AN EXAMINATION OF THE ROLE OF ATRIAL STRETCH IN THE GENESIS OF ATRIAL FIBRILLATION AND THE ANTIARRHYTHMIC EFFECTS OF DIETARY FISH OIL

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1 Table of Contents

1 Abstract

This thesis is submitted as a PhD by portfolio of publications. It explores the role of atrial stretch in the pathogenesis of atrial fibrillation and the modulating effect of dietary fish oil.

Atrial fibrillation is more common in conditions associated with atrial stretch. This relationship is thought to be due to changes in activity of stretch-sensitive ion channels and alterations in calcium handling. Increasing atrial pressure in isolated rabbit hearts shortens atrial refractoriness and enhances the inducibility and sustainability of atrial fibrillation.

The first of the publications in this thesis[1] describes the effect of pericardial constraint on the isolated rabbit heart model which uses increasing atrial pressure as a surrogate for increasing stretch. Reproducing the original description of this model but with an intact pericardium, increasing atrial pressure did not result in the electrical changes seen with marked atrial dilatation. When the pericardium was removed, the relationship between increasing atrial pressure and susceptibility to atrial fibrillation was restored.

The second publication[2] reports the effect of streptomycin and intracellular acidosis on the rabbit heart atrial fibrillation model. Stretch-activated channel blockers gadolinium and Grammostola toxin have been shown to limit atrial fibrillation with stretch in the rabbit model. We further explored the role of the non-specific cation stretch-activated channel using streptomycin. Streptomycin reduced the stretch-related vulnerability to atrial fibrillation without altering the drop in refractory period associated with stretch. We proposed that the drop in refractoriness might be related to activation of stretch-activated potassium channels. These channels have also been shown to be sensitive to intracellular pH. We therefore investigated the interaction between intracellular pH and stretch in the induction of atrial

fibrillation. Intracellular acidosis, induced with propionate, amplified changes in refractoriness and inducibility of atrial fibrillation with stretch.

The third publication[3] examines the effect of dietary fish oil on the rabbit model of atrial fibrillation. Changes in membrane fluidity and fatty acid composition could alter the stretch response. We proposed that changing the phospholipid membrane composition could alter the mechano-electric feedback in this model. Comparing rabbits fed for 12 weeks with fish oil or sunflower oil supplemented diets, we reported protection from the stretch induced vulnerability to atrial fibrillation in the fish oil fed rabbits. This was associated with an increase in n-3 omega fatty acids in the atrial tissue which was reflected in changes in erythrocyte membrane composition.

The last publication[4] measured the effect of a 12 week dietary fish oil supplement on the heart rate variability of 46 overweight adults. This was a substudy of a larger randomised double-blinded placebo controlled study of fish oil and exercise on cardiovascular health. Frequency domain analysis was performed before and after the 12-week intervention. Fish oil increased the high frequency component of heart rate variability in keeping with increased parasympathetic activity and improved autonomic function.

The outcome of this research has been to further the understanding of the complex interplay between stretch and atrial arrhythmias and to raise the possibility of using dietary fish oil to treat atrial fibrillation.

2 Signed Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holders of those works.

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Lastly I would like to dedicate my thesis to my wonderful wife, Kathryn and my three little distractions from writing: Joseph, Jeremy and Sarah.

Melius tarde, quam nunquam.

4 Statements of Contributions of Jointly Authored Papers

5 Contextual Statement

5.1 Background

5.1.1 Atrial Fibrillation

Atrial fibrillation remains the most common arrhythmia encountered in clinical practice and it has been recognised as one of the modern epidemics of this century. Closely related to the aging population and increasing obesity, it is contributing to the escalating cost of health care[5, 6]. Despite recent advances in the management of atrial fibrillation (including pulmonary vein isolation, complex left atrial ablation and new antiarrhythmic drugs), there are still major gaps in our understanding of the pathophysiology of this increasingly common arrhythmia. The relationship between atrial size and atrial fibrillation has been long recognised and in populations studies atrial size is a predictor of atrial fibrillation[7]. Increased atrial pressure and size are closely associated with atrial fibrillation both in acute illness (e.g. acute pulmonary embolus or myocardial ischemia) and chronic conditions (e.g. mitral valve disease, hypertension and heart failure). It has been suggested that the increase in obesity and resultant hypertension, glucose intolerance and vascular stiffness could help to explain the increasing rates of atrial fibrillation, even after adjusting for the aging of the population[8].

5.1.2 Mechano-Electric Feedback

The process of mechano-electric feedback, whereby mechanical forces applied to cardiac tissues can impact on the electrical properties, has been well described in animal models and is also evident in humans. Although one of the earliest clues that chamber stretch could influence cardiac electrical activity described a change in heart rate with alterations in atrial filling[9], much of the early research in this area focused on the possible role of stretch in the development of malignant ventricular arrhythmias. More recently it has become clear that these factors are also at play during atrial arrhythmias.

The effect of acute stretch on the heart appears to be dependent on the timing and the nature of the stretch, with changes in membrane potential and action potential duration recorded at a cellular level[10] and in isolated hearts[11]. In isolated hearts and in-situ animal models, acute stretch has been shown to induce depolarisation (triggering ectopic beats) and shortening of the monophasic action potential with consequent reduction in refractory period[12-14] and slowed conduction (providing the substrate for arrhythmia). The action of stretch-activated channels and altered calcium handling[15] are the most likely cellular explanations for these alterations in electrical activity with acute stretch. In situations of chronic atrial stretch, more complex mechanisms are likely to be involved.

5.1.3 Stretch-Activated Channels in the Atrium

Stretch-activated channels were originally described in chick skeletal muscle[16] and have subsequently been described across organ systems and between species. Despite their ubiquitous expression, their role in normal mammalian cardiac physiology and in disease remains unclear. Non-selective stretch-activated ion currents have been recorded in human atrial myocytes[17] and 2-pore K^+-selective stretch-activated channel expression has been reported in human heart tissue[18, 19]. While the focus of research in this area is the response to stretch of the atrial myocyte, these cells do not work in isolation with important communication across gap junctions to surrounding myocytes and fibroblasts. Cardiac fibroblasts also possess stretch-sensitive currents and the interplay between cardiac myocytes and fibroblasts adds another layer of complexity to the picture[20].

In isolated guinea-pig hearts, atrial stretch induced afterdepolarisations and changes to the monophasic action potential[21]. The stretch-activated channel blocking agents, gadolinium and streptomycin (but not calcium antagonists) limit the stretch-related proarrhythmic effects. In the isolated rat atrium[22], gadolinium blocked the afterdepolarisations and action potential changes related to stretch. In the isolated guinea pig heart, streptomycin

suppressed afterdepolarisations[23] and modified the stretch induced changes in the monophasic action potential[24].

Attempts to investigate this phenomenon in large animal experiments and in humans have yielded conflicting results, with both shortening and prolongation of atrial refractoriness being reported with atrial stretch and volume loading. The different models and approaches used, including volume loading[25-27] and dual chamber pacing[28-33], made reconciling these data difficult and the role for atrial stretch in atrial arrhythmogenesis remained unclear.

5.1.4 Acute Stretch and Atrial Fibrillation

Increased atrial pressure and the resultant atrial stretch were shown some time ago in animal models to contribute to the initiation and maintenance of atrial fibrillation[34]. Antoniou *et al* studied a group of patients with lone atrial fibrillation during high and low atrial pressures using acute changes in fluid loading[35]. They found it was easier to induce atrial fibrillation and that the atrial fibrillation was more sustained during higher atrial pressure. Acute changes in atrial electrophysiology have been recorded following the drop in atrial pressure with mitral balloon commissurotomy for mitral stenosis[36] and also spontaneously during atrial flutter [37] and with non-invasive manoeuvres[38]. Several groups have tried to demonstrate acute changes in atrial electrophysiology in humans during short-term dual chamber pacing with conflicting results[28-30, 32, 33].

Contemporary models of atrial fibrillation consider the interaction between the triggers of the arrhythmia and the characteristics of the atrial tissue (substrate) that serve to perpetuate it. The predominate triggers are thought to be rapidly firing ectopic atrial beats originating in the pulmonary veins, while the proarrhythmic substrate factors include shortened refractoriness, slowed conduction and increased heterogeneity of refractoriness and conduction. The effect of atrial stretch on both the triggers and the substrate may explain the clinical association between elevated atrial pressure and atrial fibrillation.

Afterdepolarisations and stretch related changes in atrial refractoriness and conduction velocity are probably important in the initiation of atrial fibrillation during haemodynamic stress (e.g. acute heart failure or pulmonary embolism). More complex mechanisms explain the association between chronic atrial stretch (e.g. hypertension and chronic heart failure) and atrial fibrillation. In addition to the acute effects, chronic atrial stretch leads to changes in ion channel expression and other structural changes (such as fibrosis) that serve to perpetuate atrial fibrillation. Adverse electrical and mechanical remodelling of the atrium have been reported with chronic atrial stretch (both with and without atrial fibrillation) [39-42].

In light of an accessible animal model of acute atrial stretch, we chose to focus on the mechano-electric feedback associated with acute stretch and in particular, the effects of stretch on atrial refractoriness.

5.1.5 Isolated Rabbit Heart Model

The original paper describing the isolated rabbit heart model of atrial stretch and vulnerability to atrial fibrillation drew our attention to the role of stretch in the genesis of atrial fibrillation and in particular, the changes in atrial refractoriness[43]. It elegantly described a reduction in action potential duration and atrial refractoriness with increasing atrial stretch and showed that this correlated with the increased vulnerability to atrial fibrillation. In isolated Langendorff rabbit hearts, atrial stretch was controlled by manipulating atrial pressure after ablating the atrioventricular connection and inducing ventricular fibrillation. It was assumed that the electrical changes associated with increasing atrial pressure were related to atrial stretch rather than the elevated atrial pressure itself.

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5.2 Pericardial Constraint

In our attempts to reproduce the rabbit model in our laboratory, it became apparent that the electrical changes described with increasing atrial pressure could not be demonstrated while the pericardium was intact. The first publication in this thesis reports the experiments conducted to verify this observation. Although technically challenging, it was possible to perform these experiments with an intact pericardium if great care was taken as the hearts were removed and during the necessary dissection. The experiments were only possible if the pericardium was intact, as the atria would bulge through any defects if the pericardium was incomplete or torn.

The publication confirms that atrial pressure alone is not responsible for the vulnerability to atrial fibrillation in this model but that it relies on atrial stretch. This raises the possibility that the pericardium helps to regulate atrial dilatation and the electrical consequences of stretch in vivo.

The pericardium has been increasingly recognised as an important determinant of ventricular filling and it has been suggested that ventricular constraint may protect against the proarrhythmic effects of ventricular dilatation. The pericardium has been shown to alter the pressure-volume relationship in the atrium and it is possible that it also protects the atrium from the arrhythmogenic effects of atrial dilatation. This may explain some of the discrepancies in the literature comparing the results in isolated heart, open-chested and whole animal experiments and human atrial mechano-electric feedback phenomena.

5.3 Stretch-activated Channels and the Rabbit Model

In the first report of this rabbit model, Ravelli and Allessie suggested that the action of stretch-sensitive ion channels might explain their findings[43]. Others went on to show that this stretch induced vulnerability to atrial fibrillation can be modified using agents which are known to block stretch-sensitive channels[44, 45]. It is recognised that stretch alters many calcium handling processes in cardiac cells (e.g. the affinity of the contractile proteins for calcium), and that these alterations in calcium handling can in turn have electrophysiological effects via activation of electrogenic calcium transporters such as the sodium-calcium exchanger[46]. The relative contribution of stretch-sensitive ion channels and changes in calcium handling to mechano-electric feedback remains controversial. It is likely that they both play a role. In the isolated rabbit heart model, verapamil prevented both the stretch related drop in atrial effective refractory period (AERP) and inducibility of atrial fibrillation but also changed the AERP at baseline (with minimal stretch)[47].

Gadolinium, a potent blocker of stretch-activated channels, produced a dose dependent reduction in atrial fibrillation without a change in the stretch-related drop in refractoriness[44]. Both burst pacing-induced and spontaneous atrial fibrillation were prevented by gadolinium. These authors proposed that although gadolinium was a relatively non-specific drug with calcium channel and IKr blocking activity, stretch-activated channel blockade was responsible for this effect. There was no change in refractoriness with gadolinium, in contrast to the effect seen with the L-type channel blocker verapamil. They suggested that gadolinium may have exerted its effect by limiting the stretch related dispersion of refractoriness or suppression of arrhythmogenic afterdepolarisations. Previous work in rat atria had shown similar suppression of stretch related afterdepolarisations with gadolinium but not with diltiazem[22].

A subsequent study supported the proposition that stretch-activated channels were involved by employing the Grammostola spatulata tarantula spider toxin GsMTx-4[45]. This agent was chosen as it has a more specific effect on stretch-activated channels. GsMTx-4 had a similar effect to gadolinium in this model at concentrations that had been shown to have no effect on the action potential. This provided additional evidence that the non-specific cation stretch-activated channels contributed to the stretch induced vulnerability to atrial fibrillation through mechanisms other than changes in refractoriness. It was suggested that the potassium specific stretch-activated channels (such as TREK-1 and TRAAK) could underlie the changes in refractory period with stretch.

Speculating that the non-specific stretch-sensitive channels and potassium specific channels both contribute to the atrial fibrillation model, the second publication in this thesis reports the effect of streptomycin (a blocker of non-specific cation stretch-sensitive channels) and propionate (proposed to alter the activity of the potassium selective channel TREK-1) on this model.

5.3.1 Streptomycin

To further test to the proposition that the non-specific stretch-activated cation channel played a role, we applied another agent with stretch-activated channel blocking properties to the isolated rabbit heart model of atrial fibrillation. In the absence of a commercially available stretch-activated channel agonist or antagonist, we were limited to using agents that block these channels in vitro with relative specificity at particular concentrations. Streptomycin blocks the non-specific stretch-activated cation channels at concentrations from 40ug to 200ug[23, 24, 48-50]. We studied the relationship between atrial stretch, atrial refractory period and vulnerability to atrial fibrillation in 6 rabbit hearts at baseline, with 160microM streptomycin and again following washout. Following the addition of streptomycin, atrial fibrillation was more difficult to induce and was less sustained when compared to baseline. There was no significant effect of streptomycin on the drop in atrial refractory period with atrial stretch.

These findings were similar to the effect of gadolinium and the GsMTx-4 toxin and consistent with blockade of non-specific stretch-activated cation ion channels.

The success of pulmonary vein isolation in the management of paroxysmal atrial fibrillation shifted the focus from the body of the atria to the importance of the pulmonary vein- left atrial junction in the initiation and perpetuation of atrial fibrillation. In particular, the rapid atrial ectopics arising from this region can trigger paroxysmal atrial fibrillation and also contribute to the electrical atrial remodelling which can help sustain atrial fibrillation. It is possible that stretch in this region associated with acute changes in haemodynamics could explain the onset of atrial fibrillation at times of haemodynamic stress. Enlarged pulmonary veins have been described in hypertensive individuals at risk for subsequent atrial fibrillation, indicating a possible explanation for the link between hypertension and atrial fibrillation[51]. In the isolated sheep heart model, increasing atrial pressure was associated with more rapid and organised electrical activity emanating from the junction between the left atrium and pulmonary veins[52]. In a more recent study of isolated rabbit pulmonary veins, increasing tension was associated with more rapid spontaneous activity along with early and late afterdepolarisations in the veins. Interestingly, gadolinium and streptomycin both reduced the incidence and rate of spontaneous firing from these veins[53], indicating a role for stretch-activated channels in the genesis of pulmonary vein triggers for atrial fibrillation. This could explain the observation that these drugs suppress the spontaneous atrial fibrillation that often accompanies marked atrial stretch in the rabbit model.

5.3.2 Potassium Selective Stretch-sensitive Channels

Since it appeared that the stretch-activated channels blocked by streptomycin, gadolinium and GsTMx-4 were not responsible for the drop in refractory period with stretch, Bode et al suggested that potassium selective, stretch-activated channels (that are insensitive to gadolinium[54, 55]) could underlie this effect[45]. A number of the members of the two-pore-domain potassium channel family have been detected in the mammalian heart and the activity

of one stretch-activated member (properties consistent with TREK-1), has been described in both ventricular[56] and atrial[57] mammalian cardiomyocytes.

In patch clamp experiments, potassium selective, stretch-activated channels TREK-1 and TREK-2 can also be activated by intracellular acidosis[54, 58-60]. Intracellular acidosis and mechanical stretch act on these channels synergistically such that the stretch activation is amplified at low intracellular pH[61]. We reasoned that if TREK-1 is involved in stretch-induced vulnerability to atrial fibrillation, intracellular acidosis should amplify this effect.

5.3.3 Clinical Acidosis and Atrial Fibrillation

There was some observational clinical data to support the hypothesis that intracellular acidosis could predispose to atrial arrhythmia. Postoperative acidosis was identified as a risk factor for the development of atrial fibrillation following aortic valve replacement[62]. Acute atrial fibrillation has been reported in cases of acid ingestion and carbon dioxide inhalation with systemic acidosis[63-65]. Intracellular acidosis may also play a role in the atrial fibrillation associated with other causes of systemic (e.g. acute respiratory failure) and local acidosis (e.g. myocardial ischaemia). Animal models of atrial ischaemia have described reductions in AERP[66] with right coronary occlusion and slowing of conduction with isolated atrial ischaemia which promoted persistence of atrial fibrillation[67]. An inhibitor of the Na^+/H^+ exchanger prevented short term electrical remodelling with either rapid atrial pacing or ischaemia, indicating a possible role for intracellular acidosis in the maintenance of atrial fibrillation[66].

5.3.4 Acidosis and Stretch Related Atrial Fibrillation

In the second series of experiments in the second publication in this thesis, we investigated the interaction between stretch and intracellular acidosis in the rabbit model using propionic acid. Propionic acid produces a rapid, sustained and reversible drop in

intracellular pH (pHi) in isolated rabbit myocytes. Intracellular acidosis induced with propionate was associated with an amplified stretch-induced susceptibility to atrial fibrillation in the Langendorff rabbit model. The greater stretch-induced reduction in refractory period with propionate could underlie the changes in atrial fibrillation inducibility. This is consistent with enhanced activation of stretch-sensitive potassium channels at lower intracellular pH. This hypothesis was supported by the shift to higher dominant frequencies during atrial fibrillation with propionate.

The interaction of stretch and acidosis in the rabbit model is consistent with the properties of the tandem pore potassium channel, TREK-1. Others from our laboratory have reported the expression of two-pore stretch-sensitive potassium channels TREK-1 and TRAAK in human atrial cardiomyocytes[19]. Changes in TREK-1 activity might contribute to clinical atrial fibrillation and these channels could be a target for antiarrhythmic drug development. We plan to further investigate the effect of chronic atrial stretch on the expression of these channels in pressure and volume-loaded animal models. Unfortunately, a specific stretch-activated potassium channel blocker is not currently available to further define the role of these channels.

5.4 Antiarrhythmic effects of dietary fish oil

5.4.1 Fish Oil and Cardiovascular Disease

It has long been recognised that dietary fish consumption is associated with lower cardiovascular mortality[68-71]. Furthermore, both dietary modification to increase fish consumption[72] and dietary fish oil supplementation[73] have been shown to reduce cardiac events. In the largest study, dietary fish oil supplementation reduced cardiovascular death without reducing the infarction rates, implying an antiarrhythmic effect[74]. While the mechanism for this remains unclear, it is thought to relate to a lower likelihood of malignant ventricular arrhythmias following the uptake of n-3 fatty acids into the myocardial membrane[75]. The resultant changes in membrane fluidity and ion channel function due to this altered fatty acid profile could explain the observed antiarrhythmic effect. The acute administration of free n-3 fatty acids have been shown to have antiarrhythmic effects on isolated rat ventricular myocytes [76, 77 78] and human channels expressed in HEK 293 cells[79]. Others in our laboratory have previously reported the effects of dietary fish oil on calcium handling[80], which is another possible mechanism underlying electro-mechanical feedback in the heart.

5.4.2 Fish oil and Atrial Fibrillation

Following the observation that fish oil protected against sudden cardiac death, investigators focused on possible direct antiarrhythmic effects of fish oil on ventricular myocytes. While most research exploring the effects of fish oil on the heart have focused on the ventricles, it was likely that the fatty acids are also taken up in the atrium. The antiarrhythmic effect of n-3 fatty acids on isolated cells has also been demonstrated in atrial myocytes[81]. Previous work in our laboratory had demonstrated changes in cardiomyocyte calcium handling following dietary fish oil supplementation in the rat[80].

We speculated that the antiarrhythmic effect of dietary fish oil could reduce atrial fibrillation. Having established the rabbit model in the laboratory, we set out to demonstrate the uptake of dietary n-3 fatty acids in the rabbit atria and to test whether this conferred any antiarrhythmic effect in the stretch induced atrial fibrillation model. The third publication in this thesis[3] compares rabbits fed tuna fish oil (source of n-3 polyunsaturated fatty acids) with rabbits fed sunflower oil (a control polyunsaturated oil) over a 12 week period leading up to the Langendorff experiments.

5.4.3 Dietary Fish Oil Supplementation in the Rabbit

In contrast with the past experience in our laboratory of providing fish oil soaked pellets to rats, feeding rabbits a fish oil soaked chow proved more challenging. In our initial attempts, some rabbits refused the fish oil supplemented feed and they were supplemented with standard dry feed. Feed stored at room temperature or left in the feed troughs soon smelled like stale fish and the rabbits would refuse it. Recognising that oily fish are not part of the native diet of the average laboratory rabbit, it proved critical to prepare and store the feed to minimise oxidation. The successful technique involved soaking the pellets with fresh, human grade fish oil in nitrogen-gassed bags and immediately freezing it in airtight containers for later use. The feed needed to be changed daily to remove any residual pellets. The rabbits' dietary intakes and weights were closely monitored. When prepared in this way, all but one of the rabbits accepted the feed. Despite a number of attempts to keep this animal on the fish diet, it repeatedly refused the feed and we crossed this rabbit over into the sunflower oil arm and it immediately fed well. The final weights of the rabbits in the different groups were comparable.

5.4.4 N-3 Fatty Acid Profiles

There is limited data regarding the changes at a myocardial level following dietary intervention. Analysis of atrial fatty acid profiles were performed to confirm that the dietary intervention was sufficient to impact on atrial fatty acid profiles. Prior to killing the rabbits, blood samples were taken for erythrocyte fatty acid analysis. Following the Langendorff experiments, samples of atrium and ventricles were taken for tissue fatty acid analysis. The fatty acid profiles of the erythrocytes paralleled the atrial and ventricular tissue profiles, suggesting that erythrocyte fatty acid analysis could also serve as a marker of atrial fatty acids.

Harris et al reported an association between myocardial fatty acid levels and those measured from the erythrocytes of heart transplant recipients[82]. They suggested that an "Omega-3 Index" of erythrocyte n-3 fatty acids could be used as a non-invasive measure of cardiac omega-3 fatty acids and speculated that it might be an independent predictor of cardiac events[83]. This remains to be tested prospectively.

5.4.5 Dietary Fish Oil and Atrial Fibrillation in the Rabbit

Dietary fish oil supplementation conferred protection from atrial fibrillation in the rabbit model. This was associated with attenuation in the stretch related reduction in atrial refractory period. This suggested that the incorporation of n-3 fatty acids into the atrial myocardial membrane modulates the stretch-sensitive channel function. Future studies could target the underlying mechanisms to see whether these changes relate to altered expression or activity of stretch-sensitive channels or altered calcium handling.

5.5 Dietary Fish Oil and Heart Rate Variability

5.5.1 Heart Rate Variability

Heart rate variability is a non-invasive measure of cardiovascular risk which predicts mortality in patients with established heart disease[84-86] and in the general population[87, 88]. Reduced heart rate variability is associated with a higher cardiovascular mortality and sudden cardiac death, in particular. Measures of heart rate variability quantify the beat to beat variation in heart rate recorded during a specified period of time. Time domain parameters are usually applied to longer ECG recordings and frequency domain analysis is used for short term ECG recordings[89]. Results from autonomic stimulation and blockade experiments suggested that particular measures of heart rate variability are influenced predominately by sympathetic activity, parasympathetic activity or both. It is generally accepted that the high frequency component of heart rate variability reflects the respiratory variation in heart rate which is determined by parasympathetic activity (vagal tone)[90-92]. The low frequency component is thought to be predominately influenced by sympathetic activity[90] but is also influenced by changes in parasympathetic tone[91].

A number of diseases associated with increased cardiovascular risk (such as diabetes and renal failure) have reduced heart rate variability[93-95]. Several interventions shown to improve survival have been associated with improvements in heart rate variability[96, 97], suggesting that modulation of the autonomic nervous system could reduce cardiac arrhythmias and sudden cardiac death.

5.5.2 Autonomic Nervous System and Atrial Fibrillation

There is increasing evidence of the role of the autonomic nervous system in the pathogenesis of atrial fibrillation. Both sympathetic stimulation in disease states and excessive vagal tone in young healthy individuals have been implicated in the initiation of atrial fibrillation[98]. Increasing attention has been given to the innervation of the atria and pulmonary veins and the effects of ablation on these areas [99]. It has been suggested that some of the beneficial effects of left atrial ablation may be due to cardiac denervation[100]. Other interventions that modify autonomic tone could affect the incidence of atrial fibrillation.

5.5.3 Fish Oil and Heart Rate Variability

In population studies, adults with low dietary fish consumption and low n-3 fatty acid levels have lower heart rate variability[101-106]. In some populations, dietary fish oil has been shown to improve heart rate variability but the data are conflicting. Improvements in heart rate variability with fish oil have been shown in patients following myocardial infarction, chronic renal failure, nursing home residents and healthy men with low heart rate variability at baseline[104, 107-109]. In contrast, others did not show an improvement in heart rate variability with fish oil supplementation in dialysis patients, healthy subjects and following myocardial infarction[106, 110-113]. These conflicting results may be explained by differences in the oil dose, study populations and the measures of heart rate variability used. While it is recognised that heart rate variability is reduced in overweight adults[114], the effect of fish oil supplementation on heart rate variability in this population was unknown.

5.5.4 Fish Oil and Heart Rate Variability in Sedentary Overweight Adults

In the fourth publication in this thesis, we measured the effect of dietary fish oil supplementation and exercise on heart rate variability in a group of overweight adults. This was a substudy of a larger randomised, double blind, parallel comparison of the cardiovascular and metabolic effects of tuna fish oil supplementation with and without regular aerobic exercise for 12 weeks[115]. Subjects had a body mass index greater than 25 with additional risk factors for the development of future coronary disease. Heart rate variability measures were derived from ECG recordings taken before and after the 12-week intervention.

Dietary fish oil increased the high frequency component of heart rate variability in keeping with enhanced parasympathetic activity, while there was no effect on the low frequency component. This is consistent with the effect of fish oil on other populations with a shift to a more favorable autonomic balance. There was also a reduction in resting heart rate and heart rate with exercise with fish oil. This could be a direct cardiac effect due to altered membrane composition of the heart rate regulating cells of the sinus node in the atrium. Alternatively, this could reflect improved cardiac efficiency and enhanced baroreflex sensitivity through improved vascular function[115].

Obesity has been recognised as a risk factor for atrial fibrillation[116, 117]. The risk of atrial fibrillation increases with increasing Body Mass Index (BMI)[118], features of the metabolic syndrome[119] and the severity of associated obstructive sleep apnea[120]. We speculate that dietary fish oil supplements may impact on the rates of atrial fibrillation in this population by modifying the many cardiovascular effects of obesity, including autonomic dysfunction.

5.6 Conclusion

5.6.1 Significance of the Work and Future Directions

The work presented in the portfolio of publications has furthered our understanding of the way stretch contributes to atrial fibrillation and the possible role of dietary fish oil in the management of atrial arrhythmias.

The first publication raises the novel concept of the pericardium as a stabilising influence on the electrical activity in the atrium. We are interested in extending these findings to human studies, investigating the effect of pericardial closure following cardiac surgery on the incidence of postoperative atrial fibrillation. There are currently a number of devices that are being trialled for the management of heart failure which rely on passive ventricular constraint. This technology could be extended to the atrium in whole animal models of heart failure to test whether this has an impact on the inducibility of atrial fibrillation.

The second publication provides additional evidence for the role of both the non-specific cation and potassium-specific, stretch-sensitive ion channels in the stretch related vulnerability to atrial fibrillation. These channels represent a target for antiarrhythmic drug development. Further experiments with novel compounds, which are more specific agonists and antagonists of these channels, will help tease out the mechanisms behind this intriguing interaction between atrial stretch and atrial fibrillation.

The third publication was the first to report a change in atrial fibrillation inducibility as a result of a dietary intervention. In particular, this paper suggests that the antiarrhythmic effects of fish oil are not limited to the ventricles, which had been the chambers of interest in the past. The article provoked an accompanying editorial[121] describing the paper as an important first step in evaluating the complex protective role of dietary fish oil against stretch induced vulnerability to atrial fibrillation. Population based cohort studies of fish consumption and atrial fibrillation have yielded conflicting results[122, 123]. A subsequent study has confirmed the uptake of n-3 polyunsaturated fatty acids into human atrial tissue[124] and a small study has demonstrated a reduction in perioperative atrial fibrillation with dietary fish oil[125]. Large, well-designed human studies to further evaluate fish oil as a treatment for atrial arrhythmias are currently underway.

The fourth publication was the first to describe an improvement in heart rate variability in overweight adults with dietary fish oil. It adds to the body of evidence in favour of the cardiovascular benefits of dietary fish oil not only for those with established heart disease, but also those at risk of future coronary disease. Further work will aim to understand whether the improvements in basal heart rate and heart rate with exercise could be explained by changes in baroreflex sensitivity. We are also interested in expanding this work to further understand the inconsistencies in the literature of the effect of fish oil on the autonomic function of healthy adults.

5.6.2 Concluding Statements

The work presented in this thesis provides further evidence to implicate stretch-activated channels in the triggers and substrate responsible for the atrial fibrillation associated with atrial stretch. We speculate that the non-specific cation channels aggravate the triggers through calcium overload and afterdepolarisations, while the potassium specific channels may explain the stretch related drop in refractoriness contributing to the substrate for the maintenance of atrial fibrillation. The development of specific stretch-activated channel blocking agents will provide powerful tools for further investigating the role of stretch-activated channels in health and disease states to help us better understand the origins of this very common clinical problem.

The publications included in this thesis also introduce fish oil as one of the potential therapeutic options in the management of atrial fibrillation, particularly when associated with atrial stretch. This relatively inexpensive and well-tolerated treatment should be further evaluated in the treatment of atrial fibrillation in humans. With the percentage of the population classified as overweight and obese rising, dietary fish oil may also have a role in maintaining cardiovascular health, impacting on blood pressure, lipids and autonomic dysfunction.

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