Atrial Structure and Function in Non-ischemic Heart Failure.

DISSERTATION

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Abstract

For years researchers have looked for the perfect model to study heart failure (HF), and thereby understand its mechanistic causes and consequences. Among those models, there is one, rapid pacing, that has been used for decades because it recreates the process in weeks to months, as happens in human beings. Rapid pacing has answered several questions on mechanisms of cardiac remodeling during heart failure and how the body responds to it in terms of changes in hormones and proinflammatory mediators. In this study I established in a non-invasive manner the effect of time-dependent effects of heart failure on atrial depolarization time and how this is associated with atrial size and function (structural remodeling). At the in vitro level we examined the qualitative and quantitative effects of HF on atrial connexin 43. Atrial functional and structural remodeling during HF, contributes to the generation and maintenance of the most common sustained arrhythmia found in human beings, atrial fibrillation.

In this study, HF caused electrophysiological remodeling (properties of the P wave), structural remodeling (LA enlargement), and dynamic functional remodeling (tardy contraction of the caudal portion of the LA). These findings closely mimic observations made in human patients at risk for atrial fibrillation [1, 2].
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Chapter 1: Introduction.

1.1 Definition of heart failure.

As a general definition, heart failure (HF) is a syndrome resulting from the inability of the heart to pump an adequate cardiac output and/or sustain acceptable venous and capillary pressures. This syndrome is irreversible and it often develops over months or years. When clinical signs or symptoms develop from either the reduction of cardiac output or venous congestion, the state of HF exists [3].

1.2 Epidemiology of heart failure.

Cardiovascular disease is the most common cause of mortality in industrialized countries [4]. In the United States, cardiovascular disease was found to be responsible for 35.2% of the deaths in 2008 [5]. HF and the associated changes in heart structure are linked not only to reduced force of contraction, but also to reduced filling and to several types of arrhythmias (abnormal heart rhythms). Of the arrhythmias, atrial fibrillation is the most common sustained arrhythmia [6, 7]. Once congestive HF develops, patients typically have multiple hospitalizations that affect their social and economic life, as well as cause a heavy economic burden on the health care system [8].
1.3 Pathophysiology of heart failure.

Here I consider some aspects of HF in general. Details of heart failure induced by rapid pacing will be discussed later in this work.

The main function of the heart is to pump the requisite amount of blood per minute to supply all tissues of the body with oxygen and nutrients. When this is not achieved, either because cardiac output falls, or is maldistributed, there are associated clinical signs and consequently, the syndrome of heart failure/congestive heart failure is present.

1.3.1 Main causes.

HF may be caused by valvular disease, cardiac shunts, atherosclerosis, or cardiomyopathy, that results in either pressure and/or volume overload in the presence of remodeling (e.g., gross, histopathological, ion channelopathy) [9]. Systolic dysfunction, which is found in 40 to 60% of patients with HF, results from reduced contractility and/or increased hindrance to ejection into and through the systemic or pulmonary arterial trees. Diastolic failure occurs due to failure of relaxation and/or increased stiffness of the ventricle, resulting in reduced ventricular filling [10]. Abnormal diastolic function may be a manifestation of a reduced rate of sequestration of Ca$^{2+}$ from the troponin-C to the sarcoplasmic reticulum, or from frank stiffening of the muscle due to fibrosis, edema, or biochemical changes. Energetic imbalance—imbalance between O$_2$ delivery and utilization—is a common cause for ATP depletion and leads to alterations in both systolic and diastolic function, to ion channel dysfunction, and to arrhythmia [11].
1.3.2 Progression of HF.

Initially in HF, there is a decrease in the stroke volume due to reduced contractility, decreased preload, or increased afterload. Therefore, at the end of the heart beat there is hence a higher end systolic volume. When the volume added from the pulmonary circulation reaches the left ventricle there is a higher end diastolic volume. This should not be a problem in a normal ventricle since the Cyon-Frank-Starling [12] law of the heart states that an increase in end-diastolic volume increases the force of contraction and therefore the stroke volume, with a resultant return to or toward a normal end-systolic volume. However, a failing ventricle may not react in the same manner and the end-systolic volume may remain high [13]. This can result in an increased end-diastolic pressure that is reflected to the left atrium when the mitral valve opens during diastole. This increase in left atrial pressure elevates the pressure in the pulmonary circulation inducing pulmonary edema, pulmonary hypertension, right ventricular failure, and pulmonary failure [14-16]. Additionally, the reduction in the gradient between atrium and ventricle reduces the early ventricular filling. The subsequent increase in atrial pressure initially increases the early ventricular filling, but when the ventricle becomes stiff there is a reduction in the rate of both early and late ventricular filling [17].

Pressure and volume overload increase the heart’s external work reducing its performance, increasing oxygen consumption (determined by wall tension, contractility and heart rate), which is a crucial fuel for heart function [18]. When the left ventricular chamber has to deal with an increased amount of blood per heartbeat due to volume overload its response is to dilate to accommodate the increased volume. A dilated
chamber with more blood at the end of diastole has to deal with a higher pressure and a bigger diameter. Additionally, a dilated chamber has a thinner wall. Thus, we can say that a thinner wall has to face a higher preload and hence the tension (and oxygen demand) is increased [18, 19].

1.3.3 Neurohumoral responses.

HF also elicits neurohumoral responses that activate the renin-angiotensin-aldosterone system, an increase in production in antidiuretic hormone and the increase of circulating catecholamines [20, 21]. Additionally, the initial reduction in cardiac output is detected by the baroreceptors as low pressure and this induces an increase in catecholamine release and production. This increases heart rate and oxygen consumption, reduces diastolic time and coronary flow [22]. The elicited homeostatic responses sustain blood pressure at the cost of a tremendous effect on work efficiency [23].

1.4 Models to study heart failure in the laboratory.

In order to understand the different forms of HF that affect people and animals, there is a requirement to develop models to recreate the process in the laboratory [24]. Here, I will review different available models of heart failure.

1.4.1 Acute and chronic ischemia.

Different species are currently used to generate the model of acute ischemia. Different rodents, rabbits, dogs, cats, non-human primates and sheep are the most common species used [25]. Initially, rat models of HF were created by repeated injections of isoproterenol to cause diffuse necrosis of the myocardium, or by focal injuries by
electrocautery. Years later, Pfeffer et al. developed the coronary ligation model [24, 26]. The procedure includes surgically opening the chest and the ligature of the left anterior descendent coronary artery, circumflex artery or both. The ligature of the vessel could be permanent or temporary; with temporary models using a plastic tube that allows the removal of the suture and induction of reperfusion [27, 28]. Acute occlusion of a coronary artery can also be achieved by central catheterization and the use of inflatatable balloons. Some alternative models of ischemia include the induction of hypercholesterolemia by high intake of cholesterol in the diet in pigs and non-human primates to induce atherosclerosis. However, a limitation is that the location of the subsequent infarcts is impossible to predict [29-32], as it depends on the sites of atherosclerosis. An alternative model of chronic multifocal ischemia is induced by the injection of different amounts of polystyrene microspheres or thrombin and autologous blood with fibrin into the coronary arteries by central catheterization. Some authors report a requirement for 3 to 9 injections to induce a reduction of 35% percent in ejection fraction with injection of polystyrene microspheres [29, 33]. Mortality with this model ranges between 30% and 50%, [34] reducing the cost-efficiency of this model.

1.4.2 Pressure overload:

Pressure overload induced HF is achieved by chronic banding of either the pulmonary artery or aorta. This banding induces chronic increased afterload and ventricular hypertrophy [24, 35]. Pressure overload can also be achieved by means of bands placed on the aorta in young animals. The normal growth of the animal induces a
progressive restriction with a resulting increase in afterload, inducing ventricular hypertrophy [36]

1.4.3 Volume overload:

Different models of volume overload-induced heart failure exist. Volume overload can be induced by aorto-caval shunt, or by rupture of the cordae tendinae at the mitral valve to induce mitral regurgitation [37-39] Also, for the induction of pulmonary hypertension, volume overload combined with respiratory distress can be induced by meconium in the lower respiratory ways [40].

1.4.4 Toxins and drugs:

Some substances used as therapeutic drugs as well as known toxic agents can also be used to create models of heart failure. Among the substances used to induce HF by toxicity, doxorubicin and monocrotaline are the most common. [41, 42].

1.4.4.1 Monocrotaline:

Monocrotaline is a pyrrolizidine alkaloid that induces pulmonary hypertension and right-sided HF by selectively injuring the vascular endothelium of the lung inducing pulmonary vasculitis [43-45].

1.4.4.2 Doxorubicin:

This drug is commonly used for the treatment of sarcomas and a variety of carcinomas, [46, 47] which was observed to result in cumulative dose-related HF in patients [48]. In animal models, doxorubicin injection either systemically or directly in the coronary arteries can be used for induction of HF. It induces free radical formation
and lipid peroxidation, which contribute to apoptosis [48]. Doxorubicin injection generates an unstable heart failure with high mortality rates [42, 49]. Doxorubicin can also be mixed with microsphere injection to reduce the dose of both and the amount of injections required to induce HF.

Propranolol, saponin, imipramine and snake venoms can also be toxic to the heart. These substances have been used to create models of heart failure too [42, 43].

1.4.4 Tachypacing:

Tachycardia induced heart failure (TIHF) is a commonly used model for induction of dilated cardiomyopathy. It consists of the implantation of a pacing lead at the endocardium or epicardium in different locations in the heart. Once implanted, the lead is connected to a programmable pacemaker to increase heart rate to induce chronic tachycardia. There are various protocols that use different lead position, heart rates and durations of pacing [50-52]. In dogs, most commonly, the ventricles are the chosen for this model due to the fact that dogs develop atrio-ventricular blockade even at relatively low stimulation rates [53]. Although the dog is the most common animal used for this model, sheep, pigs and rabbits are also used [51, 53].

1.5 Model Used for this study:

The model used for my dissertation research is the TIHF model. TIHF is defined as the dysfunction of either the atria or ventricles or both induced solely by the chronic increase in heart rate [54]. TIHF recreates the process of congestive heart failure, a very
common syndrome in human beings affected with heart disease [55]. A brief description of the model is presented here:

1.5.1 History:

TIHF is a model that was developed by Whipple and collaborators at 1962 based on the observation that patients with tachycardia develop HF [51]. Initially, it was observed that after the induction of atrial tachypacing, signs of HF were generated in dogs. With the passing of time the model has been used to answer several questions about the progression of congestive HF in humans [8, 56]. TIHF facilitates its study because it imitates the progression of HF in humans that usually is developed in months and not in days like other models, such as toxin-induced HF. TIHF can be induced without major surgical procedures reducing the effect of surgery on the normal intrathoracic physiology, and induces several of the clinical manifestations of HF in humans such as ascites and pulmonary edema among others [51, 57].

Several studies have used the tachycardia induced heart failure model. However, most of the information published was generated after 2 to 8 weeks of ventricular tachypacing [58-60] despite data suggesting time dependence for left ventricular (LV) remodeling and HF reversibility after pacing stoppage in the TIHF model [61]. More recently, studies using 4 months of tachypacing have found that this amount of time is necessary to induce sustainable and irreversible heart failure and atrial remodeling [62].

Chronic tachypacing generates atrial fibrosis and electrophysiological changes. Sridhar et al found that 4 months of chronic tachypacing induces changes in atrial tissue that provides a substrate for sustainable atrial fibrillation. Those include reductions in
atrial electrical refractory periods at different pacing rates, reduction in action potential
duration and reduction in resting membrane potential. Also, the resting potential in cells
from sick hearts after 4 months of tachycardia-induced heart failure is less negative
compared to normal cells [62]. Calcium leak from the sarcoplasmic reticulum –
associated to weakening of contraction through depletion of calcium from the
sarcoplasmic reticulum (SR) - is also time-dependent. Belevych et al, found increases in
calcium leak from the SR that progress in parallel with the reduction of the contractile
function in hearts in the TIHF model. Also the RyR2 channels lose the ability to become
refractory with increase in calcium release from the SR increasing the arrythmogenic
potential [63]. Therefore the rate and duration of the heart failure induction are the most
important determinants of the onset and progression of the disease [64].

1.5.2 Pathophysiology of tachycardia induced heart failure:

In human patients, heart rate is an independent predictor of cardiovascular disease
[65, 66]. High heart rate is associated with a reduction in diastolic filling time and
increased shear stress with increased oxygen consumption and reduced coronary
perfusion, increased levels of circulating catecholamines and reduced elasticity of the
vessels [67]. This, induces endothelial damage and predisposes to ventricular dysfunction
and HF [66].

1.5.3 Effect on heart function.

The effects of chronic tachycardia on the heart include but are not limited to four-
chamber enlargement including biatrial hypertrophy, systolic dysfunction measured as
reductions in cardiac output and $V_{\text{max}}$ [17, 55, 68]. Diastolic function is permanently and irreversibly affected [52, 69, 70]. Ventricular filling pressures are increased [55]. At the vascular level, there is an increase in resistance [17].

1.5.4 Cellular damage.

Chronic tachycardia induces cardiomyocyte death, altered extracellular matrix with reduced myocyte attachment to the basement membrane. Cellular elongation and changes in alignment, which conduces to thinning of the walls and changes in contractile function [71, 72]. Studies in the TIHF model have determined changes in membrane $\beta$-adrenergic receptors. Among those, there is down regulation of $\beta_1$, reduced transduction, and reduced adenylate cyclase activity. These changes are related to the high sympathetic tone and contribute to reduced ventricular function [73, 74]. On the other hand, $\beta_2$ receptors seem to be increased during heart failure however its function is reduced compared to that of $\beta_1$ as they are relatively uncoupled from the G proteins [9]. Tachycardia-induced heart failure is also known to induce ventricular and atrial fibrosis, tissue apoptosis, inflammatory cell infiltration and cell death [75]. Atrial fibrosis as well as in the case of ventricular fibrosis is associated with arrhythmogenicity [76], and the extent of fibrosis is associated to an increased likelihood of atrial fibrillation occurrence [77, 78].

1.5.5 Neurohumoral responses.

Chronic tachycardia also induces changes in the neurohormonal response [79, 80]. Previous studies demonstrated that in the first days of tachypacing there is an increase in
atrial natriuretic peptide. However values went back to normal after heart rate was reestablished [81]. Early studies did not find early increases in serum renin values. [51, 82]. However, a model in sheep by Fitzpatrick and colleagues found increases in renin inducing activation of the renin-angiotensin system soon after the beginning of the induced tachycardia. They also found increases in atrial natriuretic peptide, angiotensin II, plasma aldosterone, norepinephrine, antidiuretic hormone, and cortisol. Importantly, in this 2-week model, values went back to baseline when pacing was stopped [81].

1.5.6 Common concerns about the model of TIHF.

Some researchers have debated the possibility that this model is somehow an ischemia model. This is based on the fact that chronic tachycardia reduces high-energy-phosphates due to the imbalance between energy supply and demand [83]. However, ischemia does not seem to be the responsible for the induction of heart failure in the TIHF model. Previous studies have demonstrated that troponins I and C are not increased even after 12 months of continuous pacing [61]. Furthermore, Wilson et al did not find increases in myocardial lactate extraction and myocardial arteriovenous O₂ difference [84]. Thus, this model is most consistent with a non-ischemic model of HF. Regarding the concerns about recovery from HF after cessation of rapid pacing, it is known that in patients and laboratory animals, normalization of heart rate induces improvements of systolic function in TIHF [52, 85, 86]. On the other hand, diastolic function remains abnormal demonstrating that permanent ventricular remodeling is present [70]. This phenomenon is also observed in the dog model as reported by several authors [56, 87].
1.6 Atrial conduction and P wave generation.

1.6.1 Structures involved

The initial depolarization that generates the impulse that ultimately depolarizes the atria originates in the sinoatrial node in the right atrium, more precisely between the crista terminalis and the intraatrial septum. The sinoatrial node is an isolated structure that is connected to the myocardium of the right atrium by four exit pathways that have been identified \[88, 89\]. From here, the electrical signal travels from the right atrium to the left atrium using different pathways. It has been recognized for years that the conduction between the right atrium (RA) and left atrium (LA) uses at least three different identified pathways \[90-92\]. Those are known as margin of fossa ovalis, coronary sinus ostial region and Bachman bundle (BB). Although it is recognized that the conduction from RA to LA uses any of the above-mentioned pathways or the combination of the three it has been determined that the preferential way for left atrial activation is the BB \[90, 91, 93-95\]. BB cells are highly conductive structures with electrophysiological characteristics similar to those in both the atrial myocardium and the Purkinje fibers. Of importance, the faster phase 0 leads to a velocity conduction of 166 cm/s \[96\]. Also, the BB tissue has a longer refractory period compared to the surrounding tissue \[96, 97\] making BB a potential substrate for reentrant arrhythmias at the supra ventricular level \[98\].
1.6.2 Definition of P wave

The conduction of electricity through the atria underlies the P wave on the surface electrocardiogram. The P wave is the recording of the electrical activity that conducts the depolarizing impulse from the sinoatrial node (SAN) or more exactly through the RA to and then through the LA (since the activity in the SAN cannot be recorded from the surface electrocardiogram). The period of time between the beginning and the end of the wave is called P wave duration. In human beings any prolongation of the P wave duration above 110 msecs is indicative of interatrial partial block, and suggests a reduction in the conduction velocity in the interatrial pathways [94, 97]. Several studies support the idea that alterations in the interatrial conductions are highly associated with supraventricular arrhythmias [94, 97]. For instance, it is thought that bifid P waves in the ECG represent complete blockade of conduction in BB, one of the inter-atrial conduction pathways [94, 99, 100]. This complete block in the BB induces a deviation of the impulse to use other pathways inducing changes in the depolarization vector[97].

1.6.3 Cellular response to the SAN stimulus.

But how are those impulses in the atria transmitted to produce a change in the isoelectric line in the electrocardiogram? As in any other part of the heart, conduction is dependent on several factors for it to be normal. A voltage gradient between the cell most recently depolarized and the cells downstream are required for the impulse to propagate [101]. However, for this to happen, an action potential has to be generated in a single cell or group of cells. The action potential starts with the movement of ions across the cell membrane through voltage-dependent channels changing the voltage from
negative to less negative and producing the rapid upstroke or phase 0 of the action potential [102]. Sodium channels are responsible for the initiation of this upstroke in the action potential in non-nodal cardiac tissues. Their normal function allows a normal sodium current, $I_{Na}$, after the threshold is reached for them to open [102]. The summation of the voltage reached in every single cell in the atria, the velocity of this impulse traveling from the SAN to the left atrium and the atrioventricular node and the angle from which this impulse is recorded determines the duration, amplitude and shape of the P wave.

1.6.4 Intercellular connections.

1.6.4.1 Structures.

At the level of the gap junctions in the intercalated disks where myocytes are electrically connected, connexins 40, 43, and 45 - the known cardiac isoforms of connexins -, are responsible for rapid and coordinated impulse propagation [103, 104]. Connexins are proteins present at gap-junctional channels in groups of six [105]. They form connexons and each gap-junctional channel is formed by two hemi-connexons, with adjoining myocytes having one hemi-connexon [106]. Connexin 43 is present in the atrium, ventricle and ventricular conduction system. It is the most common connexin found in cells from these regions [107]. Connexin 40 is found in the sino atrial and atrioventricular nodes. The function of connexin 40 is controversial as some groups suggest that it is responsible for the rapid conduction of the impulse [102] whereas others suggest that when the expression of connexin 40 is high relative to the total connexin signal there
is a reduction in the velocity of conduction in the atrium [105]. The function of connexin 45 is not clearly understood either [102]. It is found mainly in the sinoatrial and atrioventricular node [108, 109]. Connexin 45 forms low conductance channels in vitro and is located at the atrioventricular node, a place where the signal velocity is reduced [108, 110].

1.6.4.2 Remodeling of intercellular structures.

Gap-junctional channels as other structures are remodeled as a consequence of disease. Among those, atrial fibrillation remolds them mainly via changes in connexins [111, 112]. This remodeling means changes in the structure of the intercalated disk with redistribution of connexins to the periphery of the cell (lateralization) leading to altered impulse propagation and block [113]. This lateralization process leads to increased transverse velocity as seen after connexin 43 redistribution in a model of atrial fibrillation[112]. Interestingly, this same phenomena was not found in the ventricle despite changes in connexin 43 expression [114].

Another process affecting connexins is phosphorylation level. Phosphorylation of connexin 43 is a very important early step in incorporation in the plasma membrane. It regulates the localization, degradation and function of the connexin 43 [115, 116]. It also regulates protein trafficking and assembly as well as in impulse conduction [117]. Several kinases are responsible for this process to occur [118]. On the other hand, protein phosphatases such as PP1 and PP2A are responsible for connexin 43 dephosphorylation and their activity are increased during HF [119]. Dephosphorylated and redistributed connexin 43 does not incorporate in junction channels [106]. Thus, connexin
phosphorylation status is important in conduction, and is therefore important in the mechanism of reentry that maintains atrial fibrillation [120].

Intercellular conduction is also affected by fibrosis [121]. As mentioned before, atrial fibrosis is a consequence of tachycardia-induced heart failure. It also can be a consequence of aging, and clinical conditions such as heart failure and hypertension [122, 123]. This induces either global reduction of conduction velocity or localized reduced velocity in any of the conduction pathways that communicate between the atria. Of these, Bachman’s bundle is the most commonly affected [124, 125].

1.7 Atrial physiology.

Atrial physiology has been studied before with the use of markers chronically inserted at different portions of the atrium, echocardiography, magnetic resonance and pressure-volume loops among others [68, 70, 126-128]. It is known that the atrium modulates the movement of blood towards the ventricle in different phases. The first phase occurs when the atrium fills and expands during ventricular systole. A second phase passively allows the flow of blood to the ventricle during diastole, and a third phase that actively pumps blood to the already relaxed ventricle, activity that accounts for almost 15 to 20% of the cardiac output [129, 130]. The process can be summarized observing the atrial pressure volume loop and its relationship to the electrocardiogram (figure 1). There is an initial increase in volume and pressure followed by a reduction in pressure and a small reduction in volume corresponding to mitral valve opening and left ventricular suction. When P wave appears and contraction of the atrium is elicited, there is a rapid decrease in atrial volume and an increase in atrial pressure followed by a
reduction in atrial pressure and the beginning of a new cycle. A family of pressure-volume loops generated with the pressure generated per unit of left atrial change can be used to measure the elastance at the end of atrial systole. The slope of the linear regression line fitted with the end systolic atrial pressure volume indicates atrial elastance [70].

Figure 1. Atrial pressure-volume loop depicting the atrial cycle. During the filling portion of the cycle the loop is directed upwards and to the right. Passive and active emptying are represented by arrows going leftwards with an initial reduction in pressure and with a subsequent increase in pressure after the P wave.
1.7.1 Atrial function during heart failure.

There is an initial increase in pumping and reservoir induced by Cyon-Frank-Starling mechanism. This becomes less effective with the progression of HF and conduit takes precedence[131]. During HF, the increased filling pressure right before the contraction of the atrium reduces the atrial component of ventricular filling. This has been elucidated by measurements of the E/A ratio using echocardiography [132]. This seems initially to be a compensatory mechanism for the increased left ventricular filling pressure, that with the further decrease in ventricular function increases the wall stress and deteriorates the atrial function [133]. Patients with systolic heart failure usually have a dilated left atrium and that is a predictive parameter of outcome that is independent of left ventricular ejection fraction [134]. On the other hand, patients with diastolic heart failure have increased left atrial volume compared to normal subjects [135]. Interestingly, in the canine model of TIHF, after cessation of tachypacing decreased atrial function persists [70]. This is due to persistent diastolic failure after chronic tachypacing, which is unlike the systolic function, which improves when tachypacing is stopped [136].

1.8 Atrial fibrillation.

1.8.1 Definition and epidemiology:

Atrial fibrillation (AF) is defined as fast and disorganized electrical activity and contraction of the atrium[137]. It induces high atrial rates that are followed by fast and irregularly irregular contractions of the ventricle due to variable impulse conduction through the atrio ventricular node (AVN) [138]. AF is a common comorbidity in
patients with HF and in those patients the risk of death is increased 1.6 to 1.9 fold [139].

AF is the most common sustained arrhythmia in human patients [6, 139, 140]. As it is more prevalent in the elderly, its incidence is increasing as the population ages [140, 141]. Atrial fibrillation also can happen in patients without structural abnormalities (“lone” AF) or with any type of heart disease that causes changes in the atrial myocardium [142]. AF has been associated with predictive risk factors, including left atrial enlargement, increased left ventricular wall thickness, and reduced left ventricular fractional shortening [143]. All these are typical findings in patients with heart failure. Also, atrial fibrillation itself can induce HF by chronic rapid ventricular response during AF episodes. Some describe AF as a new epidemic of cardiovascular disease [144].

1.8.2 AF relation to HF

Data obtained from animal models have determined the relation between heart failure and atrial fibrillation. Previous studies showed that during HF the atrium suffers structural and electrical remodeling [62, 141]. That includes reduction in action potential amplitude, a less negative resting membrane potential and reduced refractoriness [6, 145]. All these changes resemble the ones found in the TIHF model in dogs by Sridhar et al [62]. They demonstrated a use-dependent reduction in refractory periods compared to normal dogs, reduction in membrane potential, a less negative resting membrane potential and decreased action potential duration at 50 and 90 percent repolarization [62]. Also, AF itself generates electrical changes in the atrium that allows its own perpetuation [146]. This process is referred to as “AF begets AF” [147]. The phenomenon seems to be associated to the decreased action potential duration primarily by the reduction in $I_{Ca}$. 

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induced as protection against the cytotoxic effects of Ca loading. The effect is also seen in some inward rectifier potassium currents [6].

1.8.3 Mechanism of AF.

AF is often considered to result from multiple reentrant arrhythmia circuits [148]. In normal circumstances, the impulse originating in the sinoatrial node is extinct due to the relatively prolonged refractory period that exists in the normal heart tissue. However under certain circumstances, the impulse may be blocked, travel in circles or reenter the affected area [102]. Reentry requires the presence of areas with different conduction velocities as well as some level of dispersion of refactoriness for reentrant arrhythmia origination and maintenance [149] (Figure 2). There is evidence that these differences are found between the atria. LA action potential duration (APD) is shorter compared to that in the RA. Also, the refractory period is shorter possibly due to a greater density of the delayed rectifier current $I_{kr}$ [150]. These differences in refactoriness can be responsible in part, of the role of the LA as “the director” of the AF. This scenario allows an impulse to travel the areas already repolarized faster and be blocked in the refractory areas. The impulse then travels the areas that now are repolarized and since the two portions of tissue are connected in a potential circuit, it can also return to the initial area when it is no longer refractory. This completes a circuit that spins around a reentrant loop. Several foci of reentrant impulses can be connected and many impulses can travel anterograde and retrograde in a certain portion of tissue [151]. The reduction in refractory period and a possible reduction in velocity conduction in the atrium create reduced (in size) wavelengths that can “fit” better in the atrium. This is important in the case of
enlarged atriums where even longer wavelengths can be supported [138, 152]. AF can also be generated by ectopic activity that triggers extra stimuli such as early or delayed afterdepolarizations from the atrium or the pulmonary veins [6, 153].

![Substrate for Reentry](image1)

**Figure 2.** Requirements for reentry. Left panel shows two of the requirements: dispersion of refractoriness and dispersion of conduction velocity. A. An action potential with a short refractory period and slow velocity conduction. B. An action potential with prolonged refractory period and faster conduction velocity. At the right side, an unidirectional block and an impulse that travels the area with short refractory period and the circuit is closed when the same impulse reaches the same area when its refractory period is over (figure courtesy of Dr. Cynthia Carnes).

1.8.4 Atrial function during AF.

During atrial fibrillation, contractility function is lost and cardiac output is reduced [154]. There is a decrease in atrial diastolic compliance [155]. Contractile functional reductions are more pronounced in longer periods of atrial fibrillation [156]. Atrial fibrillation per se increases the size of the left atrium and left atrium increase is highly associated to atrial fibrillation [157, 158]. Also, atrial fibrillation increases atrial fibrosis, a process associated with altered conduction, reduced function and increased left
atrial size [159]. As another manifestation of atrial remodeling, atrial dilatation due to atrial fibrillation is not completely reversible after cardioversion [160].

1.9 SUMMARY:

For years researchers have looked for the perfect model to study heart failure, and thereby understand its mechanistic causes and consequences. Among those models, there is one that has been used for decades because it recreates the process in weeks to months, as happens in human beings. Rapid pacing has answered several questions on mechanisms of LV remodeling over time during heart failure and how the body responds to it in terms of changes in hormones and pro-inflammatory mediators. In this study we aim to establish in a non-invasive manner the effect of rapid pacing on the atrial depolarization time and how this is associated with atrial size and function (structural remodeling) by evaluating these in a time-dependent manner. At the in vitro level we look to observe the qualitative and quantitative effects of TIHF on atrial connexins. Ultimately we want to study what HF-induced changes generated with the rapid pacing of the ventricle during four months resemble those that facilitate the generation and maintenance of the most common sustained arrhythmia found in human beings, atrial fibrillation.
Chapter 2: Materials, methods and studies.

2.1 Materials and Methods

14 healthy mature dogs (about 20 kg) were implanted with single lead pacemakers (Medtronic) with the generator implanted at the right side of the neck. The pacing lead was affixed endocardially at the apex of the RV under fluoroscopic guidance as previously described [61]. After 15 days of recovery from surgery, baseline (0 months) echocardiogram and ECG were obtained under butorphanol sedation (0.25 mg/kg IV). This was repeated once a month for four months. Four additional dogs with similar characteristics were not implanted and were used as controls for right atrial samples. Another group of four dogs was implanted with the same protocol but only followed for 1 month. Pacemakers were programmed to stimulate the right ventricular endocardium at 180 beats per minute for 4 consecutive months. Pacemakers were turned off during 15 to 20 minutes for electrocardiography and echocardiography collection and then turned back on immediately.

2.1.1 Studies.

2.1.1.1 Echocardiography.
For the echocardiographic study, a General electric Vivid 7 was used. Images were obtained using a 5 MHz probe. The 2 dimension (2D) echocardiography included evaluation from the right parasternal window in long axis view to evaluate left atrial end systolic area (LAES), left atrial end diastolic area (LAED) and diameters, as well as the percentage difference between maximum and minimum area (e.g. fractional area change [FAC]). The left parasternal window in apical view was used to evaluate right atrial area during ventricular end systole (RAES) and end diastole (RAED), and FAC. FAC was calculated based on the difference between end systolic and end diastolic area measurements as percentage of change (figure 3).

From the apical view in the left parasternal window, an image of the left atrium was also obtained. Tissue Doppler imaging and pulse wave analysis were used to evaluate the time of maximum velocity in the free wall of the left atrium, using the apical view (this part of the study will be discussed in a different chapter).

To evaluate left ventricular function and establish the presence or absence of heart failure, M mode echocardiograms of the short axis at the level of the papillary muscles was used. The analysis included: ejection fraction (EF) and left ventricular end diastolic diameter (LVIDd).
Figure 3. 2D echocardiography of left and right atria. Left picture shows long axis view of the heart used to measure left atrial area and diameter (left arrow). Picture in the right (arrow) shows the right atrium in apical view used to measure right atrial area.

2.1.1.2 Electrocardiography (ECG).

Orthogonal ECG was obtained with dogs in right lateral recumbence. A Ponemah amplifier and a Biopac system (Biopac systems, Inc.) were used. Leads obtained included I, II, III, aVF, aVL, aVR, V3 and V10. 4 consecutive minutes were measured. The sample rate was 1000 per second and no filter was used.

2.1.1.2.1 P wave duration.

P wave duration was measured using Acqknowledge version 3.9.1. (1992-2007, BiOPAC systems, Inc). Thirty consecutive P waves were measured one by one using the points crossing the isoelectric baseline to define the beginning and end of the P wave.
(Figure 4). Lead two was selected for measurements as it has higher amplitude P waves compared to the other leads.

Figure 4. P wave measurement. Points crossing the baseline were considered as beginning and end of the wave.

2.1.1.2.2 P wave dispersion.

For P wave dispersion measurement, 5 consecutive beats were measured in leads I, II, III, aVF, aVL, aVR, V3 and V10 (Figure 5). For this part of the study, only animals having good quality traces in all the leads were selected. At the end, only two time points,
0 months and 4 months were selected to be compared, and only six dogs had sufficient quality ECG traces in all of the leads.

Figure 5. Leads measured simultaneously to obtain values for P maximum, P minimum and P dispersion.
2.1.1.2.3 P wave amplitude.

P wave amplitude at lead II was measured from 100 consecutive beats using an EMKA IOX system (EMKA technologies, France).

2.1.1.2.4 P wave signal average.

P wave signal average (figure 6) was obtained by averaging 3 minutes of ECG in lead II. Only 11 animals had good quality traces of at least 3 minutes for all the time points. For this analysis Acqknowledge software was used (Acqknowledge version 3.9.1. 1992-2007, BIOPAC systems, Inc).

Figure 6. Signal averaged of 3 minutes of P waves. The figure shows the measurement of P wave duration after signal averaging.
2.1.1.2.5 Autonomic system status evaluation based on ECG parameters.

For the evaluation of the autonomic system status the time of conduction from the SAN to the ventricle (PQ), the time of conduction through the AVN (end of P to Q) were evaluated, time between beats (RR), and heart rate variability were used. Measurements and evaluations were as follows:

2.1.1.2.5.1 PQ and end of P to Q segments.

Besides the duration and amplitude of P wave, the duration of the interval from the beginning of the P wave and the end of the P wave to the Q wave were measured using an EMKA IOX system (EMKA technologies, France).

2.1.1.2.5.2 P-Q short-term variability.

P-Q short-term variability was evaluated using Poincaré plots. Values for P-Q duration and variability were averaged and differences established by the mean orthogonal distance from the points comprising the cloud to the diagonal.

2.1.1.2.5.3 RR and heart rate variability.

Heart rate variability was determined by the standard deviation of the RR interval. Heart rate variability was measured using an EMKA IOX station (EMKA technologies, France). Heart rate variability was assessed at baseline, 1 month and 4 months.

2.1.1.2.5.4 R-R short-term variability.

R-R interval short-term variability was evaluated using Poincaré plots. Values for P-Q duration and variability were averaged and differences established by the mean orthogonal distance from the points comprising the cloud to the diagonal.
2.1.1.2.6 Vector loops.

P wave vector loops (figure 7) were generated using P waves from leads I and II to evaluate the angle of atrial depolarization in the frontal plane. Lead I in the upright position and lead II multiplied by -1 were used to generate the loop. Acqknowledge software was used (Acqknowledge version 3.9.1. 1992-2007, BIOPAC systems, Inc). PixelStick calipers for Mac were used to measure size and angle (2012 Plum Amazing).

![Figure 7. Generation of a P wave vector loop using lead I and an inverted lead II (left panel). In the right panel a vector loop of the P wave.](image-url)
2.2.1.3 Connexin 43.

Samples from right atrial appendage from control dogs (n=4) and dogs at 1 (n=4) and 4 months (n=4) were obtained, frozen and processed for Western blot analysis for measurement of connexin 43 (total, phosphorylated and non-phosphorylated). Immunoblotting was performed as previously published [161]. Samples were processed and results were generated by Dr. Peter Mohler’s laboratory at the Ohio State University. Connexin 43 expression was examined in the right atrium (RA) at 1 and 4 months TIHF, and in normal controls (n=4 per group). Total expression, phosphorylated and non-phosphorylated connexin 43 was detected using rabbit polyclonal anti-Connexin 43.

2.2 Statistical analysis.

2.2.1 Echocardiography.

Areas at end of ventricular systole and end diastole as well as fractional area change for both right and left atria were averaged from 3 measurements obtained per individual per time point. Parameters were separately analyzed and compared using a one-way ANOVA for repeated measures (with the exception RA data in which unpaired data was used) with Bonferroni’s post-test using Graphpad Prism version 4.00 for Windows. Graphpad Software, San Diego California USA.

Left ventricular ejection fraction and diameters, were also measured and 3 values averaged per time point per individual. Statistical analysis was the same used for the atrial area and fractional area change. Differences were considered significant when p<0.05.
2.2.2 P wave duration.

Thirty values for each dog at each time point were averaged. For determining P wave duration differences at all time points, a one-way ANOVA with Bonferroni’s post-test was performed using Graphpad Prism version 4.00 for Windows. Graphpad Software, San Diego California USA. Differences were considered significant when p<0.05.

2.2.3 P wave dispersion.

Five values for each dog at each time point were averaged. The difference between the maximum and minimum duration in any of the leads measured was used as the P wave dispersion for each individual. Maximum and minimum P wave durations at 0 months and 4 months were compared. For P wave dispersion, maximum and minimum P wave durations were compared with Student’s t-test was performed using Graphpad Prism version 4.00 for Windows. Graphpad Software, San Diego California USA. Differences were considered significant when p<0.05.

2.2.4 P wave amplitude.

One hundred values for each dog at each time point were averaged. For determining P wave amplitude differences at all time points a one-way analysis of variance (ANOVA) with Bonferroni’s post-test was performed using Graphpad Prism version 4.00 for Windows. Graphpad Software, San Diego California USA. Differences were considered significant when p<0.05.
2.2.5 Other ECG values.

Differences in mean values at the different time points selected for P-Q, P-Q short-term variability, RR, heart rate variability and RR short-term variability, were evaluated using a one-way ANOVA with Bonferroni’s post test using Graphpad Prism version 4.00 for Windows. Graphpad Software, San Diego California USA. Differences were considered significant when p<0.05.

2.2.6 Connexins.

Differences in the total expression and phosphorylated and non-phosphorylated amounts of connexin 43 were compared using one-way ANOVA. P value less than 0.05 were considered significant.

2.2.7 P wave vector loops.

Values of length, width and angle were averaged for all time points and differences were evaluated using a one-way ANOVA with Bonferroni’s posttest using Graphpad Prism version 4.00 for Windows. Differences were considered significant when p<0.05.

2.3 Multi-variate -linear regression analyses

This model was used to evaluate inter-variable dependencies as a function of time in the same individuals. Independent variables used were left atrial end systolic area, left atrial fractional area change, left ventricular internal diameter at diastole and ejection fraction. Dependent variables were P wave duration and amplitude. Interactions between time and predictors were tested.
The model used was as follows:

Longitudinal models using data from all time points.

a. Outcomes (2): P-wave duration, P-wave amplitude.

b. Primary predictors (3): LAES, LA FAC, LVIDD

c. Secondary predictor (1): EF

We aimed to answer the question: Is there a univariate relationship between the degree of heart failure and atrial function and P wave duration and/or amplitude? If so, does the relationship change over time?
Chapter 3 Results

3.1 Electrocardiography.

3.1.1 Effect of HF on P wave duration.

3.1.1.1 Manually measured P waves.

Initially, results for measurements obtained using manual measurements are presented. A plot comparing the mean values and standard deviation at the monthly time points is presented for all the parameters explored in this study. Figure 8 shows the increase in P wave duration observed as a consequence of TIHF. A statistical increase from baseline was found at 2 months and persisted through 4 months. An additional increase was seen after 2 months reaching a plateau value at 3 month.

3.1.1.2 Signal-averaged P waves.

Figure 9 shows P wave duration values measured from the signal-averaged ECG. The figure demonstrates prolongation at all the time points compared to those obtained with non-automated measurements. However the statistical differences between 0 and 2, 3 and 4 months is preserved as shown.

3.1.2 Effect of TIHF on P wave amplitude.

The P wave amplitude values (about 1 minute) generated automatically showed a statistically significant increase in the amplitude beginning at 1 month of TIHF, which
remained significantly increased relative to baseline through 4 months of TIHF (Figure 10).

3.1.3 P wave dispersion.

As shown in figure 11 the dispersion of the P wave duration was increased in dogs at 4 months of TIHF compared to the same individuals before starting pacing. P wave maximum (figure 12) and minimum (figure 13) duration were also prolonged in dogs at 4 month of TIHF. As explained before, only 6 dogs had sufficient quality traces in all leads and thus, only 0 months and 4 months were included for this reason.

3.1.4 PQ

3.1.4.1 PQ duration.

No differences were found in the PQ duration at any of the evaluated time points. PQ duration data was shown in Figure 14.

3.1.4.2 PQ short-term variability.

As mentioned, no changes were found in the PQ segment duration at any of the time points evaluated. However, when Poincaré plots (figure 15) were used to evaluate the short-term variability (STV) of PQ during heart failure, statistical differences were found. Figure 16 shows the difference between 0 months and the different time points evaluated. A clear reduction in variability was present at all time points evaluated consistent with an increase in the adrenergic tone.
3.1.5 RR and Heart rate variability.

With the intention of evaluating the effect on TIHF on the autonomic tone, HR and RR variability were evaluated. HR variability and RR values were compared at 0 months, 1 month and 4 months and no statistical differences were found. However when RR was compared using Poincaré plots we found a reduction in short-term variability at 4 months TIHF compared to baseline. Figure 17 shows the comparison of mean values at the different time points evaluated. Once again this reduction in variability could be attributed to an increase in the adrenergic tone and its effect on the heart rate and the rate of discharge of the SAN.

Figure 8. P wave duration is prolonged during chronic non-ischemic TIHF. No Difference was found at one month compared to baseline. * P < 0.05 vs. baseline (time 0) and 1 month. # P< 0.05 vs. 2 months.
Figure 9. Signal average of the P wave duration is prolonged after 2 months of TIHF. P waves are measured using the signal averaged P wave over 3 minutes on lead II. * P < 0.005 vs. baseline (time 0). # P < 0.05 vs. one month.
Figure 10. P wave amplitude increased after 1 month of TIHF with no further increase or decrease in its value. * P < 0.001 vs. Baseline (time 0).
Figure 11. P wave dispersion was increased at 4 months of TIHF compared to 0 months.

Figure 12. Maximum P wave duration was increased after 4 months of tachycardia induced heart failure.
Figure 13. Minimum P wave duration was increased at 4 months of TIHF.
Figure 14. P-Q duration at baseline (0 months) did not differ after 1 month and 4 months of heart failure.
Figure 15. Short-term variability of the PQ. An example of the short-term variability of the PQ interval in a representative dog. Baseline (0 months), 1 month and 4 months are compared. Y axis shows PQ in milliseconds and X axis shows PQ-1.
Figure 16. PQ short-term variability is reduced at 1 month of TIHF, and remains significantly reduced through 4 months of TIHF. * \( P < 0.05 \) vs. baseline (time 0).
3.2 Echocardiography.

3.2.1 Left atrium

The left atrial area measured at the end of ventricular diastole (Figure 18) was unchanged after one month, but increased after two months of TIHF relative to baseline. Time-dependent increases occurred between months two and three, with no further increase after month three. Left atrial end systolic area (figure 19) was also increased at two months of TIHF but not difference between two and three months was found. However, a further increase was detected at four months compared to two months.

Figure 17. Short-term variability of the RR is reduced at 4 months. * P < 0.05 vs. Baseline.
Fractional area change of the left atrium (Figure 20) was reduced significantly at 2 months of TIHF. After the second month, a further decrease occurred at 3 months, which was sustained through 4 months.

Changes in left atrial end systolic and end diastolic diameters paralleled the changes in areas. Statistical differences were observed at 2 months and plateau values were reached at 3 months of TIHF (Figures 21 and 22).

Figure 18. Left atrial area at the end of diastole was increased at 2 months of tachycardia-induced heart failure. *P <0.05 vs. baseline (time 0). # P< 0.05 vs. 1 month. + P< 0.05 vs. 2 months.
Figure 19. Left atrial area at the end of systole (maximum area) was increased at 2 months of TIHF. * P < 0.05 vs. baseline (time 0). # P < 0.05 vs. 1 month. + P < 0.05 vs. 2 months.
Figure 20. Left atrial fractional area change (FAC), a measure of atrial function, was progressively reduced by heart failure. * P< 0.05 vs. baseline (time 0). # P< 0.05 vs. 1 and 2 months.
Figure 21. Left atrial diameter at the end of ventricular diastole was increased at 2 months of TIHF. * P < 0.05 vs. baseline (time 0). # P < 0.05 vs. 1 month.
3.2.2 Right atrium.

End diastolic right atrial area was not increased at any time point during the four months of TIHF. A decrease in the area was found at 1 month. In a similar manner, no differences were found in end systolic area and fractional area change at any time point evaluated during the 4 month duration of TIHF (Figures 23 to 25). Figure 26 shows the differences in the percentage change in area size over time when end systolic and end diastolic areas from LA and RA are compared.
Figure 23. Right atrial end diastolic area was not increased at any of time during four months of HF. A small but statistically significant decrease was found at 1 month of TIHF (* P < 0.05 vs. 4 months).
Figure 24. Right atrial end systolic area. No heart failure-induced differences were found in ES area in the right atrium during 4 months of heart failure.
Figure 25. Right atrium fractional area change. No heart failure-induced changes in RA fractional area change were found during 4 months of HF.
Figure 26. Comparison of right vs left atrial areas change (Top: end of diastole. Bottom: end of systole) during TIHF. Note that only left atrial area changes were seen *P < 0.05 vs Baseline.
3.2.3 Ejection Fraction.

Ejection fraction as an indicator of left ventricular function was also evaluated. Figure 27 shows its decrease at one month of TIHF with no further change. Thus, LV function was stably reduced starting at one month for the duration of the study.

![Graph showing ejection fraction over time](image)

Figure 27. Left ventricular ejection fraction was reduced by approximately 50% after 1 month of TIHF with no further time-dependent decrease through 4 months of heart failure. * P < 0.001 vs. baseline (0 months).

3.2.4 P wave vector loops

The angle of P wave vector loops were generated and measured at all time points. Figure 28 shows that vector loop angles of the P waves did not differ at any time point.
3.3 Connexin 43 expression and phosphorylation measurement.

Connexin 43 total expression was unchanged (figure 29) at both 1 and 4 months TIHF, but there was reduced phosphorylated Connexin 43 at 4 months of TIHF (Figure 30).

P wave vector loops were unchanged during four months of heart failure. No difference in the angle of P wave vector loops was found at any time point.
Figure 28. Total expression of connexin 43 was not reduced at either one or four months of heart failure. However, phosphorylation of connexin 43 was reduced at 4 months of TIHF (p<0.05). Data was normalized to expression from GAPDH and obtained from 4 dogs at each time point.

Figure 29. Phosphorylation of connexin 43 was reduced at 4 months of TIHF (p<0.05) compared to controls or to one month of HF.
3.4 Linear regression.

When tested for the interaction between time and the main independent variable (LAES area, LA FAC, LVIDd and LVEF) there was a significant interaction between time and LA FAC ($p<0.0001$). The interaction between time and LAES was also significant ($p=0.0209$). The other predictors were not significantly time-dependant at $\alpha=0.05$ level. This means the relationship between these predictors and the outcome variables was not significant over time.

LA FAC, when compared to P wave duration over time (Figure 42. see appendices) was initially positive, which could be interpreted as when P wave duration increases, LA FAC also increases. However, at 2, 3 and 4 months the relationship becomes negative meaning that when LA FAC increases, P wave duration decreases. On the other hand, the relationship between LAES area and P wave duration is positive at all time points (Figure 41. see appendices). This means that an increase in LAES is followed by an increase in P wave duration.

The same models used to determine the relationship of P wave duration were applied to P wave amplitude. No relationship between P amplitude and any predicted variable was found indicating that none of the parameters contributed to modulation of P wave amplitude (Figure 43. see appendices).
3.4.2 Left ventricular internal diameter at diastole (LVIDd).

Figure 31 shows the increase in LVIDd at 1 month of TIHF. Values kept increasing in a time dependent manner at month two, reaching a maximum value at 4 months. Figure 32 shows the correlation between LVIDd and LA ES (maximum area).

Figure 30. Left ventricular internal diameter at diastole increased after 1 month of TIHF. * P < 0.05 vs. Baseline (time 0). # P < 0.05 vs. 1 month. + P < 0.05 vs. 2 month.
Figure 31. Correlation between LVIDd and LA ES.

LVIDd vs LA area size n=14

$r^2$ 0.53

$p<0.0001$
Chapter 4. P wave morphology

4.1 Materials and methods.

The morphology of P waves was classified as either monophasic or bifid. Monophasic waves are those with only one spike, bifid waves are those with two spikes or a notch in the P wave. The bifid P waves found in dogs at 0, 1 and 4 months of TIHF were evaluated. In dogs with variable morphologies of P waves (bifid and monophasic in the same ECG) three P waves of each were measured for duration and amplitude (Figure 33). The RR interval including the P waves measured was also measured. For this, Acqknowledge software was used (Acqknowledge version 3.9.1. 1992-2007, BIOPAC systems, Inc). The differences in RR intervals between the segments including bifid vs. monophasic P waves were compared using a T student’s test with Graphpad Prism version 4.00 for Windows. The amplitudes and duration between monophasic and bifid P waves were also compared using T student’s test. The duration of the two portions of the bifid P waves were also compared using Student’s t-test.
4.2 Results.

From the 14 dogs included in the study 8 (57%) presented with bifid or notched P waves at 4 months of TIHF during the 4 minutes of ECG. In two of them there were only bifid P waves (no monophasic P waves found). In the other 6 dogs with bifid P waves, a mix of bifid and monophasic P waves were found. At 0 months there were 4 dogs (28%) with mixed morphology P waves. At 1 month there were 8 dogs with mixed morphology P waves. Fisher’s exact test was used to establish differences in the proportion of dogs with bifid P waves at 0 and 4 months (healthy and HF dogs). No statistical difference was found.

Bifid P waves were divided into two equal duration parts. There were no statistical differences in amplitude between the two portions.
No intra-individual differences were found in the duration between bifid and monophasic P waves after averaging them (Figure 35).

Bifid P waves occur when heart rate (assessed by RR) is slower compared to the RR in which monophasic P waves occur (Figure 34).

The amplitudes of the P waves from individuals presenting only with monophasic P waves and mixed P wave morphologies (bifid and monophasic in the same ECG) were not statistically different (Figure 36).
Figure 33. RR interval measured when bifid and monophasic P waves are present in dogs after 4 months of TIHF. Note that bifid P waves occur at lower heart rates.
Figure 34. Duration of bifid and monophasic P waves in dogs after 4 months of TIHF are not different.
Figure 35. P wave amplitudes in ECG with different P wave morphologies. No statistical differences were found between the average amplitude in the lead II of the ECG from dogs presenting mixed morphologies of P waves and only monophasic P waves.

4.3 Discussion.

As mentioned before, it is usually believed that changes in P wave morphology towards a bifid P wave rather than a monophasic are associated with inter-atrial disturbances in velocity of conduction known as interatrial block [100]. This type of P wave has been named P mitrale and has been related to the increased size of the left atrium found in patients with different cardiovascular pathologies [162].

During the present study, variable morphologies of P waves on the electrocardiogram developed during chronic heart failure, suggesting variable patterns of atrial electrical
activation. Eight of 14 dogs developed bifid P waves at 4 months. This time point is one where the left (but not the right) atrium was enlarged and the duration of the P wave itself was increased. However, bifid P waves were not found consistent rather they were found at moments when the RR interval was increased, this means when heart rate slows. The reverse-rate dependence of bifid P waves was also found at baseline (0 months) in four of 14 and in eight of 14 in at one month of HF. (2 and 3 months ECG were not included for this part of the study) Since atrial size was not increased, and P wave duration was unchanged at one month relative to baseline, it is unlikely that atrial size or global atrial electrical activation caused the bifid P waves. Whether at baseline, or one or four months of HF, bifid P waves occurred during longer RR intervals, and slowed rate was the only variable found to associate with appearance of bifid P waves.

Since the duration of monophasic and bifid P waves did not differ, this suggests that simultaneous slowing of atrial conduction velocity did not result in the variable P wave morphology. One potential mechanism may be changes in the conduction pathway for activation of the right atrium by the sinoatrial node [89]. Notably, there are inferior and superior conduction pathways from the SA node to the right atrium [89]. Changes in the activation path from the sinoatrial node to the right atrium are influenced by the autonomic system [89, 163], as are alterations in the RR interval [164].

4.4 Conclusions.

Bifid P waves were present in healthy dogs, dogs in early stage heart failure (one month) without significant atrial remodeling and in dogs with four months of heart failure with documented structural atrial remodeling measured by echocardiography. This
means that for this group of dogs the presence of bifid P waves, normally associated with interatrial block [100], was not related to structural remodeling of the atrium.

The finding that bifid P waves occur when the RR is prolonged can be interpreted as this change in morphology being influenced by the sympathetic tone and/or vagal. However, one would expect that dogs with induced heart failure have higher sympathetic tones and hence reduced RR. Nevertheless, as seen in this study, heart rate was not statistically changed at any time point. Respiratory sinus arrhythmia was at least in partially preserved as changes in heart rate were present.

It is believed that changes in P wave morphology may reflect changes in the pathway used by the impulse in its travel from the SA node to the right atrium to the left atrium [100]. Theoretically, bifid P waves are the result of the impulse not conducting through Bachman’s bundle and using an alternative conduction pathway [96]. This change in the conduction pathway theoretically delays the time of conduction and changes the morphology of the P wave.

We did not evaluate changes occurring specifically at the Bachman’s bundle and hence we cannot rule out this possibility, however these changes occurred in animals considered healthy and in others with reduced ventricular function but normal atrial size and function.

Although it is known that changes in P wave morphology associated with changes in heart rate are due to shift of the pacemaker site, isolated variation in P wave morphology may also result from interatrial block [96]. We cannot determine the origin
of bifid waves in the present study, but suggest that they may reflect a change in the origin of the cardiac impulse. Interestingly this shift is not lost during heart failure.
Chapter 5. Atrial Electro-mechanical Synchrony assessed by tissue Doppler Interrogation.

5.1 Tissue Doppler interrogation of the left atrial free wall.

Tissue Doppler interrogation (TDI) of the free wall of the left atrium is a new tool used to estimate the duration of the total atrial electrical-contraction activation[165]. TDI measures the high frequency low velocity waves generated by the myocardial tissue generating systolic and diastolic waves [166, 167]. For this study, the interval between the onset of the P wave to the peak velocity of the second negative wave (generated by electrically stimulated contraction of the atrium) was measured. Prolongation of this parameter has been associated with a history of atrial fibrillation, heart failure and valve disease [168]. Prolonged P wave to A’ (PA)-TDI is associated with an increased risk of new onset of atrial fibrillation[169]. This interval has also been used to identify the risk of developing atrial fibrillation in patients with congenital heart disease [170].

5.2 Materials and methods.

A group of 14 adult hound dogs of either sex (≈20 kg) were implanted with pacemakers at the right ventricular apex as previously described [61]. They were paced at 180 beats per minute for four months to induce heart failure. Time points selected for echocardiographic evaluation were 0 months or baseline, 1, 2 3 and 4 months. 2D and M mode echocardiography were performed to evaluate left and right atrial size and function.
and left ventricular function and to establish the presence or not of heart failure. TDI images of the left atrial area were obtained and the pulsed sample was located on the left atrial free wall (figures 37 and 38). Only good quality traces with clear A’ waves were included in this study. Thus, traces were selected from nine dogs at baseline (month 0), eight at month one, 11 at month two, and five at both three and four months of HF. The duration of the total atrial electrical activation was quantified as the time interval between the beginning of the P wave on the electrocardiogram and the peak velocity of the wave generated by left atrial contraction (A’). A General Electric Vivid 7 echocardiograph with a 5 MHz probe was used. Three cardiac cycles were measured per dog and per time point, and averaged. Average values per time point were compared by analysis of variance with Bonferroni’s post-test using Graphpad Prism version 4.00 for Windows. Graphpad Software, San Diego California USA.
Figure 36. Tissue Doppler interrogation of the free wall of the left atrium in a dog at baseline. The picture depicts the time measured between the beginning of the P wave and the peak velocity of A’ (atrial contraction).
5.3 Results.

Tissue Doppler Interrogation of the left atrial free wall showed a value at baseline (0 months of 67.99 ±7.8. At 1 month and 2 months no statistical change was found. Beginning at three months, there was a significant increase in the interval relative to baseline, with an average value of 87.45 ± 3.1(p <0.01); with no further increase at 4 months (Figure 39).
Figure 38. PA-TDI time is increased at 3 months of TIHF relative to baseline. No further difference between 3 and 4 months was found.

5.4 Discussion and conclusions.

These results show that in a chronic model of tachypacing-induced HF, there are global changes in the total electrical excitation-contraction time in the atria which are detectable by TDI. Considering the context of the changes reported in the other chapters of this work, and timing of these changes, we know that the left atrium starts increasing in size at two months of TIHF, and that left atrial contractile function is also reduced at two months. This pattern is similar to that found in the P wave duration. For this reason, these changes in atrial excitation-contraction interval can be related either to the increased size of the left atrium, in which case the time of conduction would be delayed simply because it has to travel across a longer pathway. It can also be related to a
reduction in the velocity of conduction due to altered intercellular communication, interatrial block or a combination of the three. However, PA TDI changes occur later than those detected by 2D echocardiography such as area, diameter and fractional area change.

Collectively, the changes presented in the previous chapters of this work show that this model replicates the changes typically associated with atrial dysfunction during heart failure which are associated with an increased risk of atrial fibrillation.
Chapter 6: General discussion.

6.1 P-wave duration versus LA area (size):

One important finding in this study was the ability to produce an increase in P wave duration. P wave was prolonged compared to baseline and compared to previous reports [171]. Since the P wave is produced by atrial depolarization, it follows that the duration of the P wave should depend upon the size of the atrial syncytium [172] (from, SAN to the most distant portion of the LA), the atrial myocardial conduction velocity (nominally 1 to 2 m/s), and the time-order of atrial depolarization (whether originating in the SAN and spreading to the most distant portion of the LA, or possibly originating in the middle of the atrial syncytium and spreading a much shorter distance). We did not study the time order of atrial depolarization, however we did measure LA area and duration of P waves, and if we presume no change in time-order of depolarization, then a direct relationship between P wave duration and LA area and conduction velocity should exist. In a study Huang et al [173] showed an increase in the atrial regions with slow conduction during increased atrial pressure. This increases the susceptibility to AF due to increased refractoriness and increased dispersion of refractoriness [173-176]; therefore prolongation of the P wave could be due to both increased area and reduced conduction velocity. When the P wave durations for all dogs at all times during pacing-induced HF was graphed against LA area alone, the relationship appeared linear. Thus we do not
know the relative contributions of area alone and decreased conduction velocity alone, but it appears that the P wave duration could be explained only by the left atrial size.

Velocity of conduction depends upon many factors; i.e., the relative differences in $[\text{Na}^+]$ between the inside and outside of the cell [177], resting membrane potential altering the availability of the sodium channels [ref], the rate of current flux between stimulated and resting portions of the cell [highly dependent upon the cable properties (resistance, conductance, inertance)] [101, 102], facilitation of conduction by gap junction (determined in part by connexin-43) [105-107]. We determined that degree of phosphorylation of connexin-43 was reduced significantly during later stages of rapid pacing, thus this could account for reduced velocity of conduction [120] and lengthening of P wave duration even if LA area was not increased. As mentioned before, phosphorylation is an important step into the migration and assembly of connexin 43 in the gap junction [116, 120]. Considering that changes in connexin expression affects intercellular communication (see chapter 1), these results might indicate reduced conduction velocity due to reduced phosphorylation of connexin 43. However we do not know the relative contributions of area (atrial size) and velocity (connexins, Na channels) to prolongation of P wave.

6.2 P wave amplitude versus LA size:

The amplitude of the P wave depends upon many factors: the sizes and geometries of boundaries of depolarization traversing the atria (depending upon from where within the SAN the wave is emitted), the orientation of these boundaries to the electrode lead axis on the body volume, thickness of the atrial walls, differences in
resistivity between atrial myocardium and intracavitary blood (the Brody effect) [178].

During evolution of the increase in LA size from onset of rapid pacing to 4 months after rapid pacing, we showed that P wave amplitude increased between baseline and at 1 month, but not thereafter, despite the increasing LA area. Since the RA did not appear to enlarge, increased amplitude of P must be attributable to changes in the LA. As mentioned above, since the P wave duration depends upon velocities and lengths of pathways which have been shown to increase with duration of pacing, and duration of P wave lengthened continually as the LAA increased, that P wave amplitude only increased at 1st month and not thereafter may indicate that the increased area of P, during later months, arose at the expense of increased distance from the inter-atrial septum (eccentric enlargement) and not by concentric “bloating” of the atrium. Unfortunately the 2-D images from the ECHO were inadequate to identify the 3-D time-order of RA atrial enlargement during the rapid pacing.

Increased amplitude of the P wave may be, merely, a consequence of prolongation of atrial depolarization so that there is greater asynchrony between either the right atrium and left atrium or between the caudal and cranial regions of the left atrium [179]. The P wave occurs only when there is a separation of charge within the atrial syncytium, i.e., if the surface of all cells were either positive or negative, no potential difference would exist and no body surface potential would be produced. However, because there is a general spread of atrial depolarization from SAN to caudal region of LA (i.e., from cranial region of RA to caudal region of RA, and from cranial region of LA to caudal region of LA), differences of potential do exist and P waves are produced. Of course the
amplitude of the P wave is determined, principally, by how large the interface (boundary) is between depolarized and resting myocardium, and by how parallel to the lead axis the wave travels [180, 181]. The body surface P wave may be synthesized from the action potentials of the atria, because these action potentials “tell” what the sign is on the cell surface, and therefore what differences in signs contribute to the P wave obtained at the body surface (Figure 40). By retarding atrial conduction velocity and enlarging the LA—but not the time-order of activation—the caudal border of LA depolarization (red) would occur later compared to the cranial border (blue) than if the chamber were not enlarged and/or the velocity of propagation were not decreased [181]. Thus with LA enlargement or retarded conduction velocity, the differences in potential between the cranial and caudal borders (green) would be greater even though the voltages at each point were not different. In fact, in some animals with LA enlargement, larger Ta waves were observed. Again, this is not because of atrial injury, but because of greater asynchrony of global atrial depolarization as shown in the figure. This argument is consistent with tardy motion of the caudal border of the LA (see discussion of tissue-Doppler interrogation).
Figure 39. Two action potentials with same amplitudes but occurring at different times due to retarded velocity of conduction. Blue line indicates the activation of the initial portion of the LA and red line indicates the activation of the latest portion activated at the LA. Notice the increase in amplitude and duration of P waves. (Figure courtesy of Dr. Robert Hamlin).

6.3 P wave duration and amplitude versus right atrial size:

During the rapid-pacing HF paradigm, there were no differences in right atrial dimensions on echocardiograms. This could indicate either that the right atrium did not increase in size, as did the left atrium, or that the 2-D echocardiographic interrogation was insensitive to right atrial enlargement. The possible lack of sensitivity could be attributable to the elliptical geometry of the RA compared to the spherical geometry of the LA, or that the angle of interrogation was inappropriate to characterize the RA. The
hearts were harvested quickly from the euthanasia preparation to obtain viable cells for
electrophysiological study, but during rather quick and superficial post mortem
observations, the RA did not appear significantly enlarged, despite that fact that both
vena cava were clearly dilated compared to normal dogs. This finding is counterintuitive,
since both atria must have worked equally hard during rapid pacing, and one might have
expected equivalent dilatation.

Normally, the maximal height of the P wave is determined by size of the right atrium and by the location of the initial wave of atrial depolarization [182-184]. For any given right atrial size, the more craniad the wave begins in the right atrium, the less cancellation and the duration and the amplitude of the P wave are greater [185]. The maximal height of the P wave is determined by the area of the maximal boundary of the wave of depolarization traversing the right atrium. Because, in dogs (and also in humans) with respiratory sinus arrhythmia, the origin of the wave of depolarization oscillates between the cranial (during inspiration) and caudal (during expiration) portions of the SAN [186-190], the finding that P waves occurring during inspiration are more peaked than those during expiration is expected. These oscillations stem from waxing (during inspiration) and waning (during expiration) of vagal activity to the SAN [191, 192]. With signal averaging of all P waves (i.e., during both inspiration and expiration), the configuration of the “average” P wave will vary by the rate and vigor of respiration, which determines the numbers of peaked P waves and the degree of peaking. That is, breathing more rapidly produces more peaked P waves to be averaged. However with heart failure, vagal tone is decreased and the disparity between P waves occurring during
inspiration and expiration may disappear. Thus in our dogs with pacing-induced heart failure and no doubt reduced vagal efferent activity, even if the right atrium were enlarged, differenced in P waves between inspiration and expiration might be minimized, and the amplitudes of signal-averaged P waves might differ from P waves measured at any particular time. The increased durations of P waves measured by signal averaging is due to the fact that signal average may be more sensitive for small amplitude deflections in the initial and terminal portions of the P wave, relative to manual measurements of individual beats. Thus, the signal averaged beats may include O and Ta waves that were not included with the manual technique. However, we relied on the manual technique for many analyses as this most commonly used method in both research and clinical settings.

6.4 Dispersion of P wave duration:

Dispersion of P wave duration refers to differences in durations in various ECG leads [1]. If we presume that all electrodes are “distant” (i.e., more than 3 heart’s radii) from the heart, then dispersion results from differences in trajectory of component sequential vectors on lead axes. That is if a vector is oriented at right angles to a lead axis, no deflection will appear. But if the vector is oriented parallel to the lead axis, a deflection will appear. Thus if 2 lead axes (A=left to right, B=head to tail) are orthogonal (i.e., at right angle to each other) dispersion is possible but not mandatory. If atrial activation begins with a vector (1) oriented right to left and has no orientation head to tail, but ends (2) with a vector orientation head to tail and not left to right, at the outset of the complex no deflection will occur in lead B and a positive deflection will occur in lead A, and at the end of the complex no deflection will occur in lead A and a positive
deflection will occur in lead B. Thus there would be no dispersion in duration between 
leads A and B, because each lead “misses” a component, B at the onset and A at the end. 
However if we have another lead, C, with an axis midway between the axes for A and B, 
both vectors 1 and 2 will be manifested in lead C, and the duration of the complex will be 
longer, i.e., dispersion between A and, and C would exist.

The increased dispersion identified during rapid-pacing and atrial enlargement 
could occur simply because of atrial enlargement. The enlarged atrium could become 
closer to an electrode, make the electrode less than 3 heart’s radii from the heart, and thus 
be biased by proximity. That is, it might “see” activity sooner or later (i.e., for a longer 
duration) than leads placed more distantly. Alternatively all leads—in particular thoracic 
leads—may not be 3 heart’s radii from the heart, they may be biased by proximity, and 
they may “see” voltages (for a longer time) that might not be seen by truly distant leads.

6.5 General considerations on P wave morphology:

The dog normally has a very complex P wave. It often begins with an O wave of 
small amplitude (oriented, spatially, either craniad or caudad) and short duration, 
followed by a positive component (oriented much more caudad than sinistrad) of much 
greater height and duration. The positive component is often bifid, with the first portion 
greater in amplitude than the later. It is thought that the initial portion is generated by 
atrial depolarization from SAN to the caudal portion of the RA; while the latter 
component is generated by left atrial depolarization [191]. Even at best this is a gross 
oversimplification that assumes the atrial syncytium may be modeled as a pond into 
which a pebble is thrown and impacts at one end (the SAN). A wave then is emitted from
the point of impact and travels to the other end of the pond. It is known well that atrial activation (the pond) contains many islands around which the wave must travel and within which the wave may surface [191]. Furthermore, at least in the horse [193] (whose atrial activation process appears as a giant dog’s) the terminal portion of the major, positive portion of the P wave appears to be produced by activation over Bachmann’s bundle and the interatrial septum, with left atrial depolarization occurring “blind” (i.e., after the end of the P wave) to the ECG.

6.6 Consequences to atrial contraction:

Because of increased asynchrony between the initial and terminal portions of the atrial depolarization, we expected differences in the time-order of atrial contraction, i.e.; the most caudal border of the LA would be expected to contract (generate tension) increasingly later after the initial portion. This was demonstrated by interrogation of contraction using tissue-Doppler. For normal dogs, duration between onset of the P wave and maximal velocity of motion of the caudal border of the LA is approximately 65 to 70 ms; after rapid-pacing this duration is always greater and may exceed 100 ms.

Effects on atrial contraction were also identified by interrogation of LAFAC (left atrial fractional area change). After 1 month of TIHF, LA FAC did not change, despite the fact that LVEF had already decreased significantly. However after 2 months and plateauing at 3 months, LA FAC decreased significantly. The changes in LA FAC mimic closely the changes in LV internal diameter during diastole, i.e., as LVID increased LA FAC decreased. The LA FAC depends upon the degree of atrial emptying due to atrial systole, but principally due to ventricular diastolic suction (the Y-descent of the
interatrial and venous pressure pulse). During heart failure, of course, ventricular systolic and diastolic decrease. Why, after 1 month of TIHF did LA FAC not decrease when LVSF did? This may be explained by the fact that when LVEF decreased, LA preload increased, thus even a “failing” LA could generate normal force invoking the Cyon-Frank-Starling law [70]. The force of contraction depends upon both myocardial contractility and preload; contractility was reduced. But preload was increased. During later stages of TIHF, however, either the more severely failing LV or severely failing LA may have resulted in the reduced LA FAC.

Thus this preparation demonstrates electrophysiological remodeling (properties of the P wave), structural remodeling (LA enlargement), and dynamic functional remodeling (tardy contraction of the caudal portion of the LA). These finding mimic closely observations made in human patients at risk for atrial fibrillation [1, 2].

6.7 Considerations on PQ and RR short-term variability.

The PQ segment includes the impulse traveling from the SA node to the left atrium, and the conduction through the atrioventricular node. The actual PQ values did not change at any measured time point. However, the variability of the PQ was decreased after only one month of TIHF. This reduction in variability was sustained until the fourth month. This finding could be related to an increased sympathetic tone inducing not a faster conduction in the atrioventricular node but a less variable one among beats. Additional studies are required to clarify the role of the atrioventricular node on the change in short-term variability in the TIHF model. The variability of RR on the short term indicates fast changes in heart rate. This is associated to the reduced variability of
the SA node discharge as a consequence of the increase in sympathetic tone. Reduced RR short-term variability is a predictor of all-cause mortality and sudden cardiac death [194].
Chapter 7. Limitations and future directions.

The main limitation of the study was that the data was collected with dogs under sedation and in lateral recumbence which may alter autonomic balance, although this was consistent throughout. This could affect mainly heart rates and heart rate variability as well as other parameters defined by the balance between sympathetic and parasympathetic tone.

Another limitation of the study was the fact that right atrial area was measured only in one axis. As discussed previously this may have reduced the sensitivity to observe a potential increase in its internal area. Also, multiple variables changed between two and three month. The study design precludes precise determination of the time of changes. We are unable to extrapolate beyond four months.

In this study we quantified the expression of connexin 43 but not its distribution which is also an important determinant of intercellular conduction.

Fibrosis, a known determinant of reduced myocardial velocity conduction, was not measured in this study.

Some future directions of this study include the study of the role of the sinoatrial node exit pathways in the morphology of the P wave. It is necessary to establish the role of the anatomic inter-atrial pathways in the P wave morphology and how they are affected with the TIHF model over time.
Chapter 8. References


Appendix A. ECG and echocardiographyc original data.

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<tr>
<td>3</td>
<td>0.42 ± 0.09 (0.3, 0.63)</td>
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<td>0.4 ± 0.07 (0.3, 0.59)</td>
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<td>Time (months)</td>
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Table 1. Original data, mean, standard deviation, maximum and minimum values.
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Table 2. Difference from 0 months: mean, standard deviation, maximum and minimum values.
Appendix B. Statistical tables and figures.

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<th>P-Value (to 1 Month)</th>
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Table 3. Connexin 43 phosphorylation.

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<td>0.02</td>
<td>0.058</td>
<td>0.029</td>
</tr>
<tr>
<td>4 Month</td>
<td>0.70</td>
<td>0.71</td>
<td>0.158</td>
<td></td>
<td>0.07</td>
<td>0.162</td>
<td>0.081</td>
</tr>
</tbody>
</table>

Table 4. Connexin 43 total expression.
Figure 40. The relation between LA ES and P wave duration is positive over time. This is significant at 0, 2 and 4 months. $P < 0.03$ overall with a significant relationship. $P < 0.007$ at 2 months; $P < 0.04$ at 4 months.
Figure 41. Relationship between LA FAC and P wave duration changes over time (Overall p<0.0001). At later time points, increases in P wave duration correspond to decreases in left atrial contractility (FAC). P=0.0008 at two months; P = 0.0074 at four months.
Figure 42. Relationship between EF vs time and LVIDD vs time. No statistical correlation was found between P wave duration and the predictors LV EF and LVIDD.
<table>
<thead>
<tr>
<th>Predictor</th>
<th>Comparison</th>
<th>Estimated difference in P-wave amplitude</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAES</td>
<td>5 unit increase in LAES: month 0</td>
<td>0.028</td>
<td>(-0.087, 0.142)</td>
<td>0.6273</td>
</tr>
<tr>
<td></td>
<td>5 unit increase in LAES: month 1</td>
<td>0.103</td>
<td>(-0.001, 0.206)</td>
<td>0.0512</td>
</tr>
<tr>
<td></td>
<td>5 unit increase in LAES: month 2</td>
<td>0.058</td>
<td>(-0.013, 0.13)</td>
<td>0.1079</td>
</tr>
<tr>
<td></td>
<td>5 unit increase in LAES: month 3</td>
<td>0.010</td>
<td>(-0.045, 0.065)</td>
<td>0.7074</td>
</tr>
<tr>
<td></td>
<td>5 unit increase in LAES: month 4</td>
<td>-0.014</td>
<td>(-0.054, 0.027)</td>
<td>0.5051</td>
</tr>
<tr>
<td>LA FAC</td>
<td>1 unit increase in LA_FAC</td>
<td>0.001</td>
<td>(-0.03, 0.03)</td>
<td>0.9686</td>
</tr>
<tr>
<td>LVIDD</td>
<td>0.5 unit increase in LVIDD</td>
<td>0.004</td>
<td>(-0.025, 0.032)</td>
<td>0.8047</td>
</tr>
<tr>
<td>EF</td>
<td>5 unit increase in EF</td>
<td>-0.011</td>
<td>(-0.029, 0.006)</td>
<td>0.1981</td>
</tr>
</tbody>
</table>

Table 5. P wave amplitude vs. other parameters over time. No positive relationship between P amplitude and any of the predictors was found.