere semantics. Nevertheless, even with this important caveat, adipose tissue around the myocardium has been shown to be a risk factor for AF (171, 172), as well as AF severity and ablation-related outcomes in the absence of associations with standard anthropometric indices of adiposity (562). Similarly, the incremental risks of incident AF with increasing EAT are greater than those associated with increasing abdominal or whole-body adiposity (101). These observations suggest adiposity-related arrhythmic risk is at least partly explained by EAT volume.

Amongst the first to provide mechanistic insights into the association between EAT and arrhythmic risk in humans was Mahajan et al (99), who performed cardiac MRI and electroanatomic mapping of the atria in 115 AF patients prior to catheter ablation and divided this cohort based on their obesity status. In comparison to non-obese group, obese individuals had slower conduction velocity, increased electrograms fractionation, and increased area of low voltage signals that resulted in greater voltage heterogeneity. The areas of low voltage co-localised to EAT, which is particularly marked in the posterior and inferior left atria (99). Notably, the correlations between EAT and left atrial fractionation ($r^2=0.55$) and conduction velocity ($r^2=0.31$) in their study were comparable to the strength of association between EAT and ventricular RTG and atrial ATG, respectively, reported herein. Furthermore, the observation that conduction velocity is slower in obesity is also consistent with prolongation of atrial activation observed in the of the present study. The results from Mahajan et al's study should also be interpreted in the context of obesity being defined as $BMI > 27 \text{ kg/m}^2$. Although the ethnicity of their study population is unclear, this lower threshold of defining obesity is usually reserved for individuals of Asian descent (68). Nevertheless, it suggests that electrophysiological remodelling from EAT is present even at modest degrees of excess weight, in a category that is conventionally considered overweight rather than obese for Caucasian patients.

5.4.5. Mechanisms underpinning partial reversal of electrophysiological remodelling post-bariatric surgery

Adverse electrical remodelling reported in obese patients with pre-existing AF is, by definition, to be expected. Although observational studies and meta-analyses have

reported obesity is associated with incident arrhythmic risks, particularly AF (100, 101, 220), and the benefits of weight loss is compelling (253, 254, 255, 257), the literature is less clear on the characteristics of subclinical electrophysiological remodelling in arrhythmia-free obese patients and the degree to which these can be reversed to mitigate incident risks. This is partly borne from the fact that the proarrhythmic substrate cannot be mapped invasively in individuals without a clinical indication for an electrophysiological study. The present study addresses this limitation in the current understanding of obesity-related arrhythmogenesis. It shows that morbidly obese patients with no history of arrhythmias harbour a proarrhythmic atrial substrate with characteristics that are similar to those observed in patients with a more modest BMI undergoing ablation for pre-existing AF (99).

The fact that atrial activation persisted after bariatric surgery suggests that atrial remodelling may not reverse with bariatric surgery, even with regression of EAT and reverse LV remodelling. Experimental studies have provided insights into the mechanisms underpinning this observation. For instance, in an organ co-culture experimental set-up, Venteclef et al showed that human EAT induced atrial fibrosis that could be inhibited, but not reversed, by an antibody to Activin A (226). These findings were consistent with those observed clinically (563) (99). It is therefore possible that the persistent prolongation of atrial activation in the obese cohort is the consequence of adiposity-induced fibrotic remodelling that is irreversible regardless of weight loss. In the interests of safety and absence of clinical indications, non-contrast MRI was used for the participants of the current study, and therefore it was not possible to quantify fibrosis in the present study. On the other hand, post-operative ECGi was performed on average 270 days after the pre-operative ECGi,

and it is possible that some degree of reverse remodelling may be detected with a longer time lag between ECGi dates. The fact that weight, BMI and EAT volume remained greater in post-operative obese patients compared to their non-obese counterparts may offer another explanation for persistent prolongation of atrial activation after surgery, and further weight reduction and regression of EAT may induce reverse electrical remodelling.

In contrast to atrial ATG, ventricular RTG was greater in pre-operative obese patients and reversed post-operatively, such that there was no significant difference between lean and post-operative obese participants. Like atrial ATG, however, ventricular RTG correlated with EAT volume. The persistent prolongation of atrial activation and steeper atrial ATG post-surgery compared to the reversibility of ventricular RTG with weight loss suggests different mechanisms underlie ventricular remodelling. Unlike atrial activation, ventricular repolarisation is predominantly determined by potassium currents which are prolonged upon exposure to inflammatory cytokines, such as TNF- α , IL-1 β and IL-6 (1, 192). Under physiological conditions, EAT acts as a mechanical buffer against shear stress, as well providing insulation and a source of fatty acids (564). In obesity, however, epicardial adipocytes hypertrophy and become hypoxic owing to relative ischaemia and secrete proinflammatory mediators that modulate ion channels (1). It is therefore possible that regression of EAT with weight loss, which has also been described by others (232, 233, 565, 566), renders it less hypoxic resulting in a less inflammatory secretome that may facilitate functional recovery of repolarisation currents and thereby reversal of ventricular RTG.

Although it does not imply a causal relationship, the positive correlations between preoperative atrial ATG and ventricular RTG with EAT is consistent with the growing body of evidence implicating it with adverse cardiovascular physiology (533). Furthermore, the absence of a significant correlation between the change in EAT and changes in atrial ATG or ventricular RTG pre- vs post-bariatric surgery does not imply that EAT is not a modulator of the electrical properties of adjacent myocardial tissue. Any reverse remodelling is likely to be the result of a combination of complex interplay between structural and functional adaptations to weight loss. It is also possible that the characteristics of the EAT secretome, and consequently its paracrine effects on ion channels determining activation and repolarisation, may have been altered after bariatric surgery. Although EAT volume can be quantified with cross-sectional imaging in obese patients and co-localises with electrophysiological abnormalities (99), its secretome in obese patients undergoing bariatric surgery has not been studied as it is inaccessible for biopsy at the time of surgery. Nevertheless, other studies have shown that the EAT secretome has a greater concentration of cytokines IL-1 β , IL-6, and TNF- α (184) and adipokines adiponectin, resistin, and leptin (186) compared to subcutaneous adipose tissue (SAT). Similar characteristics were found in EAT compared to SAT from overweight and obese individuals undergoing cardiothoracic surgery, independent of concurrent coronary artery disease or hypertension (193). Given the results of this clinical study and the growing interest in the detrimental effects of EAT, the differential secretomes of EAT and SAT and their effects on cardiac electrophysiology is explored further in Chapter 6.

5.4.6. Resting heart rate variability improves after bariatric surgery in obese patients

In addition to the partial reversibility of adverse electrophysiological remodelling, obese patients in this study demonstrated poorer autonomic function quantified by HRV compared to lean individuals, which also partially improved after bariatric surgery. Obesity has previously been associated with reduction in HRV due to a reduction in parasympathetic tone and an increase in sympathetic drive (567, 568, 569). The autonomic imbalance in favour of increased sympathetic tone explains some of the cardiovascular adaptations observed in obese patients, such as higher resting heart rate, blood pressure, left ventricular hypertrophy and atrial dilatation (546). These adaptations at rest are partly due to chronic inflammation (570, 571, 572) which is also characteristic of obesity. In this regard, it is likely that the autonomic dysfunction in obesity is also partly attributable to the inflammatory secretome of EAT discussed above (188, 193, 573). Correspondingly, the improvement in parasympathetic tone and reduction in sympathetic tone with weight loss (574, 575) and cardiovascular adaptations to weight loss in obesity (576) may also be linked to regression of EAT reported herein.

Autonomic tone is an important modulator of atrial (577, 578) and ventricular (579) arrhythmias and constitutes one aspect of Coumel's triangle of arrhythmogenesis (40). For instance, ablation of sympathetic ganglia and cardiac branches of the vagus nerve, but not either alone, has been shown to completely mitigate paroxysmal AF (42, 580, 581) suggesting that both contribute to atrial arrhythmogenesis. More specifically, an imbalance between sympathetic activity that

prolongs action potential duration and parasympathetic activity that reduces refractory period in the atria leads to disruption of intracellular calcium homeostasis (5), thereby increasing risk of early after-depolarisations that can precipitate AF (582, 583). In open-chest dogs stimulation or inhibition of sympathetic innervation from the right and left stellate ganglia has been shown to have differential effects on ventricular repolarisation. Neutralisation of the right stellate ganglion resulted in prolongation of body surface QT interval and increased T-wave amplitude, whereas left stellate ganglionectomy caused T-wave inversion but did not prolong QT interval (584). Interestingly, the two stellate ganglia innervated different regions of the myocardium, which may result in heterogenous sympathetic innervation under pathological states and render the substrate proarrhythmic (584, 585, 586). The results described herein showing a partial improvement in heart rate variability post-bariatric surgery suggests that in addition to being less prone to ectopy, obese hearts may be less likely to sustain arrhythmias weight loss.

The observation that bariatric surgery reduces resting heart rate and improves HRV (quantified by RMSSD, nni20 and pnni20) in obese patients is in agreement with results from other studies showing lower SDRR, RMSSD, nni50 and pnni50 in obese vs non-obese individuals (587, 588). The results shown herein also concur with an improvement in SDRR post-laparoscopic sleeve gastrectomy in a comparable cohort of young (median age 36.5 years) morbidly obese (median baseline BMI 43.9kg/m²) mainly female patients recording a similar degree of weight loss (median 14.7kg/m²) (589). Despite these results suggesting an improvement in sympathetic function, Bienias et al showed that the frequency of atrial and ventricular arrhythmias were no

different before and 12-18 months after bariatric surgery (589). This was, however, based on a single 24-hour Holter recording and therefore it is possible that an extended period of remote cardiac monitoring may reveal differences in arrhythmic burden, such as ventricular ectopy, in post-operative obese patients. In the absence of ethical approval and clinical indications for implantation of remote monitoring devices, participants in the current study did not have cardiac monitoring post-operatively. Therefore, it was not possible to compare incident atrial or ventricular arrhythmias between obese and lean individuals or arrhythmic burden before and after surgery. Indeed, it is possible that the results of HRV computed from a maximum of 5 minutes of resting body surface potentials in the present study may be different with prolonged Holter monitoring that would also enable frequency-domain HRV features to be quantified.

Exercise induces changes in sympathetic and parasympathetic tone can unmask repolarisation abnormalities in high-risk cohorts that are otherwise absent at rest and not detectable on 12-lead ECG (301). In this study, there were no differences in heart rate variability between groups in the post-exercise period. Importantly, the electrophysiological differences observed between groups at rest using ECGi were also present in the post-exercise period. As such, there was no differential modulatory effect of exercise on atrial and ventricular electrophysiology in obese and lean groups or between pre- and post-surgery in the obese group. This contrasts with patients with inherited heart disease, such as Brugada and long QT syndromes, who are more vulnerable to changes in autonomic tone (301). For instance, lethal arrhythmias in Brugada syndrome frequently occur at night when vagal tone predominates (590) and in response to exertion or arousal in long QT syndrome

(591). Results presented herein suggest that, even in the context of structurally normal hearts, individuals with inherited channelopathies are more vulnerable to changes in autonomic tone than obese patients.

5.4.7. ECGi enables higher resolution characterisation of the proarrhythmic substrate than 12-lead ECG

Although ventricular RTG decreased post-bariatric surgery, dispersion of repolarisation was not different between groups. Furthermore, total ventricular RT was longer in pre-surgical obese patients compared to lean and did not change significantly after bariatric surgery. On the other hand, resting 12-lead ECG data showed no differences in P-wave and QRS durations, QTc interval and its dispersion between groups. P-wave dispersion was, however, greater in pre-surgical obese participants which persisted post-surgery compared to the lean group. By contrast, others have reported shortening of QT and QTc intervals and their respective dispersions on 12-lead ECG post-bariatric surgery to suggest faster ventricular repolarisation in larger cohorts (106, 110, 592). A number of reasons may explain these contrasting findings. First, the 12-lead ECG is a low spatial resolution modality recorded a distance away from the electrical source rendering it insensitive to subtle local abnormalities and inaccurate for global assessments of site of interest. Although QT and QTc intervals provided information on overall duration of ventricular depolarisation and repolarisation, they are not indicative of local repolarisation heterogeneity which is intrinsically linked to ventricular arrhythmogenesis (38, 313). Furthermore, even though QTc dispersion may be increased in patients with heart disease, the values overlap significantly with those from healthy individuals and between those with and without adverse outcomes (104). By contrast, ECGi utilises

a higher density of body surface electrodes and patient specific heart-torso geometry to reconstruct unipolar epicardial electrograms to study activation and repolarisation at the cardiac source. Unlike invasive catheter mapping using a roving probe approach over several cardiac cycles, ECGi can also map the entire epicardial surface in one beat(307) and has been extensively validated (290, 309). It therefore represents a higher resolution modality by which cardiac electrophysiology can be studied in greater detail compared to the 12-lead ECG. ECGi has also been shown to recapitulate in vivo data with at least a moderate degree of accuracy (417) and identify repolarisation abnormalities that are not observed on 12-lead ECG (38). Although this may be insufficient to guide ablative therapy in some cases (295), it has can faithfully recapitulate T-wave topologies and repolarisation maps in early and late repolarising regions, detect abbreviation and prolongation of global and local RT and identify local RTG (313). Indeed, ECGi method adopted in this study has been validated previously (400) and implemented to demonstrate differences in ventricular conduction and repolarisation in other at-risk cohorts (300) and to map ventricular arrhythmias (307).

Another reason for disparities may be explained by the methods by which repolarisation dispersion is quantified. In the present study, dispersion of repolarisation was quantified by its standard deviation. By contrast, dispersion of atrial activation and ventricular repolarisation is quantified by the maximum differences in QTc intervals on a 12-lead ECG (112, 539). Although they both attempt to quantify dispersion of repolarisation, they are not equivalent metrics and each are subject to error in measurement, outliers or artefact. Furthermore, the degree of QT interval shortening post-bariatric surgery is dependent on the equation

used to correct for concurrent heart rate, with the greatest change observed with the use of the Fridericia equation compared to Bazzett, Framingham or Hodges equations (593, 594). For these reasons, it is difficult to accurately extrapolate results between ECG and ECGi studies.

Like 12-lead ECG, the ECGi method used in this study assumed that electrical properties of the torso volume conductor between the cardiac source and body surface did not change pre- and post-surgery and was not different between lean and obese individuals. Furthermore, it was assumed to be homogenous in character for the computation of epicardial electrograms using unique transfer matrices of each individual's heart-torso geometry. On the other hand, obese patients experience significant changes in body morphology and composition following bariatric surgery, notably a decrease in adiposity, which may have implications for the accuracy of epicardial electrogram reconstruction. None of the participants in the study had undergone detail body composition profiling either before or after bariatric surgery for this data to be considered in the transfer matrix that relates body surface potentials to those on the epicardial surface. Nevertheless, previous ECGi validation studies have investigated the effect of introducing or omitting inhomogeneities of muscle, fat and lung volumes, into the torso volume conductor on epicardial and body surface potentials (381, 595). These demonstrated that homogeneous and inhomogenous models produce comparable activation and repolarisation patterns. In fact, introducing these inhomogeneities to reflect body composition that have not been accurately quantified a priori risks introducing computational errors that would be amplified by the inverse method and thereby adversely affect accuracy of epicardial electrogram reconstruction. For these reasons, changes in body composition are

unlikely to have significantly influenced the accuracy of electrogram reconstruction in our study. Similarly, differences in body habitus and sex have also been shown to not exert any discernible differences in ECGi results (382).

5.4.8. Limitations

This was a small study with a predominantly female cohort that may limit extrapolation to the wider obese population. An extracellular potential-based model was used as the cardiac source to reconstruct electrograms over the epicardium. Consequently, the results are limited to comparisons of epicardial and do not consider endocardial or transmural activation and repolarisation patterns. This may have been achieved by using the equivalent double layer model or 3D cardiac electrical imaging (290). Epicardial electrograms were computed onto fixed epicardial meshes captured at end-diastole. However, body surface potentials were recorded under physiological conditions when the heart and thoracic cage are in motion, which inevitably introduces error in solving the inverse problem of electrocardiography. Given that there were no clinical indications for the participants to have undergone invasive electrophysiological studies, ECGi data presented herein cannot be compared against ground-truth from contact electroanatomic mapping. Finally, owing to the absence of long-term follow-up and remote cardiac monitoring, it is not possible to ascertain any translational effects of electrophysiological remodelling on incident arrhythmic risk in this cohort.

5.5. Conclusions

Obesity is associated with adverse electrophysiological remodelling, including

delayed atrial activation and steeper ventricular RTG, even in individuals without a history of clinical arrhythmias. The electrophysiological differences between obese and lean individuals may be partly explained by EAT volume. Bariatric surgery reduces EAT volumes and is associated with normalisation of the ventricular repolarisation gradients but does not improve atrial activation. These observations were noted at rest and post-exercise and suggest differential mechanisms underlying atrial and ventricular electrophysiological changes in obesity. Fibrotic remodelling may explain the persistent prolongation of atrial activation, whereas recovery of repolarising ion channels with regression of adiposity may explain reversibility of ventricular RTG. Although HRV partly improved after bariatric surgery in the obese cohort, physiological changes in autonomic tone after exercise did not unmask any abnormalities that were absent at rest. These results provide mechanistic insights into the proarrhythmic substrate associated with obesity. Despite the absence of cardiac monitoring data to detect arrhythmias post-operatively, these results suggest that early surgical weight reduction intervention can partly reverse subclinical electrophysiological remodelling in arrhythmia-free obese patients which may mitigate incident arrhythmic risks.

Chapter 6: Characterisation of epicardial and subcutaneous adipose tissue secretomes and their effects on ventricular electrophysiology

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6. 1. Background

6.1.1. Epicardial adipose tissue: function and pathophysiology

Epicardial adipose tissue (EAT) is the visceral fat depot of the heart. Iacobellis et al were amongst the first to quantify EAT from echocardiography as a method of quantifying visceral adipose tissue and link it to a higher risk of cardiometabolic ill-health (596, 597). The growing interest in the effects of EAT on cardiovascular health is partly explained by its unique properties. It lies in direct contact and shares circulation with the underlying myocardium, thereby enabling molecular cross-talk between the two anatomical structures (533). Furthermore, EAT demonstrates characteristics of white as well as brown fat (533, 598). Under normal physiological conditions, EAT protects the myocardium from hypothermia, provides it with energy in the form of free fatty acids and buffers it against shear stress and inflammation. Indeed, under these circumstances, its protective role is reflected by its transcriptome which is characterised by high concentrations of adiponectin and adrenomedullin (599, 600). As EAT expands, it undergoes a phenotypic shift and begins to secrete inflammatory and profibrotic cytokines, also called adipokines, that exert paracrine effects on neighbouring structures (564, 601). This includes ion channel and gap junction modulation and fibrotic remodelling (152). EAT is a highly heterogenous fat depot that demonstrates distinct transcriptomic and proteomic signatures based on its anatomical location around the heart (421) and also in comparison to subcutaneous adipose tissue (SAT) (188). It is therefore unsurprising

that EAT has been implicated in a number of cardiovascular disease processes, including ECG changes and cardiac arrhythmias (422).

6.1.2. Epicardial adipose tissue as a mediator of cardiac arrhythmias

Much of the evidence linking EAT with cardiac arrhythmias relates to atrial fibrillation (AF). Indeed, EAT is an independent predictor of incident AF as well as recurrent AF post-ablation (101). The mechanisms underpinning these phenomena are incompletely understood but postulated to include fatty infiltration, fibrosis and inflammation (602). The electromechanical dissociation of cardiomyocytes as a consequence of physical fatty infiltration attenuates conduction and thereby generates a substrate that can facilitate re-entry (603). EAT has also been shown to induce atrial fibrosis (226) that can result in conduction block in myocardial tissue. Indeed, regions of conduction slowing and electrogram fractionation have been shown to be most marked in regions of EAT in ovine models of obesity (99), in which the extent of atrial remodelling is also related to degree of adiposity (220). Furthermore, atrial myocytes incubated with EAT demonstrate greater sodium and L-type calcium currents, but smaller rectifier potassium currents than control atrial myocytes, as well as a greater propensity to triggered ectopy in response to adrenergic stimulation (604). These latter observations are at least partly underpinned by the pro-inflammatory transcriptome of EAT (188, 421). These results suggest that the accumulation of EAT facilitates development of a proarrhythmic atrial substrate by causing myocardial conduction slowing, via fatty infiltration and fibrosis, and prolongation of repolarisation by paracrine modulation of ionic currents. The evidence for whether the same mechanisms underpin ventricular arrhythmogenesis remains unknown.

6.1.3. Epicardial adipose tissue and ventricular electrophysiology

Of the studies linking EAT to adverse electrophysiological remodelling mentioned hitherto in this chapter and in Chapter 1, three studies warrant particular mention to provide a rationale for the methods and context for the results presented herein. First, Venteclef et al showed that EAT-induced fibrosis in a rat atria organo-culture model was reversed by an anti-Activin A antibody (226). In this study, paired samples of EAT and SAT were harvested from patients undergoing routine coronary artery bypass surgery; SAT biopsies were taken from the parasternal region and EAT from the left-interventricular groove prior to the patient being initiated on cardiopulmonary bypass. Venteclef et al incubated rat atria with EAT- and SAT-conditioned media that had been diluted using a ratio of 1:4 with control culture media for 7 days with daily replacement. After 7 days, the atria were washed with PBS and fixed with 4% formaldehyde and then embedded in cryo-conservation medium and frozen with liquid nitrogen. Although these results from an organo-culture model provide compelling evidence for the paracrine effects of EAT as a profibrotic and consequently a proarrhythmic mediator, it is unclear how the adipose-conditioned media was generated. Specifically, the duration that EAT and SAT were culture to generate their respective conditioned-media was not explicitly stated. More importantly, however, although the study showed the profibrotic nature of human EAT on rodent atrial tissue, their experimental protocol did not extend to test the electrophysiological effects of EAT vs SAT.

Second, Ten Sande et al showed that neonatal rat ventricular myocyte (NRVM) monolayers cultured in multi-electrode arrays (MEA) had slower conduction when co-cultured with adipose-derived stromal cells from pigs, rats and humans (189).

Interestingly, no conduction slowing was recorded in NRVM incubated with the conditioned media from rat- or human-derived stromal cells, suggesting implicating an electronic rather than a paracrine effect for the conduction slowing observed in rat and human stromal cell-NRVM co-culture (189). On the other hand, porcine stromal cell-NRVM co-culture and porcine-stromal cell conditioned media exerted similar conduction slowing effects in NRVM suggesting a paracrine effect. Although this study investigated the effects of adipose-derived stromal cells, rather than whole adipose tissue, it implies a species-specific effect on NRVM that has important implications for extrapolation of experimental results to different organisms. The human stromal cells were also derived from abdominal SAT from healthy volunteers with BMI<30kgm² and therefore there were no comparative results on the effects of EAT-derived stromal cells. Similar to Venteclef et al (226), the duration over which stromal cells were cultured to generate conditioned media is not explicitly stated.

Third, Nalliah et al showed that human induced pluripotent stem cell-derived cardiomyocytes incubated with EAT fragments from sheep for 24 hours in MEA had prolonged field potential durations and slower beating rate compared to cells cultured in non-adipose tissue media (188). Furthermore, they also showed that HL-1 atrial cardiomyocytes cultured with rat pericardial fat-conditioned media had prolonged activation time and slower conduction velocity compared to cells treated with rat inguinal fat-conditioned media (188). In contrast to Venteclef et al and Ten Sande et al, Nalliah et al state that their rat pericardial fat-conditioned media was generated by incubating the pericardial fat for 4 hours in culture media. Although the results of this study also compellingly demonstrated the proarrhythmic effects of pericardial fat, the effect of sheep SAT was not reported. Furthermore, pericardial fat

exists in very small volumes in rodents, rendering such harvest not only arduous but it is also difficult to establish whether the biopsy comprises epicardial (enclosed within the visceral pericardium), paracardial (outside the parietal pericardium) or pericardial (combination of epicardial and paracardial) fat. This differentiation is important given that EAT is most likely to exert paracrine or electronic effects owing to its intimate contact with myocardium unlike paracardial fat which is anatomically distant. Indeed, the interchangeable use of these terms in the literature makes comparisons between studies challenging, particularly attempting to ascertain the specific effect of EAT on myocardial function.

6.1.4. Aims and hypothesis

In the context of these studies, and to address the limitations of current understanding regarding the effects of human EAT vs SAT on ventricular electrophysiology and consequently arrhythmogenesis, the aims of this study were to:

1. To culture human EAT and SAT harvested from patients undergoing cardiothoracic surgery to generate adipose-conditioned media
2. To characterise and compare human EAT and SAT adipokine secretomes
3. To study the effect of human EAT- and SAT-conditioned media on the conduction velocities of NRVM cultured in MEA

The hypotheses were:

1. EAT exhibits a more proinflammatory profile than SAT
2. EAT causes greater conduction slowing in vitro than SAT

6.2. Methods

Chapter 2 describes fully the experimental protocols used for NRVM isolation and culture, adipose tissue harvest and culture, generation of adipose-conditioned-media, human adipokine assay, ELISA and recording of conduction velocities in response to pacing in NRVM. Pertinent details are included below and statistical methods for data analyses are described alongside methods in the following sections. Technical assistance with adipose tissue culture, human adipokine array assays and ELISA were provided by Danya Agha-Jaffar.

6.2.1. Epicardial and subcutaneous adipose tissue harvest and culture

EAT and SAT were harvested from 22 patients that provided informed written consent for these biopsies to be taken during their cardiothoracic surgical procedures at Hammersmith Hospital. The biopsies were provided courtesy of Professor Prakash Punjabi and Dr Alessandro Viviano. SAT samples were taken from the parasternal region and EAT from around the left ventricle. Upon harvest, EAT and SAT samples were transported over ice in transport media Medium 199 (M199, Gibco, UK) containing Earle's salts, L-glutamine, 2.2 g/l sodium bicarbonate, 25 mM HEPES (all from ThermoFisher Scientific, UK) and supplemented with 1% penicillin-streptomycin (Sigma-Aldrich, UK) to a sterile cell culture facility for immediate processing. Adipose tissue culture was performed with Mr Curtis Se Liebers (undergraduate BSc student) and Mr Joseph Coghlin (Master of Research student) at Imperial College London under my supervision. All fat culture practices were performed in a microbial safety cabinet (BioMat2 Class II) according to protocol outlined by Carswell et al (425). A detailed explanation of these methods is provided in Chapter 2.

6.2.2. Testing adipose tissue viability

The CyQUANT™ LDH Cytotoxicity Assay Kit (Thermo Fisher Scientific, UK) was used to test EAT and SAT viability following culture using manufacturer's instructions. The methods for this assay are described in Chapter 2. Curtis Se Liebers (undergraduate BSc student) and Joseph Coghlin (Master of Research student) at Imperial College London performed this assay under my supervision during their respective research projects, and assistance was provided by Ms Danya Agha-Jaffar. The premise of the assay is that apoptotic cells release LDH which catalyses the conversion of pyruvate and reduction of NAD⁺ to NADH. The diaphorase enzyme contained in the assay solutions oxidises NADH back to NAD⁺ while simultaneously converting a tetrazolium salt to formazan. The concentration of formazan produced is therefore proportional to the concentration of LDH secreted in the EAT or SAT sample and is a surrogate metric for the degree of cell apoptosis of the samples. The optical densities of tested EAT and SAT samples and their respective conditioned-media were recorded at 680nm and 490nm wavelengths and normalised to control-media. Normalised values for EAT- and SAT-conditioned media were divided by the normalised values for EAT and SAT samples (indicative of maximal cell death) and expressed as a percent to estimate the extent of cell apoptosis in the adipose-conditioned media.

6.2.3. Proteome Profiler Human Adipokine Array assay

The Proteome Profiler Human Adipokine Array (R&D Systems, USA), referred to herein as Human Adipokine Array assay, has been previously used to characterise depot-specific adipokine profiles (426). It enables rapid characterisation of adipose tissue secretome by providing a semi-quantitative measure of the adipokines within the

samples being tested. It is advantageous over other techniques, such as ELISA, because many adipokines can be tested simultaneously and therefore represents a more cost- and time-effective option. On the other hand, unlike ELISA, it does not provide exact concentrations of adipokines. The assay consists of a nitrocellulose membrane that contains spots pre-loaded with antibodies to 58 adipokines in duplicate. These membranes were incubated with EAT- or SAT-conditioned media mixed with biotinylated detection antibodies overnight, after which Streptavidin-HRP and chemiluminescent detection reagents were applied to the membrane. Images of the membrane were analysed using FIJI open-source software by manually drawing circles around the signal over each blot representing an adipokine on the membrane. Each adipokine was quantified in duplicate and the pixel density was normalised to control spots on the membrane that contained no antibodies. The intensity of the signal over each of the blots was measured in arbitrary units (pixel density) and the mean of two blots for each adipokine was calculated. Thus, the average signal of a pair of duplicate spots for each adipokine was calculated and used to compare the profiles of EAT- and SAT-conditioned media. Further technical and methodological details about the Human Adipokine Array assay are provided in Chapter 2.

6.2.4. ELISA

Given that the Human Adipokine Array only enabled semi-quantitative characterisation of adipokines, Quantikine Human ELISA immunoassays for TNF- α , IL-1 β , IL-6 and Activin A (R&D Systems, Abbingdon, UK) were also performed to determine their respective concentrations in EAT and SAT. The choice of these adipokines was informed by their recognised role in a range of inflammatory processes and by previous studies investigating their effects on electrophysiology

(195, 197, 226, 486, 605, 606). ELISA were performed using the manufacturer's instructions and are detailed in Chapter 2. Briefly, standard concentrations were made using serial dilutions following the manufacturer's instructions to generate a standard concentration curve. The concentrations of adipokines in EAT and SAT were determined by interpolation along the standard concentration curve. The concentrations of the standards and samples were measured in duplicate and averaged. The averages for the standards were normalised to the zero standard, and the results for EAT and SAT samples were normalised to control, i.e. fat-free media

6.2.5. NRVM isolation and culture

NRVM were isolated from Sprague-Dawley rats aged 0 to 3 days old as described previously (435). NRVM isolations were performed by Dr Ivan Diakonov and Ms Danya Agha-Jaffar using a neonatal heart dissociation kit (Miltenyi Biotec GmbH, Germany) according to manufacturer's instructions. This process involves the mechanical dissociation and enzymatic degradation to isolate cells and is described in Chapter 2.

NRVM were cultured directly onto multielectrode array (MEA) to enable calculation of conduction velocities in response to external pacing under experimental conditions.

The MEA plate (MultiChannel Systems MCS GmbH, Germany) contains an 8x8 matrix of gold electrodes in its centre. Each electrode is 100 μ m in diameter with an interelectrode distance of 700 μ m. Electrodes in the corners of the grid are absent, resulting in a total of 60 electrodes in a single MEA. A plastic ring encircles the electrode matrix to create a 3ml capacity well. MEA can therefore be used to culture cells and retain sufficient culture media to enable them to mature. Following

isolation, 200,000 NRVM were cultured onto a drop of type IV human placental collagen (Sigma-Aldrich, UK) pipetted under visual inspection over the electrode matrix in the MEA under sterile conditions. NRVM were incubated at 37°C 1%CO₂ in M199 media containing Earle's salts, L-glutamine, 2.2 g/l sodium bicarbonate and 25 mM HEPES (ThermoFisher Scientific, UK) supplemented with 10% neonate calf serum for the first 24 hours. This was replenished with fresh media with 5% neonatal calf serum 24 hours later. Details on preparation of the collagen, MEA plates and NRVM culture are included in Chapter 2.

6.2.6. Testing conduction velocity in NRVMs using multielectrode arrays

The effects of EAT- and SAT-conditioned media and control media were tested on NRVM cultured in MEA. Assistance for this was provided by Mr Taesoon Hwang during this research project under my direct supervision for his intercalated BSc student at Imperial College London. The methods and the apparatus used for this purpose have been described fully in Chapter 2 and by others (435). Briefly, MEA containing NRVM were placed on a heater set at 37°C and connected to an amplifier. Pacing stimulations were conducted using a STG stimulus generator programmed by MC Stimulus II software (version 3.4.4, Multi-Channel Systems). NRVMs were paced with a biphasic stimulus lasting 2ms at 1000mV, usually from the topmost row of six electrodes of the MEA, to achieve 1:1 electrical capture. All signals were recorded at a sampling frequency of 25kHz. 10-second recordings of unipolar electrograms across the electrode array were recorded and analysed offline using MC Rack (v4.6.2, Multi-Channel Systems) and a customised MATLAB code (R2020b, The MathWorks Inc, Massachusetts). For each local electrogram recorded over the electrode matrix, activation time was determined as the interval from the

stimulation artefact to the maximum negative derivative of the QRS complex and used to construct activation maps. Conduction velocity was determined along lines perpendicular to isochronal lines by dividing the distance by the difference in local activation times (607).

Baseline conduction velocities of NRVM were recorded 48 hours after their culture into MEA plates. MEA in which NRVM recorded a conduction velocity $<25\text{cm/s}$ were not used experimentally. After establishing baseline conduction velocities, NRVMs were randomly allocated one of three groups, EAT, SAT or control, and their culture media correspondingly replaced with 1ml EAT- or SAT-conditioned media or control media, ensuring that the media was exchanged quickly under sterile conditions to avoid NRVM drying. NRVM were then incubated at 37°C $1\%\text{CO}_2$ for 2 hours before conduction velocities in response to adipose-conditioned media or control were recorded again. Conduction velocities were also recorded at 4 hours and 24 hours after baseline, with NRVMs being returned to a 37°C $1\%\text{CO}_2$ incubator in between recordings. Thus, conduction velocities of NRVM were calculated at baseline as well as 2-, 4- and 24-hours following exposure to EAT-, SAT- or control media. The effects of EAT and SAT from each patient, as well as control media, on NRVM conduction velocities were tested in triplicates, i.e. three MEA in each group. Given that the baseline conduction velocities of NRVM were different within and between groups, the average differences in conduction velocities from baseline at 2-, 4- and 24-hours were calculated first and then compared between EAT, SAT and control groups. Thus, NRVM conduction velocities are presented as change from baseline at 2-, 4- and 24-hours in cm/s .

6.2.7. Statistical analysis

LDH concentrations from tissue viability testing were tested for normality using Shapiro-Wilk test, and samples from the same patient were compared using paired two-tailed Student's T-test or non-parametric equivalent.

Given that the number of patients from whom paired samples of EAT and SAT were biopsied (n=22) was less than the total number of adipokines (n=58) that could be analysed from a single biopsy using the human adipokine array assay, principal component analysis (PCA) was used to determine differences between EAT and SAT secretomes. This statistical technique avoids performing multiple pairwise comparisons to identify differences between EAT and SAT, that may result in a significant difference being detected by chance simply by virtue of the number of the individual tests performed, which theoretically in this study would have been 58. Thus, PCA is a more appropriate analytic method for datasets in which there are many more variables per sample than the number of samples collected (608). The premise of PCA is that it reduces the dimensionality of the data, which in the current study is the number of adipokines, while simultaneously retaining variation between groups, i.e. EAT vs SAT. It does so by identifying the principal components, which are the directions in which there is maximum variation in the variables of interest (adipokines). Thus, by using principal components to compare samples, rather than the variables themselves, PCA analysis offers a reductionist method by which tens of variables can be represented by relatively few numbers (608). Pro-inflammatory, anti-inflammatory and neutral adipokines were analysed separately using PCA by Dr

Arunashis Sau (clinical research fellow, National Heart and Lung Institute) to identify any differences in EAT and SAT secretomes. The outputs of PCA were normalised to values between 0 and 1, and results between EAT and SAT compared using Wilcoxon matched-pairs signed rank test.

Adipokine concentrations in EAT and SAT determined by ELISA were tested for normality using Shapiro-Wilk test. All data from these experiments were not normally distributed and therefore results for ELISA were compared using Wilcoxon matched-paired signed rank test and are presented as median $\pm$ interquartile range (IQR). NRVM conduction velocities are presented as change from baseline and differences between EAT-, SAT- or control-media were determined using two-way ANOVA. $P < 0.05$ was considered statistically significant. All tests were performed using Graph Pad Prism (v9.1.1, GraphPad Software, La Jolla, California). Ns, non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

6.3. Results

6.3.1. Baseline characteristics

Paired EAT and SAT samples were harvested from 22 consenting patients undergoing cardiothoracic surgery at Hammersmith Hospital between 30 January 2020 and 24 January 2022. Their baseline characteristics are provided in Table 6.1. There was a male preponderance (19/22) and the mean age was 64.0 ± 10.0 years. 15/22 patients identified with a non-Caucasian ethnicity. 20 patients were in sinus rhythm and 2 patients had recorded AF at the time of surgery. Patients were scheduled to receive coronary artery bypass grafting (CABG) for ischaemic heart

disease (n=12), or valve surgery for stenotic or regurgitant valve disease (n=9); one patient underwent concurrent CABG and valve repair. In-keeping with the clinical indications for surgery in this cohort, hypertension, diabetes and dyslipidaemia were the three most prevalent co-recorded morbidities in the patients' past medical histories. The mean BMI was $25.1 \pm 3.5\text{kg/m}^2$. Blood pressure and biochemistry results shown were those recorded on the day of, but prior to, surgery. Owing to multiple co-morbidities, almost all patients had an extensive drug histories, which included aspirin (n=11), HMG-CoA reductase inhibitors (statins, n= 20), anti-hypertensive agents (including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and calcium channel blockers, n=18) and anti-glycaemic agents (oral medications and subcutaneous insulin injections, n=9). One patient with AF was prescribed warfarin and the other apixaban.

Characteristics	n (%)
Male	19 (86.3)
Age (years)	64.0 ± 10.0
Ethnicity	
Caucasian	7 (31.8)
Non-Caucasian	15 (68.2)
Surgery	
CABG	12 (54.5)
Valve repair/replacement	9 (40.9)
CABG and valve surgery	1 (4.5)
12-lead ECG	
Sinus rhythm	20 (90.9)

Atrial fibrillation (AF)	2 (9.1)
Resting heart rate (beats per minute)	73 $\pm$ 13
Blood pressure	
Systolic (mmHg)	142.0 $\pm$ 23.7
Diastolic (mmHg)	74.8 $\pm$ 13.4
Biochemistry	
White cell count ($\times 10^6/\text{ml}$)	9.1 $\pm$ 3.4
Estimated glomerular filtration rate (ml/min)	59.9 $\pm$ 25.0
C-reactive protein (mg/L)	28.9 $\pm$ 19.6
Total cholesterol (mmol/L)	3.26 $\pm$ 0.72
High-density lipoprotein (mmol/L)	1.47 $\pm$ 0.53
Low-density lipoprotein (mmol/L)	2.03 $\pm$ 0.74
Co-morbidity	
Hypertension	15 (68.2)
Diabetes	9 (40.9)
Dyslipidaemia	10 (45.5)
Peripheral vascular disease	5 (22.7)
Smoking status	
Current	1 (4.5)
Former	9 (40.9)
Never	12 (54.5)
Body mass index (kg/m^2)	25.1 $\pm$ 3.5

Table 6.1: Baseline characteristics of patients (n=22) from whom epicardial and subcutaneous adipose tissue samples were harvested. Data are

presented as mean $\pm$ standard deviation where appropriate. Data in parentheses represent percentage of the total cohort. CABG, coronary artery bypass grafting.

6.3.2. Adipose viability remains stable during 96 hours of culture

To investigate the effect of culture duration on tissue viability, EAT and SAT samples from the first 11 patients in whom these were harvested were divided between 24, 48, 72 and 96 hours of culture. Tissue viability testing with performed with Mr Curtis Se Liebers (intercalated BSc student at Imperial College London under my supervision). LDH release was not significantly different between EAT and SAT at 24hours ($40.5 \pm 5.7\%$ vs $45.7 \pm 1.3\%$, $p=0.36$), 48hours ($46.0 \pm 3.0\%$ vs $48.0 \pm 0.8\%$, $p=0.96$), 72hours ($46.4 \pm 6.5\%$ vs $38.8 \pm 5.2\%$, $p=0.57$) or 96 hours ($47.4 \pm 19.5\%$ vs 41.9 ± 10.5 , $p=0.87$). Similarly, LDH secretion did not change significantly with EAT ($p=0.14$) or SAT ($p=0.66$) culture between 24- and 96-hours. These results were in-keeping with the culture protocol suggesting that whole adipose tissue can be cultured up to 96 hours in vitro(425). Given these results, conditioned media generated from the culture of EAT and SAT over 96 hours was used to investigate their differential effect on NRVM electrophysiology in MEA experiments.

6.3.3. Epicardial and subcutaneous adipose tissue have comparable secretomes

Table 6.2 provides the list of adipokines stratified by the inflammatory status that could be detected by the human adipokine array assay. Figure 6.1 shows how much of the variance in adipokine profiles between EAT and SAT is captured by each PCA feature. The first principle component captures approximately 40% of the variance in

pro-inflammatory, anti-inflammatory and neutral adipokines. Conventionally, the principle component(s) preceding the greatest difference in their respective explained variance ratios are used to compare groups. In Figure 6.1, it can be seen that the greatest difference in variance occurs between the first and second principle components. Therefore, the first principle component was used to compare adipokine profiles between EAT and SAT. Figure 6.2 shows the results of PCA analyses using the first principal component to determine differences in the pro-inflammatory, anti-inflammatory and neutral adipokine profiles of paired EAT and SAT samples. There were no observed differences in the adipokine profiles between EAT and SAT (pro-inflammatory adipokines $p=0.09$, anti-inflammatory adipokines $p=0.35$, neutral adipokines $p=0.77$).

Pro-inflammatory			Anti-inflammatory	Neutral
Angiopoietin-1	BAFF	ICAM-1	Adiponectin	Cathepsin D
Angiopoietin-2	BMP-4	IGFBP-2	Cathepsin L	FGF-basic
Angiopoietin-like 2	IL-6	IFBP_6	Fetuin B	IGFBP-7
Cathepsin S	IL-8	Leptin	IGFBP-3	Nidogen-1
Chemerin	IL-11	VEGF	IGFBP-4	Serpin A8
Complement factor D	IL-1 β	Resistin	IL-10	Serpin A12
Fibrinogen	CRP	RANTES	TGF-beta 1	PAI-1
Myeloperoxidase	CD26	RAGE	LIF	TIMP-1
Oncostatin M	Endocan	PCSK9	Pentraxin-3	
Visfatin	EN-RAGE	Pref-1		

Lipocalin-2	MIF	PAPP-A		
Table 6.2: Adipokines detected by the Proteome Profiler Human Adipokine Array (R&D Systems, USA) stratified by their inflammatory status. BAFF; BMP-4; ENRAGE; FGF; IBGBP; IL; interleukin; LIF; MIF; PAI, plasminogen tissue inactivator-1; PAPP-A; PCSK-9; Pref-1; RAGE; RANTES; TGF-beta, tissue growth factor beta; TIMP; VEGF, vascular endothelial growth factor.				

6.3.4. Concentration of Activin A is greater in epicardial than subcutaneous adipose tissue

Examples of standard concentration curves used to determine concentrations of adipokines in EAT and SAT samples are shown in Figure 6.3. Results of ELISA for three adipokines (Activin A, TNF- α and IL-6) are shown in Figure 6.4. The concentration of Activin A was greater in EAT vs SAT (n=22): 97.73pg/ml (IQR: 45.40-168.5pg/ml) vs 45.00pg/ml (IQR 33.05-138.7pg/ml), p=0.0014. There were no significant differences in the concentration of TNF- α between EAT and SAT: EAT 11.93pg/ml (IQR 9.87-14.76pg/ml), SAT 8.58pg.ml (IQR (6.00-15.74pg/ml), p=0.39. Similarly, there were no differences in concentrations of IL-6 between EAT and SAT: EAT 288.9pg/ml (IQR 77.82-350.2pg/ml), SAT 159.2 (IQR 73.47-324.2pg/ml), p=0.10. IL-1beta could not be detected in sufficient samples to enable analysis.

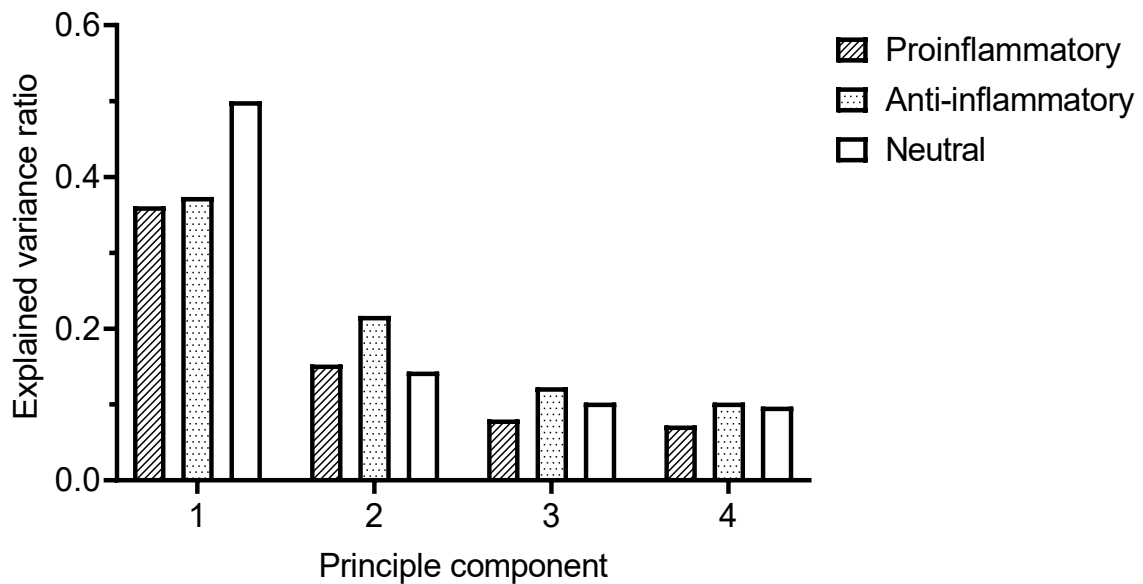


Figure 6.1: Variance between EAT and SAT secretomes. The first principle component (x-axis) explained up to approximately 40% of the variance (y-axis) observed in proinflammatory, anti-inflammatory and neutral adipokines between EAT and SAT and was used to compare EAT vs SAT secretomes (Figure 6.2).

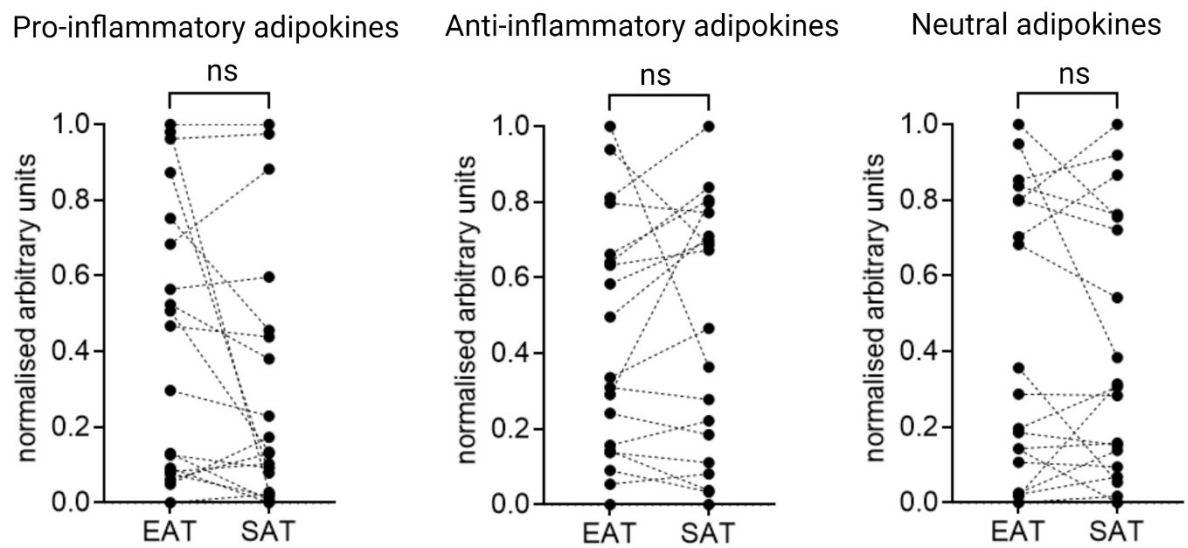


Figure 6.2: Principal component analysis of EAT vs SAT adipokines profile stratified by their inflammatory status. There were no statistically significant differences between EAT and SAT secretomes.

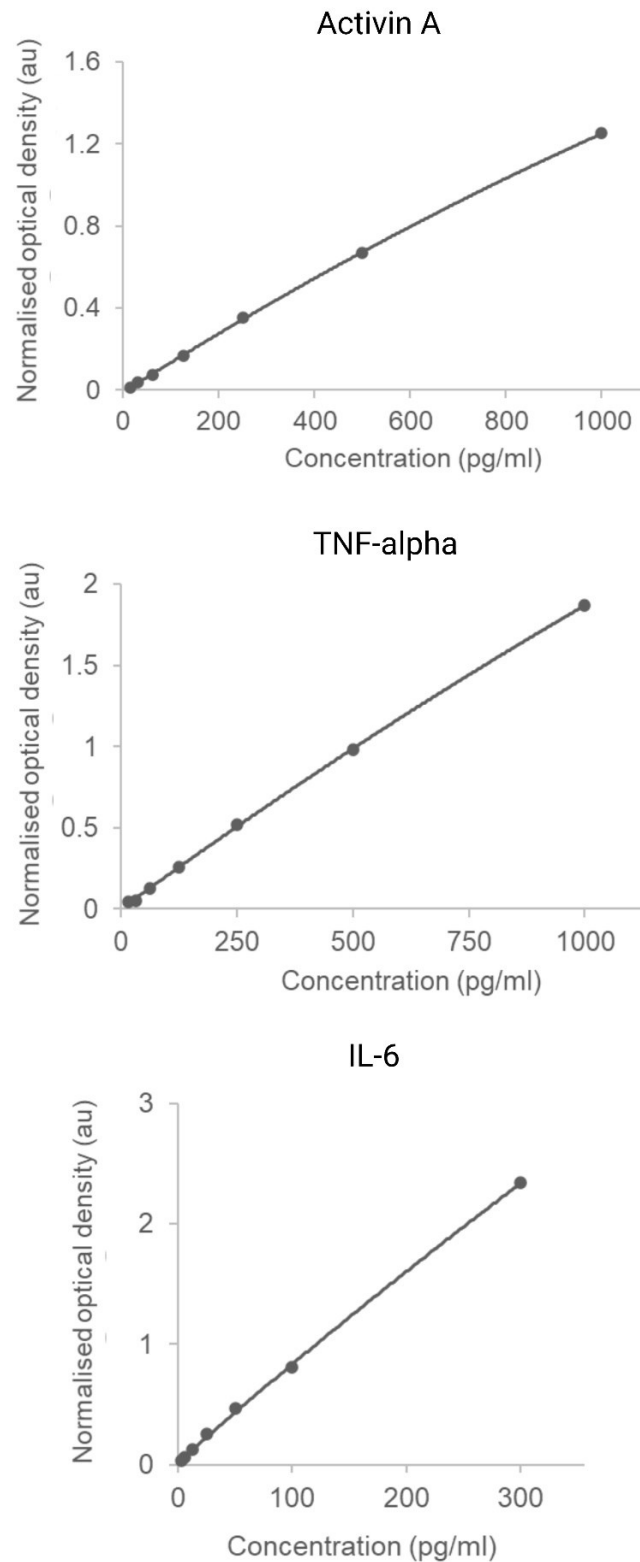


Figure 6.3: Representative examples of standard concentrations curves for ELISA. Concentrations of adipokines for EAT and SAT samples were determined by interpolation along the standard concentration curves.

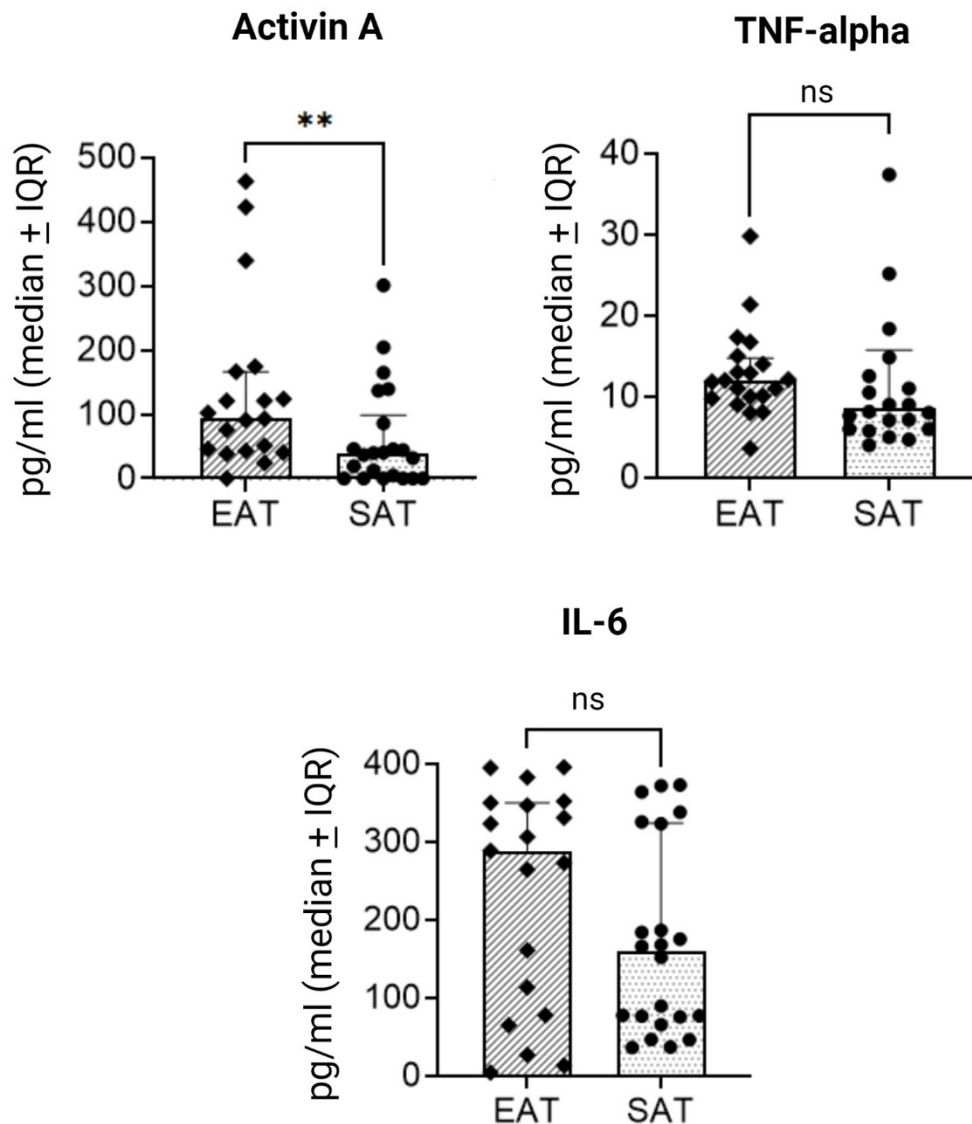


Figure 6.4: Concentration of Activin A is greater in EAT than SAT. There were no significant differences in the concentrations of TNF- α and IL-6. Bars represent median and interquartile (IQR) ranges. EAT, epicardial adipose tissue; SAT, subcutaneous adipose tissue.

6.3.5. Optimisation of electrical recordings in NRVM

The optimisation of recording electrical activity in NRVM was performed with Dr Taesoon Hwang (intercalated BSc student under my supervision). The results

presented in this section have previously been submitted in his BSc research project to Imperial College London in May 2021.

In contrast to NRVM treated with control media, experiments revealed that no spontaneous electrical activity in NRVM could be recorded within 2 hours of incubation with EAT- or SAT-conditioned media. To investigate the timepoint at which electrical activity ceased, NRVM were incubated with EAT- and control-media (n=1 MEA each) and paced at 1Hz every two minutes. It was observed that electrical activity ceased in NRVM incubated with EAT-conditioned media within 30 minutes. To ensure that electrical activity could be recorded over a longer duration to facilitate comparisons between NRVM incubated with EAT-, SAT- and control media, the effects of diluting adipose-conditioned media was tested in a series of dose-response experiments over 4 hours incubation. EAT-conditioned media was diluted with control-media to achieve concentrations of 50%, 25%, 10%, 5%, 2.5% and 1.25%. The change in conduction velocity from baseline in NRVM cultured at each of these dilutions (n=1 MEA each) is shown in Figure 6.5. NRVM incubated with 50% and 25% EAT-conditioned media showed a change in conduction velocity from baseline of -12cm/s and -8.70cm/s, respectively. No electrical activity could be recorded in beyond 30 minutes incubation in these groups. NRVM incubated with 10% EAT-conditioned media recorded a change of -1.5cm/s from baseline at 1 hour before electrical activity ceased. Electrograms were recorded in NRVM incubated with 5%, 2.5% and 1.25% EAT-conditioned media recorded electrograms at 4 hours; change in conduction velocity from baseline in these groups were -12.0cm/s, -6.7cm/s and -6.6cm/s, respectively. These results suggested that dilution of EAT-conditioned media to 5% yielded the greatest change in conduction velocity from

baseline whilst simultaneously ensuring that electrical activity could be recorded up to 4 hours into culture. Informed by these results, 5% EAT- and SAT-conditioned media (and control media) were tested in NRVM (n=1 MEA each) and shown in Figure 6.5. A significant difference in change in conduction velocity from baseline at 4 hours was observed between these three groups (EAT -12.0cm/s, SAT -1.68cm/s, control 0.19cm/s, $p<0.0001$). Thus, the results of these optimisation studies suggested that electrical activity could be recorded in NRVM incubated with 5% EAT- and SAT-conditioned media up to 4 hours after incubation with a notable decrement in conduction velocity by this timepoint. Therefore, 5% EAT- and SAT-conditioned-media were used to investigate their differential respective effects on NRVM electrophysiology in subsequent experiments.

6.3.6. Epicardial fat induces greater conduction slowing than subcutaneous fat in NRVM

Figure 6.6 shows the effects of 5% EAT-, SAT- and control-media (n=5 patients) on NRVM conduction velocities relative to baseline at 2-, 4- and 24-hours incubation. At 2 hours, NRVM incubated with EAT recorded the greatest decrease in conduction velocities from baseline compared to SAT and control (EAT -6.15 ± 2.83 cm/s, SAT -4.51 ± 2.34 cm/s, control -1.21 ± 2.00 cm/s). The difference between EAT and control was statistically significant ($p=0.035$), but not between EAT and SAT ($p=0.60$) or SAT vs control ($p=0.10$). A similar trend was observed at 4 hours: EAT -10.96 ± 3.86 cm/s, SAT -4.01 ± 3.11 , control -0.800 ± 1.05 cm/s, with significant differences between EAT and control ($p=0.007$) and EAT vs SAT ($p=0.035$), but not between SAT and control ($p=0.17$). Finally, at 24 hours, NRVM incubated with EAT demonstrated further decrease in conduction velocities (EAT -15.22 ± 3.34 cm/s, SAT

-7.07±5.76, control -1.93±1.68cm/s; EAT vs control p=0.0006, EAT vs SAT p=0.071, SAT vs control p=0.24). A progressive decline in conduction velocities was noted with time in all groups, but was most marked in the EAT group. Thus, in response to pacing at 1Hz under identical experimental conditions, EAT induced the greatest conduction slowing in NRVM compared to SAT and control media at 2, 4- and 24-hours post-exposure. Representative examples of isochronal activation maps of NRVM incubated with EAT-, SAT- or control-media are shown in Figure 6.7.

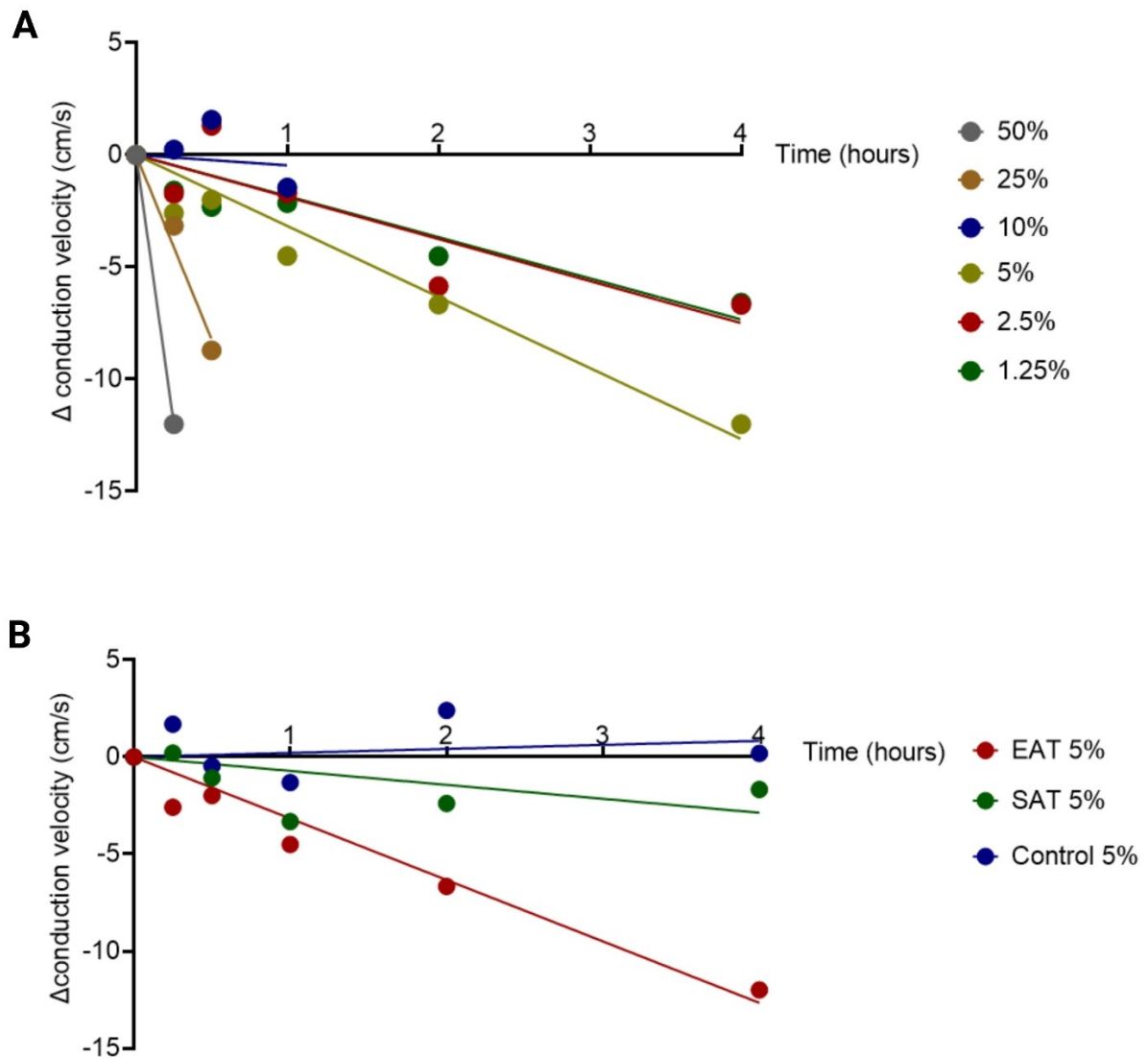


Figure 6.5: Optimisation of electrical recordings in NRVM. (A) EAT-conditioned media was diluted with control media to enable electrical activity to be recorded beyond 24 minutes. NRVM incubated with diluted EAT-conditioned media (1.25%-50%) revealed a decline in conduction velocity from baseline. The most rapid decrease in conduction velocity occurred in NRVM incubated with 50% EAT. More dilute concentrations of EAT-conditioned media (1.25-5%) resulted in a comparable decline in conduction velocity over a longer duration of incubation (4 hours vs <30 minutes). **(B)** NRVM incubated with 5% EAT exhibited slower conduction velocities compared to cells treated with 5% SAT or control media.

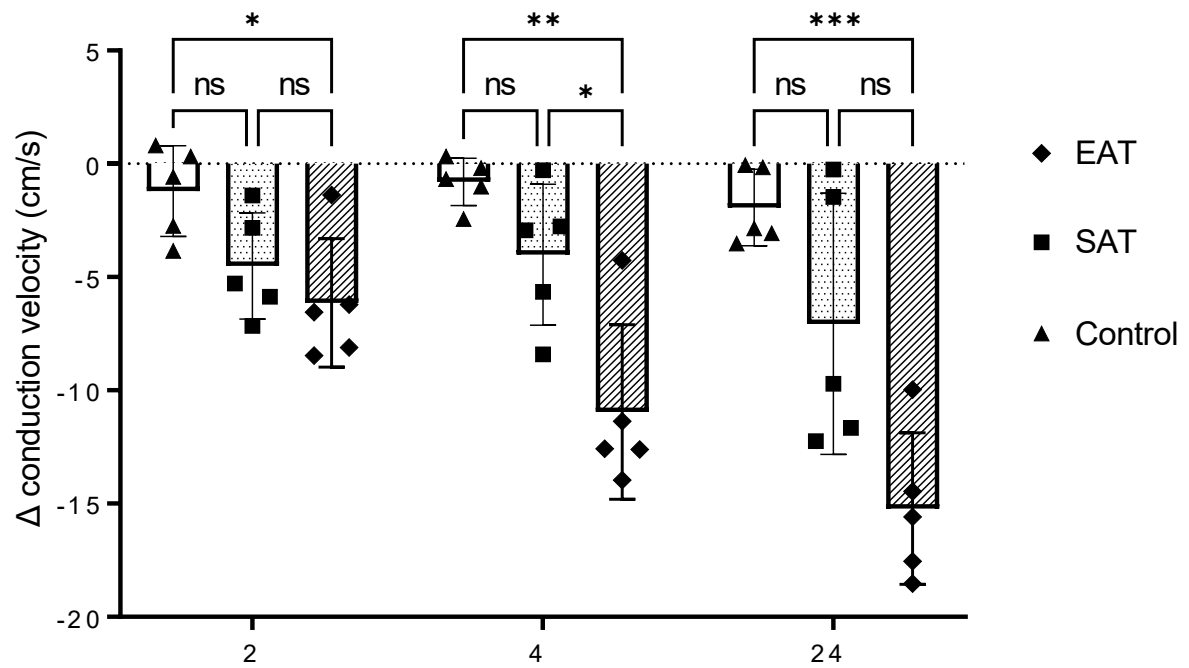


Figure 6.6: EAT induces greater conduction slowing in NRVM compared to SAT

or control. Conduction velocities are presented as mean changes from baseline.

Two-way ANOVA was used to compare groups at each time point.

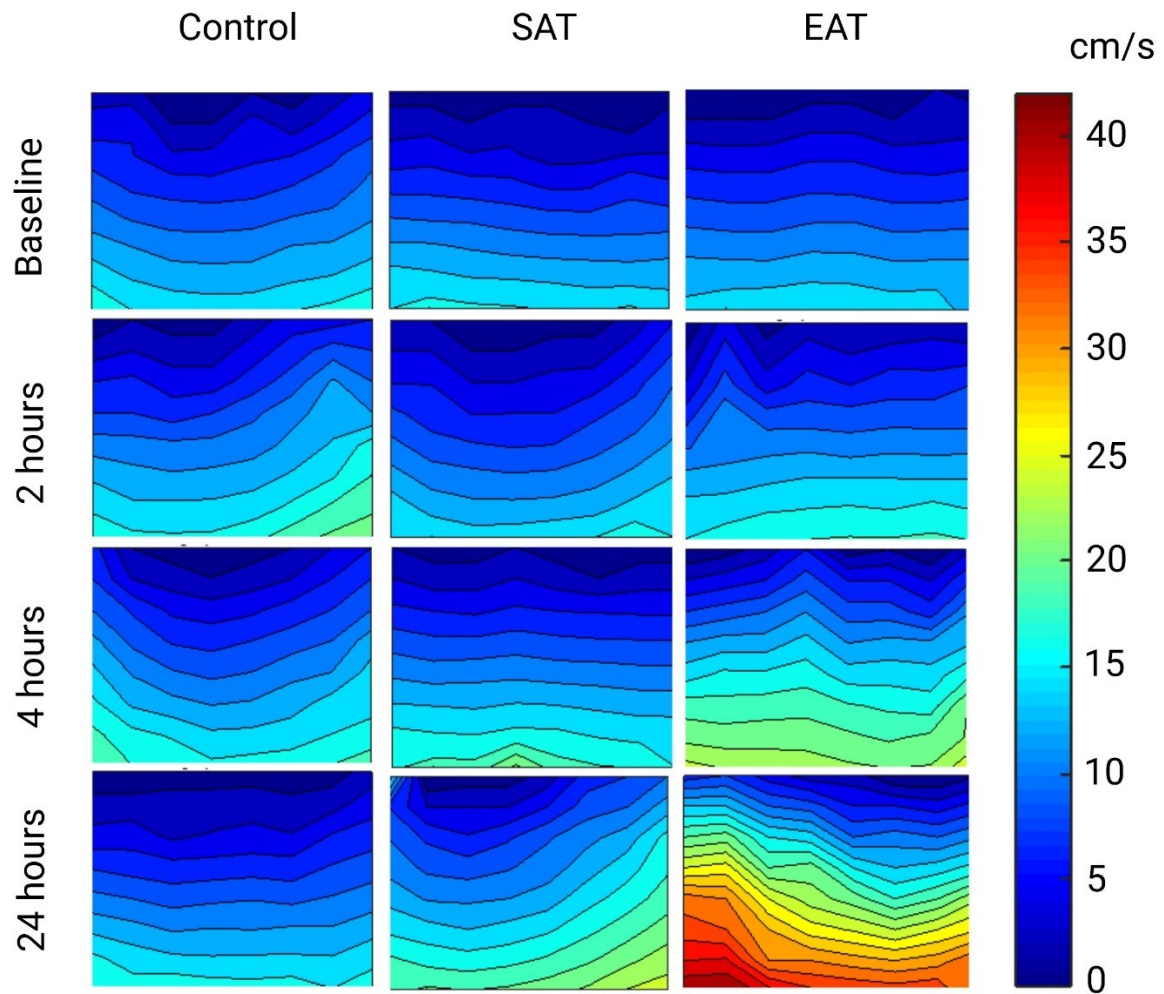


Figure 6.7: Isochronal activation maps from NRVM treated with control, SAT and EAT conditioned media. Cells in these examples were paced from a row of electrodes in the topmost aspect of the isochronal maps. Thus, the activation wavefront is seen to propagate from north to south. Blue denotes relatively faster conduction, whereas yellow and red denotes slower conduction. Conduction velocities are relatively preserved in control group between baseline and 24 hours. By contrast, NRVM incubated with EAT demonstrate the greatest degree of conduction slowing over the experimental time course compared to SAT or control. This figure was created during the work described in this chapter but has been

previously submitted as part of an assessment by Taesoon Hwang under my supervision (intercalated BSc at Imperial College London, May 2021).

6.4. Discussion

The aim of the present study was to investigate the differential effect of human EAT and SAT in NRVM electrophysiology to provide mechanistic insights to reasons why obesity confers higher risk of cardiac arrhythmias. The main findings from the experimental work described in this chapter may be summarised as follows:

1. EAT and SAT viability was not different between 24, 48, 72 and 96 hours of duration of culture in vitro;
2. There were no differences in pro-inflammatory, anti-inflammatory and neutral adipokine profiles of EAT and SAT that were semi-quantitatively measured using the Human Adipokine Array assay;
3. Concentration of Activin A was greater in EAT compared to SAT;
4. NRVM were exquisitely sensitive to exposure to adipose-conditioned media, resulting in no electrical activity being recordable within 30 minutes of incubation with EAT-conditioned media;
5. NRVM cultured with EAT-conditioned media demonstrated greater conduction slowing compared to SAT- or control-media, with progressive decrement in conduction velocities over 24 hours of exposure.

6.4.1. Adipose tissue viability remains stable over 96 hours of culture

In the present study, EAT and SAT biopsies harvested from comparable anatomical landmarks to those described by Venteclef et al and were cultured using the protocol described by Carswell et al (425), The culture reagents used were also informed by

those described by Venticlef et al in their methods. The protocol by Carswell et al suggests that adipose tissue can be maintained in culture for up to 9 days in vitro, that the inflammatory cytokines may be secreted in higher concentrations in the first 2 days of culture and that media should be replenished every 2-3 days to remove metabolites that accumulate in vitro. Given that the aim of the present study was to characterise the differential secretome profiles of EAT and SAT to explain any observed differences on their effects on NRVM conduction, the media was not replenished during the period of EAT or SAT culture in the present study. This study did, however, seek to establish whether adipose tissue viability changed with duration of culture that may have adverse effects on the EAT and SAT secretomes and rendering them less akin to their vivo characteristics. The results presented herein show that the adipose viability was similar between EAT and SAT cultured between 24 and 96 hours. For practical reasons relating to availability of biopsies and adipose culture protocols, conditioned media was generated from EAT and SAT that was cultured for 96 hours, and these were used experimentally to study their differential effects on NRVM electrophysiology.

6.4.2. Activin A is more highly expressed in epicardial than subcutaneous fat

EAT and SAT adipokines were measured using the Human Adipokine Array assay and PCA used to compare their respective profiles, to test the hypothesis that EAT is has a more pro-inflammatory secretome than SAT. Amongst the cohort of this study, that were no significant differences in EAT and SAT secretomes. This is in contrast with some evidence demonstrating EAT is characterised by a relative abundance of inflammatory and dearth of anti-inflammatory proteins compared to SAT (184, 185, 186, 188, 609). On the other hand, as in the present study, others have also reported

a modest difference, if any at all, between EAT and SAT profiles (610, 611). A number of explanations may reconcile these contrasting results. First, it is important to acknowledge that the difference in the pro-inflammatory adipokine secretome between EAT and SAT narrowly missed statistical significance ($p=0.09$). This is likely due to inadequate statistical power resulting from a relatively small sample size. Indeed, a recent study comprising a similar albeit larger cohort of patients ($n=152$) demonstrated EAT was associated with a more pro-inflammatory profile than SAT (193). Therefore, it is possible that a significant difference may have been demonstrated with a greater number of paired adipose tissue biopsies. The practical challenges of harvesting sufficient samples is discussed in section 6.4.5 of this chapter. Aside from sample size, the assay used in the present study was semi-quantitative, and although this does not preclude comparisons of EAT vs SAT, it does not provide accurate measurements of their respective adipokine concentrations. It is possible that statistically significant differences may have been observed had these adipokines been measured using quantitative methods, such as ELISA. This is however a more expensive and time-consuming technique and the Human Adipokine Array assay was chosen to enable a broad assessment of EAT vs SAT secretomes. Second, adipose tissue is characteristically heterogenous, even within the same depot in the same patient, and its secretome is heavily influenced by the prevailing physiological conditions at the time of harvest. Indeed, human EAT has been shown to exhibit a specific transcriptomic signature depending on its anatomical location (421). These factors render comparisons of EAT and SAT secretomes between studies difficult, particularly given that biopsies are unlikely to be harvested from identical anatomical locations. Furthermore, compared to the patient population in the study by Mazurek et al, which was the first to describe EAT

as a source of inflammatory mediators (573), the cohort of the present study had a lower mean BMI (25.1 vs 31.0kg/m²), lower prevalence of coronary artery disease (59% vs 100%) and diabetes (40.9% vs 57.0%). Given that obesity, coronary artery disease and diabetes alter EAT physiology resulting in upregulation of pro-inflammatory and downregulation of anti-inflammatory adipokines (66, 193, 612, 613, 614), heterogeneity in patient cohorts may also explain the inconsistent results of adipokine profiles between studies. Indeed, Vyas et al showed that obesity and diabetes influence the inflammatory profile of EAT more than the type of cardiovascular disease (i.e. ischaemic, valvular or otherwise) (193). Specifically, when comparing EAT and SAT secretomes of 152 patients stratified by their weight (overweight/obese vs lean) and diabetic status, EAT from overweight/obese and diabetic patients had 5-10 fold higher concentrations of IL-1, IL-6, TNF- α and IFN- γ compared to their lean and non-diabetic counterparts, despite exhibiting similar cellular composition (193). Furthermore, these differences between groups were not observed with inflammatory mediators in SAT or serum, and interestingly were not influenced by concurrent hypertension or coronary artery disease either (193). The mean BMI in the present study was 25.1 $\pm$ 3.5kg/m² which represents a narrower range than the cohort in the study by Vyas et al. Although the present study sought to determine differences between adipose depots irrespective of co-morbidity, it is possible that the relatively homogenous cohort of the present study with a high prevalence of diabetes may have masked any differences between EAT and SAT secretomes. Indeed, for obvious practical reasons, studies comparing EAT and SAT profile inherently comprise of patients undergoing cardiothoracic surgery and concurrently receiving pharmacological modulation to address cardiovascular risk factors. On the one hand, although this might limit the heterogeneity of patient

cohorts between studies, pharmacological agents may also introduce variation in the adipokine profiles reported between studies. For example, Parisi et al. showed that patients who had been prescribed 40mg atorvastatin daily for a mean of 24 months prior to cardiothoracic surgery was associated with lower EAT volume and a less inflammatory profile (63). Similar anti-inflammatory effects of HMG-CoA reductase inhibitors on EAT have also been reported by others (615, 616, 617). Likewise, expression of IL-1 β in EAT amongst patients with diabetes taking 25mg pioglitazone therapy for a mean duration of 24 months was less than in those without treatment (618). Incubation of EAT in vitro with 100 μ M dapagliflozin also resulted in a reduction of IL-8, CCL2 and CCL5 concentrations (619). Over 90% of patients from whom EAT and SAT were harvested in the present study were prescribed statin therapy and it is likely that this would have influenced the adipokine profiles of tissues. Thus, the site of biopsies, population characteristics, concurrent pharmacological modulation and prevailing physiology at the time of harvest are all likely to contribute to the variations in adipose secretomes between studies.

Despite these inherent variations, ELISA results of the present study showed that concentration of Activin A was greater in EAT than SAT, which is consistent with results reported by Venteclef et al (226). Activin A is a member of the tissue growth factor-beta (TGF- β) superfamily and has been implicated with pulmonary, renal, pancreatic and hepatic fibrosis (620, 621, 622, 623) and adverse remodelling in heart failure by exerting a negative inotropic effect (624, 625). In their study, Venteclef et al showed that atrial fibrosis induced by EAT was mitigated by Activin A neutralising antibody (226). Activin A is particularly highly expressed in EAT of patients with diabetes which may explain the higher risk of arrhythmias in these

patients (626). In-keeping with these observations, Abe et al showed that fibrosis was more profound in human left atrial tissue adjacent to EAT compared to fat-free tissue (16.5% vs 8.4%) (221). Furthermore, regions of fibrosis were characterised by inflammatory macrophages located between the adipocytes and the myocardium with fibrosis (221). These lines of enquiry are particularly relevant to understanding how adiposity and cardiometabolic ill-health contributes to arrhythmogenesis, given that fibrotic remodelling results in anatomical conduction block and thereby facilitates re-entry in a vulnerable substrate. Indeed, these results and those reported by Nalliah et al (188) were the stimuli for investigating the effects of human EAT and SAT on cell electrophysiology in the present study. As described herein, exposure of NRVM to EAT resulted in rapid cessation of recordable electrical activity (<30 minutes) compared to cells incubated with control media. It is conceivable that this effect is at least partly explained by the EAT secretome. Furthermore, results in this chapter also show that the decrease in conduction velocities from baseline was greatest in NRVM incubated with EAT-conditioned media compared to SAT or control media at 2, 4 and 24 hours incubation. Although these results are consistent with those from Nalliah et al, there are a number of important differences between the respective studies. First, the study by Nalliah et al utilised HL-1 cells that were incubated with rodent pericardial fat- and inguinal SAT-derived conditioned media and therefore do not constitute a comparison of EAT vs SAT (188). Furthermore, the conduction velocities recorded in HL-1 cells was considerably modest (<1cm/s) (188), compared to the those reported herein (baseline values were >25cm/s with decrements of up to 15cm/s by 24 hours in the EAT group). Conduction velocities as low as those reported by Nalliah et al renders it practically challenging to detect any significant differences between groups and represents unphysiologically slow

conduction that limits extrapolation of these results to the study of human electrophysiology. NRVM therefore represent a more electrophysiologically relevant model in which to study the effects of EAT on arrhythmogenesis.

6.4.3. Epicardial fat causes greater conduction slowing than subcutaneous fat in NRVM

The main finding of this study was that NRVM incubated with EAT-conditioned media recorded greater conduction slowing over 24 hours than cells exposed to SAT-conditioned or control media. Given that conduction slowing is one of the requisites for re-entry (21, 26), these results provide a mechanistic insight into how EAT induces a proarrhythmic substrate in vivo. They are also in-keeping with in-human studies showing that EAT co-localises with electrophysiological abnormalities. For instance, the left atria of obese patients ($\text{BMI} > 27 \text{ kg/m}^2$) with AF have slower conduction velocities compared to AF patients with $\text{BMI} < 27 \text{ kg/m}^2$ ($0.86 \pm 0.31 \text{ m/s}$ vs $1.26 \pm 0.29 \text{ m/s}$; $p < 0.001$) and EAT volume correlates with conduction velocity ($r^2 = 0.31$) (99). The present study shows that EAT from a mainly non-obese ($25.1 \pm 3.5 \text{ kg/m}^2$) non-AF ($n=20/22$) cohort can also cause conduction slowing in vitro. Considering the pathophysiological effects of increasing EAT (Chapter 1), it may be postulated from these results that EAT has the potential to induce an arrhythmogenic substrate independent of proarrhythmic cardiovascular adaptations associated with obesity (546) in lean individuals without a history of cardiac arrhythmias. Although it was not possible to quantify EAT volume in study cohort due to an absence of cross-sectional imaging around the time of surgery, these results also provide an explanation for conduction delays on 12-lead ECGs that are associated with EAT

volume (627, 628, 629) and the observation that EAT increases risk and burden of arrhythmias (101, 562, 602).

The MEA-NRVM experimental design and results of the present study is remarkably akin to a recent study by Ernault et al, which showed that compared to SAT, EAT secretome causes conduction slowing in NRVM and facilitates re-entry arrhythmias (607). Notable differences between respective studies, however, include the fact that in their study, Ernault et al harvested atrial rather than ventricular EAT from 30 patients with AF, and generated conditioned media after 24-hour incubation, compared to 96 hours as in the present study. NRVM were cultured with EAT- or SAT- conditioned media for up to 72 hours without dilution, rather than 24 hours and dilution to 5% in the present study.

The decision to dilute EAT- and SAT-conditioned media to 5% was informed by the observation that NRVM treated with EAT-conditioned media without dilution resulted in cessation of recordable electrical activity within 30 minutes of incubation (Figure 6.5). Although the reasons for this are not clear, it may be hypothesised that the 96-hour duration of EAT culture may have caused accumulation of mediators that adversely affected NRVM viability and/or electrophysiology. It may be argued that dilution of media renders it unphysiological and unlike the EAT secretome in vivo.

There are two counterarguments to this: first, in vitro culture of adipose tissue irrespective of duration is unlikely yield a secretome that directly resembles its in vivo counterpart; second, and more importantly, this does not invalidate this experimental method to study the effects of adipose tissue or its extrapolation to human cardiac

electrophysiology. Paired samples of EAT and SAT were harvested, transported and cultured simultaneously under identical conditions using a validated method (425) to obviate any environmental or experimental differences in the generation of their respective conditioned media that may bias their effects on NRVM conduction velocity. Nevertheless, the limitations inherent in this study, like other similar studies, are acknowledged in section 6.4.6 and Chapter 7. To enable an unbiased comparison between EAT and SAT groups, conditioned media from both sets of biopsies were therefore diluted to 5% before NRVM incubation. This decision was also informed by the results of optimisation demonstrating that electrical activity could be recorded in NRVM cultured with 5% media up to 4 hours into incubation (Figure 6.5). This length of time was chosen for practical experimental reasons relating to the time taken to record data from NRVM in each MEA across all three groups (EAT, SAT and control) in triplicates.

Given that the baseline conduction velocities are different in each MEA and between groups, the differences in conduction velocities between EAT, SAT and control groups presented in Figure 6.6 are shown as change from baseline to account for this and thereby enables a fairer comparison between groups. Importantly, only NRVM recording a baseline conduction velocity, i.e. prior to incubation with EAT, SAT or control media, of $>25\text{cm/s}$ were used experimentally to minimise the influence of baseline characteristics on experimental outcomes between groups. By contrast, Ernault et al reported the actual conduction velocities in EAT, SAT and control groups after 72 hours incubation, rather than change from baseline. Conduction velocity in EAT-treated NRVM was $13.1 \pm 1.8\text{cm/s}$ vs $19.7 \pm 3.8\text{cm/s}$ and $21.8 \pm 5.9\text{cm/s}$ in control and SAT groups, respectively (607). Although it is not

possible to compare conduction velocities at 72 hours compared to the results at 24 hours presented herein, and despite the methodological differences between studies, the conduction slowing in NRVM with EAT- vs SAT-conditioned media shown in Figure 6.6 is comparable to that reported by Ernault et al (607).

To provide further mechanistic insights into adiposity-related arrhythmogenesis, in patch-clamp experiments Ernault et al also showed that NRVM incubated with EAT had blunted expression and function of the inward rectifier K⁺ currents resulting in a less negative resting membrane potential compared to cells incubated with SAT (607). Furthermore, EAT-exposed NRVM also had reduced expression of Cx43, slower conduction velocities, greater conduction heterogeneity and could sustain re-entry arrhythmias compared to cells incubated with SAT or control media (607). The mechanisms underpinning these results and those presented in the present study are discussed in the following section.

6.4.4. Mechanisms that may explain epicardial fat-induced conduction slowing

The differences in conduction velocities observed between EAT and SAT described herein and corroborated by Ernault et al may be partly explained by paracrine effects on NRVM electrophysiology, despite the results of the Human Adipokine Array assay showing no quantitative differences between EAT and SAT. This is because these tests do not constitute a comprehensive or wholly quantitative assessment of EAT or SAT secretomes. Indeed, previous studies have shown that adipokines and cytokines secreted by EAT and SAT have proarrhythmic effects on cellular electrophysiology. These has been reviewed comprehensively by Patel et al (1), and

also outlined in Chapter 1. For example, exposure of rat cardiomyocytes to IL-1 β for 24 hours has been shown to stimulate release of calcium from the sarcoplasmic reticulum, thereby prolonging APD and increasing vulnerability for delayed afterdepolarisations (195). Similarly, mice overexpressing TNF- α also exhibit prolongation of APD and increased conduction heterogeneity compared to their wild-type counterparts (197). Indeed, TNF- α and IL-1 β synergistically increase sarcoplasmic Ca²⁺ leak which increases the frequency of spontaneous intracellular Ca²⁺ waves (196). Even transient (<60 minutes) exposure of murine and guinea pig ventricular myocytes to IL-6 in vitro results in augmentation of L-type calcium currents and repolarisation prolongation (486). The results presented herein are nonetheless consistent with those from Venteclef et al (226); EAT exhibits a greater concentration of Activin A than SAT, and this may contribute to greater conduction slowing in NRVM incubated with EAT vs SAT. Just as Activin A neutralising antibody mitigates atrial fibrotic remodelling (226), whether conduction slowing with EAT-conditioned media in NRVM can be prevented with the same is the subject of ongoing experimentation and is also proposed as future work in Chapter 7. Although these constitute an important set of experiments that had been planned from the outset, the practical difficulties of establishing a stable NRVM culture preparation during the study precluded these from being completed. This is discussed further in section 6.4.5.

In addition to their effects on ion channels, inflammatory mediators and free fatty acids (FFA) have been associated with modulation of gap junctions. The relationship between altered connexin expression and propensity for atrial and ventricular arrhythmias is well-established (630, 631, 632, 633). Connexin proteins in neonatal myocytes have a short half-life of approximately 1.5 to 2 hours (634) and therefore it

is possible that their expression and function in NRVM may have been modulated during incubation with adipose-conditioned media. Indeed, Nalliah et al. demonstrated lateralisation of Cx40 in HL-1 atrial myocytes following exposure to pericardial fat (188) and Benova et al showed that high-sucrose diet in rats induced weight gain and an increase in EAT volume that was associated with downregulation of Cx43 and greater inducibility of ventricular arrhythmias (635). Chronic high-fat diets also alter connexin distribution from intercalated discs to the longitudinal membranes of cardiomyocytes, which correlates with EAT volume and slower conduction velocity (188, 636). Adipokines, such as IL-6 and TNF- α , have been shown to cause gap junctional changes in experimental settings. For instance, Lazzerini et al demonstrated an inverse relationship between circulating concentrations of IL-6 protein, and Cx40 and Cx43 mRNA in patients undergoing cardiothoracic surgery (637). Moreover, increasing concentrations of IL-6 was associated with greater P wave dispersion, a predictor of AF (638). HL-1 atrial myocytes incubated with IL-6 for 24-48 hours also demonstrate reduced Cx40 and Cx43 expression, an effect that was reversed by pre-incubation with monoclonal antibody against IL-6 (637). Transgenic mice overexpressing TNF- α also have reduced expression of Cx40 that is associated with slower atrial conduction and increased incidence of supraventricular arrhythmias (639). Although 90-minute exposure of TNF- α in guinea pig heart did not suppress Cx43 protein expression, it was nonetheless associated with its distribution away from intercalated discs (639, 640). Furthermore, TNF- α exposure may alter ephaptic coupling, in which conduction relies on the close spatial arrangement of adjacent cardiomyocytes and high density of sodium channels in the sarcolemma (640). Conduction via this mechanism occurs as depolarisation of myocyte sequesters sodium ions in the cleft, rendering the

voltage of the cleft more negative and creating a steep voltage gradient between the adjacent myocytes. This stimulates the opening of sodium channels in the adjacent myocyte allowing propagation of electrical activity from one myocyte to the other. In this way, narrow perinexi enable cardiomyocytes to remain electrically coupled in instances where gap junction coupling may be compromised (641, 642). TNF- α has been shown to widen the perinexus between adjacent cardiomyocytes and consequently reduce ephaptic coupling resulting in slower conduction velocity (640). Indeed, perinexal width has been shown to be greater in patients with AF (643). Along these lines, other inflammatory proteins expressed by adipose tissue, such as vascular endothelial growth factor (VEGF), may also disrupt gap junctions resulting in conduction slowing predisposing normal hearts to arrhythmias (644). Langendorff-perfused guinea pig heart preparations have also been used to investigate the effects of perinexal width variation on arrhythmia susceptibility and how this may be modulated by extracellular sodium and calcium concentrations experimentally (645, 646, 647). It is therefore possible that the inflammatory secretome of EAT may cause conduction slowing and exert proarrhythmic effects by modulating gap junctions.

6.4.5. Addressing experimental difficulties

Experimental methods and decisions in this study were influenced significantly by the availability and volume of EAT and SAT samples. The first sample was collected on 30 January 2020. The coronavirus pandemic, and other practical reasons relating to clinical services at Hammersmith Hospital, resulted in no samples being available between March 2020 and April 2021. Although biological sample harvesting for research purposes resumed thereafter, only SAT and not EAT samples were made available to the research team from April to October 2021. Given that the study aim

was to investigate the differential effects of EAT and SAT on cell electrophysiology, no adipose tissue samples were accepted during this period. Paired EAT and SAT sample harvesting resumed in October 2021 and continued until February 2022 with the last sample harvested on 24 January 2022. Other factors that affected the availability of samples included cancellations of elective surgical procedures and patients not consenting for biopsies during their surgery. Therefore, despite the intention at outset to harvest paired adipose samples from 150 patients based on a similar study (193), the present study is likely to have been under-powered to detect significant differences between EAT and SAT secretomes at a threshold of 80% ($\alpha=0.05$). In instances when paired samples were available from the same patient, the volume of SAT was invariably much greater than that of EAT. This can be explained by the fact that SAT was harvested from the parasternal region is generally more plentiful than EAT, and given that the latter lies in intimate contact with the myocardium, its harvest is associated with a higher risk of inadvertent clinical injury. After determining with the method of culture described herein that there was no statistical difference in viability of adipose tissue between 24, 48, 72 and 96 hours of culture, the decision was made to culture all subsequent biopsies for 96 hours. Culturing adipose tissue at a single -time point would ensure sufficient yield of conditioned media to permit technical replicates in experimental tests. Indeed, this consideration was particularly important in the early stages of this project, as the original intention had been to test the effects of conditioned media on rat ventricular slices rather than NRVMs. Myocardial slices are ultra-thin (300 μ m) sections that are generated using a high-precision microtome. Their advantage over cell culture is that they better preserve the native tissue's electromechanical properties and structure and are therefore a better representative of *in vivo*

physiology (648). These slices are maintained and cultured in organ baths which necessitated a circulating volume of approximately 100ml (648). Even in the smallest chambers, developed by Fotios Pitoulis (unpublished work, personal communication), slices require a culture media volume of 20ml to remain viable. Given that the typical EAT biopsy acquired for the present study weighed approximately 100-150mg, yielding conditioned media of approximately 3-5ml, it became clear that studying the effects of adipose conditioned media on slices was not practically feasible. Given that the cell culture in MEA had been used to investigate mechanisms underpinning arrhythmogenesis previously (435), and NRVM were readily available, these were adopted to achieve the aims of the present study.

One of the major practical challenges, and indeed limitations, in this project related to the ability to reliably and consistently culture NRVM and record their electrical activity. Consequently, the effect of EAT and SAT secretomes on NRVM conduction were only tested using biopsies from 5 patients. Furthermore, it was not possible to establish whether conduction slowing observed with EAT could be mitigated with pharmacological inhibition with, for example, Activin A neutralising antibody (226). For context, NRVM were isolated and cultured once a week during this study. Attempts at recording baseline electrical activity and after treatment with adipose conditioned media were made approximately 48-72 hours later. Typically, 20-25 MEA were used every week, with 200,000 NRVM cultured in each MEA, and based on previous experience (personal communication with Dr Rasheda Chowdhury) it was expected that two-thirds of these could be used experimentally. Thus, the study had aimed to culture and test the effect of adipose conditioned media on NRVM on a

regular (weekly) basis. Work for this study began in June 2019, and aside from approximately six weeks in March-April 2021 during which time electrical activity and experimental data presented herein, no meaningful data was otherwise recorded until April 2022 despite several attempts at optimising different aspects of the experimental protocol. These difficulties were encountered in series and in tandem with the challenges of acquiring EAT and SAT biopsies. For this reason, and despite eventually acquiring paired biopsies from 22 patients, the effects of EAT and SAT conditioned media on NRVM electrophysiology could only be studied using 5 paired EAT and SAT samples. Some of the notable challenges experienced during culturing of NRVM on MEA, and the attempts to optimise the experimental set-up, are described hereafter.

First, spontaneous electrical activity of cultured NRVM was rarely observed and these cells could not be paced due inability to acquire electrical capture, even at baseline. Initially in the early stages of this study, attempts to record baseline conduction velocity in NRVM were made 72 hours post-culture on MEA. However, the absence of electrical activity at this timepoint may have been explained by NRVM denaturation. Heuristically, it was observed that no spontaneous electrical activity could be detected at 24 post-culture either, and this may be explained by the NRVM being too immature to record spontaneous electrical activity. Rather, NRVM were found to beat spontaneously, albeit inconsistently and unreliably, at 48 hours post-culture. Informed by this observation, NRVMs were cultured for 48 hours before attempting to record baseline activity. Second, under light microscopy it was observed that inadequate coverage of electrodes by NRVM, and thereby a lack of contact with electrodes, may also contribute to the inability to pace the cells or record

electrical activity. To address this, collagen was applied liberally with a margin around the electrode matrix on the MEA plate to ensure NRVM seeded across the matrix. 24 hours post-culture, MEA were observed under light microscopy to check coverage of NRVM over the electrode matrix. Only those MEA in which there was adequate coverage over the majority of electrodes were used for experimental purposes. Third, the absence of electrical activity in cultured NRVM may have been attributed to the absence of a confluent monolayer. Cell-to-cell connections in NRVM are integral to the propagation of an electrical impulse in vitro. An inadequate number of cells with such connections will result in failure of an electrical impulse to propagate across the cultured layer of cells. On the other hand, a higher density of NRVM may result in a source-sink mismatch and similarly result in failure of propagation. To identify the optimal density, NRVM were cultured in densities ranging from 50,000 to 250,000 cells/30 μ L collagen in increments of 50,000 cells. Observationally, a monolayer of NRVM was most consistently found with 200,000 cells/30 μ L collagen which was consistent with the experience of others (607). Fourth, it was observed during the latter stages of the study (November 2021 onwards) that NRVM adhered poorly to the MEA surface, to the extent that there were no confluent monolayers. Indeed, in many instances, it appeared that no NRVM had adhered to the collagen over the electrode matrix. As described in Chapter 2, MEA were routinely placed under ultraviolet light for sterility and to ensure complete dryness prior to NRVM culture. For reasons that are not understood, it was observed that MEA did not dry sufficiently under UV light despite having done so previously and the fact that the experimental protocol and reagents had remained unchanged. Thus, it was felt that the residual moisture had diluted the collagen droplet on to which the NRVM were cultured thereby preventing adequate adherence

of NRVM onto the MEA surface. To address this, MEA were dried using a drying and thermal processing oven (Baird and Tatlock) prior to NRVM culture. Nevertheless, despite adequate drying of the MEA, electrical activity from NRVM still could not be recorded consistently on a regular basis impeding data acquisition. Fifth, these challenges were further complicated by fungal contaminations of MEA and cell culture incubators and facilities which rendered them inaccessible between November to December 2021. New MEA were also purchased from to address the possibility that the shelf-life of existing MEA had expired and may be a contributory factor. The study was delayed in this regard by delays in acquisition of new MEA from August to November 2020 and did not address the shortcomings associated with data acquisition.

Given the similarities with the study by Ernault et al,(607), it is possible that some of the difficulties described herein may have been addressed by the methods used in their study. For instance, in their study Ernault used fibronectin (125 µg/mL BD Biosciences, Breda, The Netherlands) to culture NRVM rather type IV human placental collagen. NRVM culture media or adipose-conditioned media was replaced 10 minutes before electrical measurements with a modified Tyrode's solution at 36.5°C containing 140mM NaCl, 5.4mM KCl, 1.8mM CaCl₂, 1.0mM MgCl₂, 5.5mM glucose and 5.0mM HEPES at pH 7.4 that was achieved with NaOH. Furthermore, rather than electrically stimulating NRVM by the electrodes onto which they were cultured, Ernault et al employed external pacing electrodes that would have ensured more consistent electrical capture to pace cells.

6.4.6. Limitations

Aside from these practical considerations, there are a number of limitations to the current study. First, in comparison to others (193), EAT and SAT were harvested from a relatively small number of patients and therefore was underpowered to compare EAT and SAT secretomes between groups of patients, for example, between those with and without diabetes or hypertension. Second, although consistent with the methods used by others(607), this study only explored the acute effects of EAT and SAT secretomes on the NRVM electrophysiology in vitro in which it is difficult to replicate the effects of chronic adiposity and the dynamic changes that may occur with concurrent disease processes, for example, diabetes and hyperglycaemia. Third, due to the practical challenges of safely harvesting EAT, it was not possible to determine regional differences reported by others (421) and consequently any differential effects on NRVM conduction velocities. Indeed, as with other studies, it was not ethically possible to obtain EAT and SAT from healthy individuals, and therefore not possible to study differences on cell electrophysiology between adipose tissue harvested from healthy individuals compared to patients with established cardiometabolic disease. Fourth, although NRVM are an established model by which to study human electrophysiology (427) in which conduction velocities are more akin to human electrophysiology than in HL-1 myocytes (188), there are nonetheless important difference inter-species differences in the ion channels determining the action potential (649, 650). Furthermore, in vivo adipose tissue secretome is influenced by prevailing physiological conditions which cannot be reproduced experimentally. Although the in vivo secretome is therefore likely to be different to that generated in vitro, and consequently may bear different effects on electrophysiology, the fact that EAT and SAT were cultured and utilised under

identical experimental conditions mitigates any bias arising from comparisons between these groups. Finally, given the challenges of NRVM culture discussed herein, attempts at quantifying differences in connexin expression between NRVM incubated with EAT- and SAT-conditioned media was not possible but has been reported by others (607).

6.5. Conclusion

In summary, using adipose tissue from 22 patients undergoing cardiothoracic surgery at a single centre between January 2020 and January 2022, the results of this study demonstrated that the concentration of Activin A, determined using ELISA, was greater in EAT- vs SAT-conditioned media. There were, however, no differences noted in EAT and SAT secretomes according to a semi-quantitative human adipokine assay. Using samples of the conditioned media generated from EAT and SAT biopsied in this study, conduction velocities of NRVM in response to 1Hz pacing decreased significantly in cells incubated with EAT- compared to SAT- and control-media over a 24-hour period. Although the mechanisms underpinning this phenomenon could not be determined in the present study, it is postulated that ion channel and gap junction modulation by inflammatory mediators and fatty acids, fibrotic remodelling may explain these observations. These results are in-keeping with recently published studies demonstrating the pro-fibrotic nature of EAT vs SAT and its association with conduction slowing in vitro, and provide an insight into how EAT may contribute to a proarrhythmic substrate in vivo.

Chapter 7: Discussion and conclusions

7.1. Summary of findings

The work presented in this thesis aimed to investigate the effects of increasing adiposity and obesity on electrophysiological remodelling to explain the higher risks of atrial and ventricular arrhythmias in obese patients. This was achieved by first studying the associations between QTc interval and increasing adiposity quantified by a range of anthropometric indices and metabolic ill-health at a population-level using data from the UK Biobank. Additionally, the work presented in Chapter 3 sought to establish whether any QTc prolongation in obese individuals translated into higher prevalence of ventricular arrhythmias. Second, although obesity and cardiac arrhythmias are increasingly common medical problems, not all obese patients have cardiac arrhythmias. In this respect, our understanding of how obesity causes subclinical adverse electrophysiological remodelling in arrhythmia-free patients, and more importantly the extent to which it may be reversed with weight reduction, has been limited. This is clinically important as demonstrable reversibility of a proarrhythmic substrate may inform and guide primary preventative strategies for managing cardiac arrhythmias and healthcare resource allocation. The paucity of studies investigating and characterising the proarrhythmic substrate in arrhythmia-free individuals is at least partly due to the practical challenge of undertaking this task in those that do not have a clinical indication for invasive electroanatomic mapping. Therefore, the work presented in this thesis includes the optimisation and standardisation of non-invasive electroanatomic mapping (ECGi) by which atrial and ventricular activation and ventricular repolarisation can be studied in obese patients. It also includes the results of a clinical study in which morbidly obese patients underwent ECGi before and after bariatric surgery, and a comparison of these results with those from age- and sex-matched non-obese individuals. Third, given

the increasingly compelling evidence linking EAT to a range of adverse cardiovascular processes, including cardiac arrhythmias, the work in this thesis compared the secretomes of EAT and SAT of patients undergoing cardiothoracic surgery using quantitative and semi-quantitative molecular biological methods. Additionally, the effects of EAT- and SAT-conditioned media on conduction velocities in NRVM was compared to gain mechanistic insights into adiposity-related arrhythmogenesis.

The main findings with respect to the hypotheses are:

1. Amongst a large sample of the UK population, increasing adiposity prolongs QTc interval even after adjusting for sociodemographic factors and co-morbidity, and that the effects of obesity and metabolic ill-health on QTc interval prolongation is additive. Despite this, metabolic-ill health appears to be associated with a higher prevalence of ventricular arrhythmias than obesity alone.
2. Obesity is associated with subclinical electrophysiological remodelling, even in individuals without a history of cardiac arrhythmias, that can be characterised using ECGi
3. Atrial activation is prolonged in morbidly obese patients which persists up to 9 months after bariatric surgery. In contrast, spatial ventricular repolarisation gradients completely reverse over the same duration. These observations were made in the context of a reduction in HbA1c, LV mass and EAT volume after bariatric surgery and a partial improvement in HRV. The adverse

electrical remodelling in obese patients, specifically atrial activation and ventricular repolarisation gradients, correlate with EAT volume.

4. EAT has a greater concentration of the pro-fibrotic adipokine Activin A compared to SAT and causes greater conduction slowing in NRVM in response to pacing

The following sections will contextualise these findings by considering notable or contemporaneous studies related to the work contained in this thesis.

7.1.1. Increasing adiposity is associated with QTc interval prolongation

The results presented in Chapter 3 show that increasing adiposity and metabolic ill-health independently prolong QTc interval. Although ventricular repolarisation is at least partly genetically-determined (462, 607), the QTc prolonging effect of increasing adiposity was not modulated by individuals' genetics. Importantly, metabolic ill-health appeared to be associated with a greater prevalence of ventricular arrhythmias than increasing adiposity. These findings provide insight into the links between increasing adiposity and cardiometabolic ill-health and abnormal ventricular repolarisation in the UK population and are in agreement with a number of other studies (106). According to one report, when extrapolated to the general population, the rate of unexpected fatal cardiac arrests amongst morbidly obese patients may be as high as 40 times the rate observed in non-obese individuals (651). The higher rate of cardiac arrests amongst obese patients in this report was noted in absence of concurrent organic heart disease, or indeed other pathology, except QTc interval that was longer than 430ms prior to cardiac arrest (651). Although this does not imply causation, a number of the studies have subsequently

shown that obesity is a risk factor for abnormal ventricular repolarisation (106) and sudden cardiac death (116). The QTc interval denotes total depolarisation and repolarisation of cardiomyocytes in the surface ECG. Although changes in either may influence QTc interval, its prolongation is more frequently the consequence of a prolonged repolarisation phase that may be congenital or acquired. Prolongation of ventricular repolarisation, and thus an increase in dispersion of repolarisation, is one of the hallmarks of a substrate that can sustain re-entry (652, 653). A number of the factors have been shown to prolong QTc interval, including age (654), sex (466), dyslipidaemia (655), and cardiometabolic disease (656, 657). The results presented in Chapter 3 show that increasing adiposity prolongs QTc independent of these factors. Furthermore, the degree of QTc prolongation with increasing adiposity reported therein is in-keeping with other studies (107, 112, 658, 659, 660, 661). For example, Carella et al. showed QTc prolonged by 5ms for every 50% increase in fat mass percentage quantified by hydrodensitometry (660). Uniquely, leveraging genetic data of individuals in whom 12-lead ECG and anthropometric data was also available in the UK Biobank, the results in Chapter 3 show that the QTc-prolonging effects of adiposity are not off-set by heritable traits. This is important for two reasons. First, given that up to 10% of variability in QT prolongation in the general population is genetically-determined (662), the modulatory effects of genetics on the association between adiposity and QTc interval has not been reported in previous studies. The results presented herein serve to address this question amongst a large sample of the UK population. Second, the fact that increased adiposity prolongs QTc, in spite of any opposing effects of genetics, suggests that modifiable risk factors are more important determinants of ventricular repolarisation, and thus any consequent risks of ventricular arrhythmias. The results from the UK Biobank

analyses also show that ventricular arrhythmias were more prevalent amongst those with cardiometabolic disease rather than obesity. Therefore, the QTc prolonging effects of increasing adiposity, which do not appear to be modulated by genetics, may not translate into a clinically higher risk of ventricular arrhythmias. Although this may initially appear contrary to studies suggesting that obesity confers higher risk of sudden cardiac death, it is noteworthy that this evidence often comes from populations in whom existing cardiovascular disease, particularly ischaemic heart disease, is highly prevalent (116). Therefore, although obesity may contribute to a higher mortality risk, in the general population the prevalence of ventricular arrhythmias is more likely to be explained by the natural history of other more common medical conditions, such as ischaemic heart conditions and complications arising from chronic diabetes.

7.1.2. ECGi outputs are influenced by pre-processing of body surface potentials and post-processing of electrograms

The study of obesity-related electrophysiological changes presented in Chapter 3 is only practically meaningful if it could be shown that they reverse with weight reduction. In the absence of a clinical indication for electroanatomical mapping, ECGi was used to test this hypothesis non-invasively in morbidly obese individuals. Although ECGi has been validated in several cohorts (289, 375, 378, 379, 388, 399, 406, 416), and notably with invasive epicardial mapping in humans (663), variations in ECGi methods necessitated optimisation prior to its application to an obese cohort in which it has not been implemented previously.

The work presented in Chapter 4 shows that ECGi outputs are significantly influenced by pre-processing of BSPs and post-processing of electrograms. Given the paucity of studies describing ECGi methods and their rationale in a format that is accessible and comprehensible to the non-expert, it is envisaged that the results presented in Chapter 4 will be published and shared with the scientific community, particularly the ECGi Consortium (291), to enable others to consider implementing the techniques described therein. Utilising a standardised approach for signal processing across studies would ensure that results from ECGi studies are reproducible and comparable between cohorts.

The need for optimisation of ECGi methods became evident during data acquisition in the clinical study. For example, the large body habitus of a mainly female obese cohort rendered BSPs vulnerable to artefact due to poor skin contact, particularly over the anterior chest wall. This in turn resulted in significant artefact in a significant number of electrograms rendering annotation of local AT and RT inaccurate. For this reason, all 256-lead body surface potentials were manually reviewed for each participant and any artefact removed prior to solving the inverse problem. Optimising the post-processing of reconstructed electrograms constituted the other aspect of the work presented in Chapter 4. The results of developing a standardised framework for signal pre- and post-processing may be summarised as follows. First, body surface potentials with the least RR variability were the most stable and least affected by artefact, and therefore least likely to introduce error in the computation of unipolar electrograms. Second, signal averaging over 10 beats for ventricular data and 50 beats for atrial data further ensured robustness against noise. Third, defining total activation and repolarisation as the smallest range encompassing 95% of their

respective local times resulted in results that were least affected by artefact. Fourth, a method that reliably annotates the steepest upslope of the T-wave was implemented to ensure accurate annotation of the local RT. Fifth, T-wave upslope gradient (dV/dt) <1.2 consistently identified T-waves with low amplitude in which local RT could not be accurately determined using automated methods. Finally, a spatial median filter using a radius of 5mm enabled sufficient smoothing of the repolarisation patterns without comprising the spatial gradients of repolarisation. Although the significant manual input necessitated by these methods means that they are unlikely to be adopted in regular clinical applications of ECGi, they nonetheless ensured the robustness of results of the clinical study presented in Chapter 5.

7.1.3. Bariatric surgery partly reverses adverse electrophysiological remodelling in morbidly obese patients

The results of the single-centre prospective ECGi study in morbidly obese patients and age- and sex-matched lean counterparts showed that prolongation of atrial activation in obesity is not reversed by bariatric surgery, and may explain observations from other studies that risk of atrial arrhythmias is not mitigated after surgical weight loss (240, 278). On the other hand, ventricular repolarisation gradients reversed with surgical weight loss. Although total repolarisation time did not change significantly post-bariatric surgery, the reversal ventricular repolarisation gradients is nonetheless consistent with 12-lead ECG studies showing shortening of QTc interval with weight loss (107, 109, 664, 665). Furthermore, where previous studies have suggested that QTc prolongation in obesity is attributable to left ventricular hypertrophy (113, 666), the reversal of repolarisation gradients was observed in its absence amongst the cohort of the present study. These differences

between studies demonstrate how although the 12-lead ECG may demonstrate electrophysiological adaptations it cannot discern subtle changes owing to its limited spatial resolution. The regression of EAT with bariatric surgery is also in agreement with at least one other recent study (592). The same study, however, concluded that ventricular conduction improved post-surgery based on a significant decrease in QTc interval, not QRS duration, with weight reduction. The results presented in this thesis show the total ventricular activation time was in fact not different between lean and obese groups. This is unsurprising given the absence of any conduction disease in either group. In addition to the electrophysiological changes, time-domain analyses of body surface potentials demonstrated an improvement in autonomic function in obese patients following bariatric surgery.

The finding that ventricular repolarisation gradients are steeper in obese individuals and reverse with weight reduction is particularly noteworthy in the context of recent studies investigating the mechanisms of ventricular arrhythmias. This is particularly relevant to the current cohort given that repolarisation abnormalities are arrhythmogenic even in the absence of structural abnormalities (667). Noting that steep repolarisation gradients in long QT and Brugada syndromes lead to unidirectional block and thereby re-entry (27, 36, 37), Rivaud et al showed that ventricular arrhythmias are initiated and maintained as a consequence of re-entrant circuits along the border zones of long and short repolarisation regions in porcine hearts and in silico models (33). Given that the activation of arrhythmias is often a focal mechanism, most commonly an ectopic beat (668), a substrate characterised by ventricular repolarisation heterogeneity that favours re-entry is more likely to be able to sustain it. Furthermore, the fact that EADs are often generated in regions with

prolonged action potential duration and re-entry is initiated by a premature stimulation arising from a region of short action potential duration, the co-existence of both in obese hearts suggests that the substrate may be prone to initiating as well as sustaining ventricular arrhythmias. Similarly, using explanted hearts, ECGi and in silico modelling, Cluitmans et al demonstrated the electrical features that rendered a substrate vulnerable to VF (38). Using data from survivors of idiopathic VF, they showed that arrhythmic vulnerability increased with increasing ventricular repolarisation heterogeneity, premature beats were initiated in regions of early ventricular repolarisation and that these individuals exhibited a bimodal distribution of repolarisation times (38). These results are in-keeping with another study in Langendorff-perfused rabbit hearts showing that premature ventricular ectopics arise from areas of steep voltage gradients in the border zones of regions of prolonged repolarisation as a consequence of the electrotonic stimulation of voltage-gated calcium channels (558). Although the differences in RTG between pre- and post-bariatric surgery obese individuals and with non-obese individuals were less stark than those observed between VF survivors and control individuals and voltage gradients were not calculated, the results of the present study nonetheless provide an insight into why obese hearts are vulnerable to ventricular arrhythmias (45). Repolarisation times and patterns reconstructed non-invasively using ECGi have been shown to correlate strongly with contact-mapping data in Langendorff perfused pig hearts and donor humans hearts ($r^2 > 0.92$) (313). Thus, in the absence of ground-truth data with which to validate results presented herein, repolarisation abnormalities and their reversal observed in obese patients are likely to be a reasonably accurate representation of epicardial electrophysiology.

7.1.4. Epicardial adipose tissue causes conduction slowing in NRVM

The results in Chapter 6 shows that the concentration of Activin A is greater in EAT vs SAT, and that EAT-conditioned media causes greater conduction slowing in NRVM compared to SAT- or control-media. Characterisation of the EAT- and SAT-conditioned media revealed no other significant differences in adipokine profiles between the two depots.

During the course of this study, two other groups working independently reported on the effects of adipose tissue on cell electrophysiology using experimental methods and agents that resemble those described in Chapter 6. In 2020, Nalliah et al published results showing rodent pericardial fat caused greater slower conduction compared to inguinal SAT in HL-1 atrial cardiomyocytes in response to pacing (188). In 2022, Ernault et al showed that human EAT caused greater conduction slowing than SAT in NRVM (607). Indeed, some of the aims and methods of the study in Chapter 6 and that by Ernault et al are identical: both harvested paired EAT and SAT from patients undergoing cardiothoracic surgery to generate conditioned media using comparable biological methods and tested their effects on conduction velocity in response to pacing in NVRM using similar cell culture techniques and recording apparatus. Notable differences between these studies are that the study Ernault et al harvested EAT and SAT from a cohort in which AF was more prevalent (30 vs 2 patients) and did not include characterisation of EAT and SAT secretomes. They did, however, undertake a more comprehensive analysis of NRVM electrophysiology in response to EAT and SAT exposure, which included ion channel function using patch-clamp, as well as ion channel and connexin protein and mRNA expression using Western blotting and quantitative real time polymerase chain reaction.

Unfortunately, despite multiple attempts at optimisation, NRVM culture was inconsistently successful throughout the study presented in Chapter 6 which limited data acquisition. It was therefore not possible to test the effects of EAT and SAT secretomes on NRVM conduction using samples from 22 patients. For the same reason, it was not possible to compare Cx43 expression in NRVM following incubation with EAT- and SAT-conditioned media using immunohistochemistry to explain their differential effect on conduction velocity. Ernault et al however showed that Cx43 mRNA and protein expression decreased in cells incubated with EAT-conditioned media by 30% and 50%, respectively, and explained up to 25% of the reduction in conduction velocity (607). There was no difference in Cx43 expression between SAT and control, and Cx45 expression was not different across EAT, SAT or control groups (607). These results are in-keeping with others' showing lateralisation of Cx40 in regions of EAT (188). A reduction in Cx43 compromises electrical coupling between adjacent cardiomyocytes and may lead to conduction block and re-entry. Indeed, following their demonstration that EAT caused conduction slowing in vitro, Ernault et al using in silico modelling also showed that re-entry was sustained when EAT covered between 50% and 75% of left atrial epicardial surface, but not with total coverage (607). The authors conclude that this "emphasises the importance of heterogeneity for the initiation of re-entry" (607) and, given that <50% EAT coverage terminated atrial re-entry, these results also suggests that a critical threshold of epicardial adiposity is required for it to be proarrhythmic.

Given the differential characteristics of EAT based on anatomical location (421), one may argue that results of electrophysiological remodelling in *ventricular* myocytes in response to peri-*atrial* EAT that was used for atrial computational simulations may

not faithfully recapitulate the effects of adiposity on atrial electrophysiology in the study by Ernault et al. However, although from a smaller number of patients (n=5), the results of the present study demonstrates conduction slowing in NRVM in response to peri-ventricular EAT is in-keeping with those from Ernault et al, and suggest that differences in EAT harvest is unlikely to significantly influence the conclusions from both studies that EAT adversely affects myocardial conduction. Indeed, other studies have also shown that EAT increases QRS duration in pluripotent stem cell-derived cardiomyocytes (422, 669). Furthermore, sites of adipose infiltration can be used to identify sites of VT ablation post-infarction as part of growing trend towards personalised medicine (670). Thus, there is mounting evidence that EAT confers higher risks of atrial and ventricular arrhythmias.

As stated in Chapter 6, Ernault et al also postulated that any differences in conduction velocities between NRVM incubated with EAT and SAT may be explained by their respective adipokine profiles (607). Indeed, others have shown that EAT of AF patients exhibits a more inflammatory profile than that from individuals without AF (669). However, despite detailed in vitro electrophysiological assessments, their study did not characterise the adipokine profiles of these fat depots. Given the similarities between the respective studies, the results presented in Chapter 6 are complementary to those published by Ernault et al. The present study showed that, despite the concentration of Activin A being greater in EAT vs SAT which is consistent with other reports (226), no other significant differences were noted between these fat depots. The fact that EAT from AF and non-AF donors results in comparable conduction slowing (Ernault et al., 2022), however, does not rule out a paracrine effect. Rather, it may reflect how EAT is a highly heterogenous

fat depot and that its secretome is influenced by the site, concurrent morbidity, pharmacological modulation and prevailing physiology at the time of biopsy (63, 151, 533). Furthermore, implementing an experimental set-up similar to that described in Chapters 2 and 6, Ten Sande showed how NRVM co-cultured with human and rat adipose-derived stromal cells in MEA recorded slower conduction velocities than NRVM incubated with conditioned media (189). On the other hand, NRVM incubated with conditioned media of porcine-adipose-derived stromal cells displayed comparable conduction slowing as NRVM in co-culture (189). The authors concluded that conduction slowing in vitro is a species-specific phenomenon that may be influenced by electronic as well as paracrine interactions.

7.2. Clinical relevance

In the context of evidence incriminating increasing adiposity, particularly EAT, with a range of adverse healthcare outcomes (533), the results presented in this thesis may have the following clinical implications. First, they show that obesity is associated with subclinical electrophysiological remodelling that should not be considered benign. These adaptations are present on the 12-lead ECG at a population level, as demonstrated in Chapter 3, and although this may not translate directly into a higher risk of ventricular arrhythmias, obese individuals are more likely to develop metabolic disturbances over the natural history of the disease that are associated with a higher prevalence of the ventricular arrhythmias. Second, the subclinical electrophysiological, structural and autonomic remodelling in obesity is at least partly reversible with weight reduction. These observations from chronically morbidly obese patients without a history of arrhythmias is in-keeping with the reversibility of obesity-related metabolic complications with bariatric surgery. These findings advocate early

invasive weight reduction interventions, particularly with bariatric surgery in patients for whom lifestyle and pharmacological interventions may not have been successful. Ultimately, timely surgical interventions are more likely to be economically advantageous in the long term despite their higher short-term costs. Third, results presented in Chapters 5 and 6 incriminate EAT with proarrhythmic remodelling and conduction slowing, which is in agreement with evidence showing visceral adiposity is detrimental to health. This is particularly important as higher volumes of visceral adiposity, even in individuals who do not appear visibly overweight or obese, is likely to have deleterious effects on cardiac electrophysiology and has implications for screening of at-risk individuals.

7.3. Limitations

The limitations of individual studies are detailed in their respective chapters. Notable limitations from the results in Chapter 3 include the fact that the effects of pharmacological agents influencing QTc interval could not be accounted for given that the drug history of individuals participants could not be discerned from the UK Biobank. It is also possible that the absence of any modulatory effects of genetics on the associations between QTc interval and increasing adiposity and metabolic ill-health, respectively, may be influenced by unidentified SNPs influencing QTc or those that could not be included in the PRS as they were not available in the UK Biobank genomic dataset. Socioeconomic status is an important risk factor for cardiovascular disease (671), and although TDI was included in the multiple regression analyses of the UK Biobank study, the participants were largely Caucasian and restricted to a geographical distance within 22 assessment centres. The results may not, therefore, extrapolate to the wider UK population and in regions

of higher or lower socioeconomic levels. Increasing adiposity is known to influence electrocardiographic markers other than QTc interval (422). The effect of increasing adiposity on P-wave duration, for example, were not investigated given that the primary aim of this thesis was to investigate its effects on ventricular arrhythmogenesis.

The results of the clinical ECGi study in Chapter 5 was predominantly female. This is a common trait amongst obesity-related and bariatric surgical studies, and therefore sex-related differences could not be explored. The electrophysiological characteristics reported in Chapter 5 pertain to the epicardium only. Transmural and endocardial electrophysiology was not characterised, which may be achieved by utilising other cardiac models. Body surface potentials were recorded with the participants in an upright sitting posture, although heart-torso geometries were acquired in the supine posture. These differences may have introduced error in computing epicardial electrograms from the body surface potentials, although every effort was made to ensure that the MRI-safe markers corresponded as closely as possible to the anatomical positions of the electrodes. Similarly, body surface potentials were recorded throughout the cardiac cycle with concurrent ventilatory movement of the chest walls. The heart-torso geometries used to solve the inverse problem were derived, according to clinical convention, from end-diastole. This may have also introduced some error in the reconstruction of epicardial electrograms corresponding to the timing of the recorded body surface potentials. The absence of prolonged follow-up and the study design meant that it was not possible to determine the effect of reverse remodelling on incident arrhythmias.

In Chapter 6, EAT and SAT biopsies were obtained from patients presenting with multiple age-dependent comorbidities which may have influenced their respective secretomes. The main limitation of the study, however, were the practical difficulties of culturing NRVM in which electrical activity could be consistently recorded to study the effects of the EAT vs SAT on conduction velocities. It was unknown at the start and during the course of the study, that two other research groups were independently conducting similar experiments investigating the effects of EAT and SAT on cell electrophysiology. Although the ECGi study in Chapter 5 demonstrated repolarisation abnormalities in obese individuals, these could not be quantified in vitro with NRVM as the signal indicating repolarisation, and therefore field potential duration (188), could not be easily discerned owing to its low amplitude. On the other hand, local activation times and therefore conduction velocities are more readily identified owing to the larger amplitude of the signal indicating depolarisation. As discussed in Chapter 1, one of the general limitations of studies investigating the pathophysiological effects of EAT is the definition of this fat depot. The work in this thesis aimed to investigate the effect of EAT, that is the fat deep to the visceral pericardium, on cardiac electrophysiology. The terms pericardial fat and EAT are, however, often used interchangeably even though anatomically these may be considered to have different definitions (152, 533). The lack of a consensus definition of EAT vs pericardial fat in this regard means that results may not be readily extrapolated between studies (561). Indeed, one of the limitations of the study in Chapter 6 is that some EAT samples may have included paracardial fat owing to the practical challenges of harvesting adipose tissue intimate with the myocardium and discerning fascial separations by eye.

7.4. Future directions

7.4.1. Quantifying adipose volume and its distribution to predict cardiovascular outcomes

There has been growing interest the role of adipose tissue in the genesis of cardiovascular disease, fuelled by the rising prevalence of obesity in the UK and internationally (672). Indeed, the expansion and increasing availability of data from national biobanks has made it possible to quantify adipose volume in large numbers of individuals and relate this to health outcomes. For example, Bard et al developed a fully automated convolutional neural network-based tool to quantify pericardial fat from cMRI data amongst the UK Biobank imaging cohort (491). This enabled the authors to acquire pericardial fat volumes from over 40,000 individuals that was validated against standard reference CT measurements. This is particularly advantageous given that it overcomes the arduous task of manually segmenting regions of adipose tissue on cross-sectional images and can be acquired from a non-ionising imaging modality. Furthermore, the tool can be implemented on standard four-chamber cine images which are routinely acquired during standard cMRI protocols, and therefore can be applied to other MRI datasets aside from the UK Biobank. Applying this tool to the UK Biobank population, Ardissino et al showed that pericardial adiposity is associated with adverse cardiovascular function, even after PCA and mediation analyses accounted for concurrent co-morbidity, traditional cardiovascular risk factors, serum inflammatory markers and anthropometric indices of systemic obesity (492). Specifically, higher pericardial fat was associated with poorer LV structure (greater wall thickness, greater mass and concentric hypertrophy) and LV function (lower global function index and stroke volume),

reduced left atrial ejection fraction and reduced aortic distensibility (492). The authors of the study suggest that adipose quantification in cross-sectional imaging may constitute a novel biomarker to assess cardiovascular risks. Whether pericardial fat volume quantification using this tool can be integrated into standard cMRI protocols represents a current line of enquiry. This adipose quantification tool may also be implemented as a risk predictor for incident arrhythmias and other cardiovascular endpoints.

Similar to the rationale for the study by Ardissino et al (492), neural network models have been developed using almost 40,000 resting 12-lead ECGs from the UK Biobank to identify overweight and obese individuals from lean counterparts (321). In this study, a neural network-predicted BMI that was greater than clinically measured BMI was indicative of underlying visceral adiposity and cardiometabolic disease and therefore may serve as a cheaper and more accessible screening tool to identify individuals at higher risk of ill-health than MRI (321). An extension of this work would be to delineate the determinants of predicted-BMI and investigate how this correlates cardiac structure and function using MRI data. Similarly, a comparison of predicted- and calculated-BMI related cardiovascular outcomes, for example, incidence of myocardial infarction, stroke and arrhythmias, is currently work in progress.

Recently, leveraging genetic data to understand the genetic basis for body fat distribution, Agrawal et al identified 250 SNPs associated with adiposity, of which 39 were novel, amongst 40,000 individuals from the UK Biobank imaging cohort (506). They showed that common variants in the PPARG gene were associated with a

reduction in gluteo-femoral fat, a SAT depot, that conferred a higher risk of cardiometabolic disease and inactivating variants in the PDE3B gene had the opposite effect. Indeed, using a PRS in a similar way to the UK Biobank study herein, they also show that a genetic predisposition for accumulating visceral rather than subcutaneous adiposity conferred higher risks of diabetes, dyslipidaemia, hypertension and coronary artery disease (506). Extending this to include cardiac arrhythmias and related-outcomes would be an interesting line of enquiry, and other variant-to-function studies may yield better understanding of fat distribution and its relation to pathophysiological processes.

7.4.2. In silico modelling of atrial and ventricular arrhythmias using ECGi data

The standardised framework for signal processing presented in Chapter 4 will be shared with the ECGi community to enable others to adopt these methods in future studies in due course. Furthermore, the ECGi data recorded from obese and lean individuals is being pooled with a larger dataset in a collaboration with Matthijs Cluitmans and Job Stoks (Maastricht University) to validate the methods described herein with contact mapping data. Given that it was not possible to study arrhythmic incidence in the ECGi study, the results presented in Chapter 5 may be used to develop in silico models, as described in other studies (38, 558, 607, 670), to test the hypothesis that atrial and ventricular arrhythmias are less likely to be initiated and sustained in the hearts of obese patients post- vs pre-bariatric surgery, and also compared to non-obese hearts. For example, Vigmond et al have described computational methods for modelling electrical activity in cardiac tissues and these may be applied to the clinical and in vitro data contained in this thesis (673).

ECGi enables beat-by-beat reconstruction of activation and repolarisation patterns. This is particularly helpful to study atrial and ventricular ectopy. Although patients with frequent premature ventricular contractions exhibit mechanical and function abnormalities of the left ventricle in the preceding sinus beat (674), there is an absence of electrophysiological characterisation of the same. Performing ECGi on ectopic and their preceding beats amongst the obese cohort to study how patterns of activation and repolarisation change pre- vs post-bariatric surgery, and compare these to non-obese hearts, to test the hypothesis that ectopy in obese hearts are more likely to be proarrhythmic could be another insightful endeavour.

7.4.3. The effect of pharmacological- vs surgical-weight loss on reverse electrophysiological remodelling

The rationale for investigating the effects of surgical weight loss on cardiac electrophysiology was partly based on the fact that bariatric surgery is most likely to result in the greatest change in weight than lifestyle or pharmacological modulation. Orlistat is the only pharmacological agent recommended as an adjunct to lifestyle modification in the NICE guidelines for obesity management (541). Its effects on mitigating incident arrhythmic risk or reducing arrhythmic burden is not well described. Similarly, although GLP-1RA have been approved as weight reduction agents for obesity in the USA, their effects on reverse electrophysiological remodelling and incident arrhythmias in the context of weight management are unknown. Future studies may consider utilising ECGi to compare the effects of pharmacological weight reduction interventions on atrial and ventricular electrophysiology between obese groups, as well as before and after weight reduction, and compare these results with those presented in Chapter 5. This would

provide interesting insights into the extent to which drug-induced weight loss reverses electrophysiological remodelling compared to bariatric surgery and may help to inform the healthcare resource allocation as well public health strategies.

7.4.4. Characterisation of epicardial adipose tissue from different cohorts

Considering the overlap between Chapter 6 and related studies (607), a comparison of EAT and SAT secretomes from patients with and without AF and their respective effects on NRVM conduction would be a natural progression of this work. Given that pharmacological modulation has been shown to alter EAT characteristics (63) and EAT modulates cardiac electrophysiology(190, 675), one of the outstanding questions is whether drug therapy can reverse the conduction slowing associated with EAT described in Chapter 6 by others (188, 607). Indeed, although EAT-induced fibrosis may be mitigated by Activin A antibody in rat atria, its effects on cardiac electrophysiology were not described (226). The results presented herein show greater concentrations of Activin A in EAT vs SAT, therefore the corollary of the current evidence would be to test the effects of EAT-, SAT- and control-media on NRVM conduction alone and in combination with Activin A antibody, to test the hypothesis that the latter reverses conduction slowing when it is observed with former. Similar experiments may be conducted with HMG-CoA reductase inhibitors and cytokine (for example TNF-alpha and IL-6 antagonists) based on previous studies (63, 197, 486, 605, 614).

7.5. Conclusions

The work in this thesis presents evidence for adiposity-related electrophysiological remodelling across the bench-bedside-population spectrum. Notably, morbidly obese individuals without a history of cardiac arrhythmias exhibit subclinical electrophysiological, structural and functional remodelling that can be partially reversed with bariatric surgery. Mechanistically, obesity-related abnormalities may be partly explained by epicardial adiposity, and correspondingly, their reversal by the regression of EAT with weight loss. These findings concur with recent contemporaneous studies investigating the mechanisms underpinning cardiac arrhythmias and the adverse effects of EAT on cardiovascular outcomes, and incriminate obesity as an independent risk factor for atrial and ventricular arrhythmias. The results presented in this thesis support a timely and aggressive clinical approach for tackling the global obesity pandemic to mitigate the economic, psychosocial, and public health burden that it represents.

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Hari Aum Tat Sat

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