DIURNAL DIFFERENCES IN COMMON ELECTROCARDIOGRAPHIC INDICES OF ARRHYTHMIC LIABILITY IN NORMAL TELEMETERED DOGS AND TELEMETERED DOGS WITH FAILING HEARTS: IMPLICATIONS FOR SAFETY PHARMACOLOGY AND VETERINARY CARDIOLOGY.

DISERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

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Abstract

The assessment of the electrocardiogram (ECG) liabilities is a key component in both clinical medicine and safety-pharmacology. However, despite the known circadian dependence of both pro-arrhythmic substrates and prevalence of arrhythmias, the optimal time for the ECG evaluation remains undetermined. In fact the time of day of the recording is seldom considered in the interpretation. This study assessed circadian changes in ECG parameters in a well-defined telemetered canine model. Diurnal differences were sought between normal dogs and dogs with failing hearts (i.e., with reduced ejection fraction and elevated NTproBNP) but not in heart failure (i.e., asymptomatic). Methods of analyzing ECGs from (the relatively few) dogs with clinical heart failure are not of great concern, since dogs so afflicted have clear echocardiographic and other clinical evidence of disease. So this study was directed more at (the much greater number of) dogs with subclinical disease, where standard methods are more equivocal, difficult to perform, and expensive.

Healthy male dogs (n = 9) and male dogs with failing hearts (n=10) were instrumented for telemetered ECG recordings at rest (1-hour epochs) during periods of low (2AM) and high autonomic activation (6PM and 6AM); heart rates (HR), indices of cardiac
conduction (PQ, QRS) and repolarization duration (QT, QTcF) as well as heart rate variability (RRSD), repolarization instability (QTSTV) and steepness of restitution (QT/TQ) were evaluated at each time-point. Data are mean ± SD and were compared (ANOVA). The number of dogs entered into this study was determined a priori to produce a power of ~0.8 to detect 15% differences in parameters having coefficients of variation of 25% with an alpha of 0.05.

In both groups, at 2 AM, heart rates were slower and more variable while the PQ interval was longer, indicating strong parasympathetic control. No notable circadian differences in ventricular conduction (QRS duration) were found in either normal dogs or dogs with failing hearts. For normal dogs, no notable differences were found in ventricular repolarization (QT, QTcF) or repolarization variability (QTSD, QT-STV). Though in normal dogs, ventricular restitution (QT: TQ) showed some differences, these are not considered to be of clinical relevance. For dogs with failing heart, no notable differences were found in repolarization variability (QTSD, QT-STV) or ventricular restitution (QT: TQ). The QT-interval was longer at night, but rate-corrected QT duration did not change.

These data strongly indicate lack of notable diurnal differences in ECG markers of arrhythmic liability and their underlying physiology between normal dogs and dogs with failing hearts but no clinical signs of cardiac disease. Dogs with failing hearts had significantly longer QTc(F) and higher short term variability in QT, both indicators of a potential for a torsadomorphic ventricular arrhythmia. Furthermore, TQ interval was
significantly shorter for dogs with failing hearts, but QT only had a tendency to prolong, thus the QT: TQ only had a tendency to prolong. This is an indicator for a non-torsadomorphic, reentrant ventricular arrhythmia, which is known to develop in more severe heart failure. It may be postulated, then, that millions of dogs with failing hearts may develop a risk for ventricular arrhythmia, and may be candidates to receive compounds (e.g., β-blockers, ACE inhibitors) prophylactic against the development. Although there were no differences of significance in parameters of HRV between normal dogs and dogs with failing hearts, dogs with failing hearts tended to have: higher heart rates at 2 AM and 6 AM, and had higher coefficients of variation of almost all parameters. This finding, also, might indicate value for prophylaxis against the pathophysiology that occurs with more advanced heart disease and leads to morbidity and mortality.
Dedication

Dedicated to Pedro and my family, for their constant support and love during these years
Acknowledgments

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I also want to thank my friends here in Columbus for making my life easier, for being here when I needed you, in summary for being my family here at the US: thanks for the patience and support; and to my friends in Colombia for their support and encouragement.

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<tbody>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>ANP</td>
<td>Atrial natriuretic peptide</td>
</tr>
<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
</tr>
<tr>
<td>APD</td>
<td>Action potential duration</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosin triphosphate</td>
</tr>
<tr>
<td>AVN</td>
<td>Atrioventricular node</td>
</tr>
<tr>
<td>BNP</td>
<td>Brain natriuretic peptide</td>
</tr>
<tr>
<td>bpm</td>
<td>Beats per minute</td>
</tr>
<tr>
<td>CV</td>
<td>Conduction velocity</td>
</tr>
<tr>
<td>DI</td>
<td>Diastolic interval</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EF</td>
<td>Ejection Fraction</td>
</tr>
<tr>
<td>ET-1</td>
<td>Endothelin 1</td>
</tr>
<tr>
<td>FS</td>
<td>Fractional Shortening</td>
</tr>
<tr>
<td>HF</td>
<td>High frequency power</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>ITO</td>
<td>Transient outward current channel</td>
</tr>
<tr>
<td>LF</td>
<td>Low frequency power</td>
</tr>
<tr>
<td>N</td>
<td>Normal</td>
</tr>
<tr>
<td>NE</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>NN</td>
<td>Normal beat-to-beat intervals</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>N-terminal fragment of pro brain natriuretic peptide</td>
</tr>
<tr>
<td>PQ</td>
<td>PQ interval</td>
</tr>
<tr>
<td>QRS</td>
<td>QRS complex</td>
</tr>
<tr>
<td>QT</td>
<td>QT interval</td>
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<td>QT LVT</td>
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<td>QT corrected for heart rate by Fridericia</td>
</tr>
<tr>
<td>RAS</td>
<td>Renin-angiotensin system</td>
</tr>
<tr>
<td>RMSDD</td>
<td>Root mean square successive difference of normal beat-to-beat intervals</td>
</tr>
<tr>
<td>RR</td>
<td>RR interval</td>
</tr>
<tr>
<td>RSA</td>
<td>Respiratory sinus arrhythmia</td>
</tr>
<tr>
<td>SA</td>
<td>Sinoatrial</td>
</tr>
<tr>
<td>SAN</td>
<td>Sinoatrial node</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDNN</td>
<td>Standard deviation of the normal beat-to-beat intervals</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the means</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------</td>
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<tr>
<td>SERCA</td>
<td>Sarco/endoplasmic reticulum ATPase</td>
</tr>
<tr>
<td>TdP</td>
<td>Torsades de Pointes</td>
</tr>
<tr>
<td>TQ</td>
<td>TQ interval</td>
</tr>
<tr>
<td>ULF</td>
<td>Ultra low frequency power</td>
</tr>
<tr>
<td>VLF</td>
<td>Very low frequency power</td>
</tr>
<tr>
<td>VVTI</td>
<td>Vasovagal tonus index</td>
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<tr>
<td>XSDNN</td>
<td>Mean of standard deviation of the normal beat-to-beat intervals</td>
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</table>
1.1 The Electrocardiogram and Safety Pharmacology

Electrocardiography (ECG) studies the electrophysiological properties of the heart by analysis of voltages and intervals recorded at the body surface, the voltages generated by waves of depolarization and repolarization traversing the heart. However ECG in safety pharmacology is concerned principally with (1) heart rate and rhythm, (2) duration of depolarization and repolarization, (3) effects of heart rate on various intervals, (4) drug-induced rhythm disturbances.

Initial waves of depolarization leave the sinoatrial node (SAN) and, at a velocity of 1-2 M/s, traverse first the right atrium and then the left atrium giving rise to the P wave of the ECG and stimulating the atria to contract. The waves reach the head of the atrioventricular node (AVN) where they slow to <0.025 M/s, then traverse the His-Purkinje system at 2.5 to 5 M/s, and finally traverse the ventricles at ~0.5 M/s. As they traverse the ventricles they produce the QRS complex of the ECG and stimulate the ventricles to contract. Finally, waves of repolarization traverse the ventricles and produce the ST-T of the ECG, as the ventricles return to their resting state.
RR interval (Figure 1.1) is the interval from the peak of a QRS complex to the peak of the next. It is used to assess the ventricular rate (heart rate). Heart rate has an inverse relationship with the RR interval (HR=1/RR). Therefore, as RR interval prolongs, the heart rate decreases (Gauvin, Tilley et al. 2006).

The PQ interval is the time elapsed between the beginning of the P wave and the beginning of the next QRS complex. It corresponds to the AV conduction time, and ~70% of the interval arises from slow conduction through the head of the AV node.

The QRS complex is the interval from the beginning of the Q wave to the termination of the S wave, representing the time of ventricular depolarization. Following the end of QRS and occasionally confused with the QRS or depolarization, is the J wave produced by early, brief ventricular repolarization occurring over the ITO channel.

The QT interval extends from the onset of the Q wave to the end of the T wave, representing the durations of ventricular depolarization (QRS) and repolarization (JT).
Since 1957 several syndromes that produce Torsades de Pointes (polymorphic ventricular tachycardia) and sudden death have been predicted by prolongation in the QT interval. (Shaffer, Singer et al. 2002).

In 1986, it was found that a drug (terfenadine) was associated with sudden death and that it lengthened the QT. This heralded the onset of the discipline called Safety Pharmacology. There are several risk factors for Torsades de Pointes (TdP) besides drug-induced prolongation of QT: female, non-black, increasing age, slow HR, low serum potassium and magnesium, heart failure, ventricular enlargement, concomitant use of prescribed drugs for more than 3 days, comorbid diseases, having a congenitally long QT interval, drinking grapefruit juice (Rodriguez, Kilborn et al. 2001; Shaffer, Singer et al. 2002; Zitron, Scholz et al. 2005; Takahara, Sugiyama et al. 2006; Piccirillo, Magri et al. 2008; Farkas and Nattel 2010).
Several drugs (antibiotics, antineoplastics, antihistamines, antidepressants and antiarrhythmics) have been associated with QT prolongation, ventricular arrhythmias, TdP and sudden death (Shaffer, Singer et al. 2002; Farkas and Nattel 2010). Of the antibiotics supposed to alter the QT interval, macrolides (e.g., erythromycin, oleandomycin) have shown the greatest potential for causing QT prolongation, ventricular arrhythmias and TdP (Shaffer, Singer et al. 2002).

This proarrhythmic potential of drugs is now a major medical and pharmaceutical concern. Some drugs have been removed from the market and other drug warnings have been added to the product labels. Unfortunately, there is usually a long interval between the initial drug approval and the first identification of proarrhythmic potential in patients. This demonstrates the urgent need to recognize this safety hazard early in the process of producing new drugs (Hanton, Nahas et al. 2001).

Safety Pharmacology is the science that studies the potential for undesired pharmacological effects of a specific drug related to its therapeutic dose. These studies are designed to define a dose-effect relationship for adverse events. Safety pharmacology studies use several different animal models in order to mimic conditions for the purpose of studying pharmacokinetics, pharmacodynamics, dose range and toxicological effects of drugs and devices. Since disease potentially alters both therapeutic and toxic effects, it is reasonable to search for toxicity in surrogates that mimic as closely as possible the substrate present in patients for whom then drug is destined.
Safety pharmacology focuses on ECG, systemic arterial blood pressure, respiratory rate and depth, and neural function, but drug-induced changes in the ECG have resulted in more drugs being removed from the market than all other parameters measured.

Changes in cardiac conductivity or rhythmicity induced by drugs can be monitored by recording and studying the ECG, making it an important and irreplaceable tool when studying drugs in clinical and toxicological studies in animals (Hanton, Nahas et al. 2001).

ECGs provide, non-distructively and relatively inexpensively, critical information about the electrophysiological properties of the heart, principally rhythmicity and conduction (depolarization and repolarization). The preclinical assessment of the effects of drugs on cardiac repolarization and the potential risk to induce arrhythmias in humans is of particular importance. Though the exact significance of the QT interval prolongation as a risk factor remains unclear, many drugs have been removed from the market, due to their causing QT prolongation, arrhythmia and sudden death. Also, evaluation of the drug effect on QT in dogs is required (S7A, S7B) by the regulatory guidelines (Hanton and Rabemampianina 2006).

1.2 Heart Rate Variability

In dogs, the rhythm of the heart is irregular (actually regularly-irregular) due to changes in the rate of discharge of the sinoatrial node (SAN) under the influence of waxing and waning of parasympathetic efferent activity. “Heart rate variability (HRV) is a
physiological phenomenon that occurs as a result of changes in the cardiac autonomic tone” (Pereira, Woolley et al. 2008). HRV occurs at varying frequencies depending upon what factors alter it. A relatively high-frequency component (\( \cdot 0.35 \) to 0.5 Hz) occurs due to irradiations from the medullary ventilator centers, and to ventilation itself, that affect parasympathetic traffic; relatively low-frequency components (\( \cdot 0.001 \) to 0.01 Hz) occur because of sympathetic activity arising from time of day (i.e., diurnal), arousal just before feeding at around 5 AM, and again at 5 PM when most animals hunt for food. In addition if stressful or environmental (e.g., time of year) conditions occur at regular periods, low frequency (0.001 Hz) and ultra-low frequency (i.e., daily or monthly) components may occur. HRV is calculated in regard to beat-to-beat changes with regard to normal (N) beat-to-beat intervals (NN). (Calvert and Wall 2001; Calvert and Wall 2001). It has been proposed that HRV is an excellent prognostic indicator of adverse outcomes in humans after myocardial infarction and with idiopathic dilated cardiomyopathy, since parasympathetic activity protects against ectopic ventricular activity that may evolve into ventricular fibrillation and sudden death. Lower values of HRV have been related to increased risk of cardiac and all-cause deaths and increased hospitalizations after myocardial infarction and dilated cardiomyopathy (Pereira, Woolley et al. 2008).
HR is determined by temperature (it accelerates when warmed and decelerates when chilled), exercise, physical and mental stress, age, gender, blood pressure (baroreceptor reflex: rate speeds when pressure lowers and slows when pressure elevates), respiration (cardiopulmonary reflex and stretch receptors in the lungs and thoracic wall, and irradiations from medullary respiratory centers to juxtaposed cardioregulatory centers), renin-angiotensin system, time of day (diurnal variation), degree of stretch of the right atrium (Bainbridge reflex produced by a ctivation of stretch receptors), and degree of pulsation of the SA nodal artery, but is principally and rapidly influenced (figure above) by autonomic efferent activity (accelerated by sympathetic, decelerated by parasympathetic). Of course agonists or antagonists of this autonomic traffic affect heart rate (Haggstrom, Hamlin et al. 1996; Doxey and Boswood 2004; Pereira, Woolley et al. 2008).
The heart rate in dogs appears to be independent of body mass: it is slow during sleep (40 to 60/minute), and speeds up during excitement or activity (180 to 250/minute). In addition, quiet dogs manifest a large change in heart rate with breathing; heart rate speeds up during inspiration and slows down during expiration. This is termed respiratory sinus arrhythmia (RSA). RSA is modified by psychological state, diseases, and drugs, and it has been considered to be a sign of cardiac health (Hamlin, Smith et al. 1966; Haggstrom, Hamlin et al. 1996; Doxey and Boswood 2004).

It is also influenced by the dog breed (explained by the morphology of the head and the relative hindrance to ventilation and need for more vigorous effort) where it has been demonstrated, using a vagal tone index, that brachycephalic breeds have a more pronounced RSA than non-brachycephalic breeds (Doxey and Boswood 2004; Hanton and Rabemampianina 2006). In 2004, Spier and Meurs found different results by using conventional heart rate variability measures in 10 healthy Boxers and 10 Boxers with heart failure. This variability can suggest that differences between brachycephalic dogs vs. mesocephalic/dolicocephalic dogs may be obfuscated by the presence of heart disease (Pereira, Woolley et al. 2008).

Sinus arrhythmia in dogs appears to be secondary to fluctuations in vagal efferent activity associated with the respiratory cycle (Haggstrom, Hamlin et al. 1996; Miyazaki and Tagawa 2002; Gauvin, Tilley et al. 2006).
Since the heart rate variability is the result of a balance of the autonomic input to the SAN (sympathetic activity increases heart rate and parasympathetic tone decreases heart rate), it is a useful indirect measurement of the autonomic nervous system function (Doxey and Boswood 2004; Spier and Meurs 2004). During rest, fluctuations in the vagal tone resulting from respiration dominate fluctuations due to sympathetic activity in dogs, and fluctuations in heart rate due to respiration occur at the frequency of respirations at approximately 1 cycle every 3 to 4 seconds (a frequency of 0.35 to 0.55 Hz). Contrariwise, fluctuation in heart rate due to altering sympathetic efferent activity occur at a much lower frequency (0.001 to 0.01 Hz) that represent altering sympathetic tone due to feeding and its anticipation, or to interactions with people and other animals (Doxey and Boswood 2004).
Many investigators have reported that an increased sympathetic activity decreases the variability of the RR intervals and promotes cardiac arrhythmias, mainly malignant ventricular arrhythmias, whereas an increased parasympathetic tone induces an increased HRV and has a protective role on the onset of cardiac arrhythmias. However it is not always completely cardioprotective (Matsunaga, Harada et al. 2001; Spier and Meurs 2004). Cardiac arrhythmias and sudden death in humans have been related to autonomic imbalance manifested as increased sympathetic and decreased parasympathetic activities (Adabag, Grandits et al. 2008).

There are two main ways to assess HRV: time domain and frequency domain. The time domain analysis depends on mathematical quantification of the variability of RR intervals (e.g., SD, SEM, longest minus the shortest) and is the simplest method. Though time domain measures are used to evaluate short-term HRV occurring during brief recordings, they are best used for long-term recordings. On the other hand, the frequency domain analysis, also known as power-spectral analysis, evaluates the periodicity of variations in RR intervals attributable to varying frequencies, and it resolves more precisely the influences of both sympathetic and parasympathetic activities attending respiration or any other determinant of heart rate (Calvert and Wall 2001; Calvert and Wall 2001; Matsunaga, Harada et al. 2001; Pereira, Woolley et al. 2008)

Commonly HRV has been measured as the standard deviation of several RR intervals and the ratio between maximal RR interval during expiration to the minimal RR interval during inspiration (Haggstrom, Hamlin et al. 1996). However, there are several different measurements that can be performed using the time domain method, such as calculating the
standard deviation (SD) of NN intervals (SDNN) at different epochs, mean of the standard deviation of NN intervals (XSDNN), root mean square successive difference of NN intervals (RMSSD), percentage of NN interval that varies from the previous NN interval by more than 50 milliseconds, among others (Haggstrom, Hamlin et al. 1996; Calvert and Wall 2001).

The vasovagal tonus index (VVTI) is a non-conventional time domain analysis of the heart rate variability and is considered influenced predominantly by parasympathetic activity (Pereira, Woolley et al. 2008). It is calculated as the natural logarithm of the variance of the RR intervals for 20 consecutive beats (Haggstrom, Hamlin et al. 1996). Heart rate variability evaluated by VVTI is unable to evaluate other variables such as thermoregulation, renin-angiotensin system, blood pressure, etc., since it mainly reacts to modulations of the autonomic nervous system and respiratory-related variations (Pereira, Woolley et al. 2008).

It is known that the frequency domain analysis (power spectral analysis) determines both, parasympathetic and sympathetic influences, better than the time domain method (Calvert and Wall 2001).

High frequency variations (HF) (>0.15 Hz) are a result of the variations of the parasympathetic tone. Dogs at rest breathe approximately 14 times a minute or at a frequency of 0.23 Hz (Doxey and Boswood 2004). Since the RSA is mediated predominantly over the parasympathetic nervous system, HRV at this frequency estimates parasympathetic (vagal) efferent activity. Therefore, it has been determined that, in humans
and dogs, the high frequency power (>0.1Hz) is predominantly parasympathetic activity and its frequency is controlled by the respiratory rate or frequency (Eaton, Cody et al. 1995; Calvert and Wall 2001; Pereira, Woolley et al. 2008)

Besides the high frequency components derived from respiration, frequency domain measures have shown there are fluctuations that occur at low frequencies (Haggstrom, Hamlin et al. 1996). The low frequency component (LF) (<0.1Hz) is a mixture of parasympathetic and sympathetic activities (β-adrenergic), and has been attributed to fluctuations of the vasomotor tone associated with thermoregulation and baroreceptor functions. The physiologic interpretations for the lower frequency components are still unclear: the very low band (VLF) and the ultra low band (ULF) (Eaton, Cody et al. 1995; Haggstrom, Hamlin et al. 1996; Calvert and Wall 2001).

Several studies have evaluated HRV in dogs by using the frequency domain analysis. Usually, it has been considered that the frequency of ventilation occurs between 0.10Hz and 1.0Hz (Haggstrom, Hamlin et al. 1996).

In 2001, Calvert et al used 0.04Hz to 0.15Hz for the low frequency band and 0.15Hz to 0.4Hz for the high frequency band. However, they conclude that an upper limit of 0.5 to 1.0Hz would be more proper in dogs, since 0.4 can eliminate portions of HF data, because the typical respiratory rate may go above that of humans (Calvert and Wall 2001). In a different study, Matsunaga et al (2001), found that the peak for the high frequency bands was between 0.15Hz to 0.25Hz (Matsunaga, Harada et al. 2001)
In the study reported by Calvert and Wall in 2001, they found that HRV in normal dogs was greater during the night than during the day, as a result of the parasympathetic dominance during sleep hours. On the other hand, the ratio between LF and HF bands did not change over time (Calvert and Wall 2001).

Correlations between time domain measures and frequency domain have revealed a strong relationship ($r > 0.8$) between SDNN and RMSSD and the HF power, indicating that these variables can be used interchangeably when evaluating long-term recordings. On the other hand, correlations between LF power and XSDNN were not as strong (Calvert and Wall 2001).

1.3 Telemetry System

Heart rate can be measured by palpation of the arterial pulsations or by auscultation of heart sounds, but the ECG measures it most accurately. The ECG may be obtained by wires leading from the body surface to the ECG recorder, but most often in studies of safety pharmacology, it is measured by radiotelemetry to minimize perturbation of the subject. By the use of remote radio-telemetry it is possible to obtain physiological measurements from awake, conscious, freely moving dogs (trained and untrained), avoiding distortions and deleterious effects induced by physical or chemical restraint.

There are several advantages of radiotelemetry when collecting long-term, continuous physiological variables: more humane; ability to record from conscious, freely moving
animals; decreased stress related to animal handling; less expensive; more reliable; more user-friendly; more accurate than most non-invasive methods; allows for high resolution and continuous measurements for longer durations of data collection; allows to exclude any human contact to the animals preventing interference; augments legibility and the accuracy of the data, allows for the reduction of numbers of animals used (i.e., sample size); increases the likelihood of detecting QT prolongation due to a drug since it allows having lower heart rates because of decreased stress due to restraint (Miyazaki and Tagawa 2002; Gauvin, Tilley et al. 2006).

1.4 Circadian Rhythms and Heart Rate

Circadian rhythms have been reported in biological systems for many years. These diurnal variations include changes in heart rate, blood pressure and core temperature among others (Ashkar 1979; Miyazaki, Yoshida et al. 2002; Gauvin, Tilley et al. 2006). The autonomic nervous system (ANS) is responsible for these circadian rhythms since it is well known that both components (sympathetic and parasympathetic systems) fluctuate in a daily pattern such that parasympathetic tone increases during the night, whereas the sympathetic activity decreases, depending on the phase of sleep. These changes affect heart rate, which slows down during the night and speeds up during day. There is also a circadian variation in levels of endogenous circulating catecholamines and myocardial contractility decreasing during night (Tzivoni and Stern 1973; Ashkar 1979; Bexton, Vallin et al. 1986; Soloviev, Hamlin et al. 2006).
Besides circadian rhythm, heart rate is affected by exercise and the state of rest or excitement, changes in environmental temperature and altitude, body functions such as digestion and muscular activities, emotions and disease states (Boas and Weiss 1929).

It is known that the function of the ANS has circadian rhythms and its responses to autonomic (sympathetic or parasympathetic) blockade in dogs are completely or partially similar to those observed in other animals (Matsunaga, Harada et al. 2001). During sleep, there is a decrease in the respiratory rate, a narrowing of the pupils and bradycardia. These changes are considered to be the result of an increase in the parasympathetic tone and/or a decrease in the sympathetic activity; therefore changes in the ECG during night are expected (Tzivoni and Stern 1973).

In 1973, Tzivoni and Stern found that in humans, there is a sharp decrease in heart rate during the first hour of sleep, followed by a slight progressive drop. They attribute the first sharp decrease to a sudden reduction of the oxygen demand and a decrease of the cardiac output. The following slight decrease was attributed to a gradual increase in vagal tone (Tzivoni and Stern 1973).

It has been reported that the maximal heart rate in dogs is achieved when there is a change from light to darkness, and the minimal heart rate is related to the change from darkness to light (Ashkar 1979). However some studies show no large differences in heart rates recorded during day from those recorded during night (Ashkar 1979; Gauvin, Tilley et al. 2006; Soloviev, Hamlin et al. 2006).
Soloviev et al in 2006 found that there was a pattern in which heart rate decreased at night (6pm-6am) and peaked (7.4% lower) at the end of the dark period, showing a weak diurnal pattern in Beagle dogs (Soloviev, Hamlin et al. 2006).

Several studies performed in awake and conscious dogs have reported an increase of heart rate just before and during feeding. After feeding, heart rate decreases gradually during the rest of the day (Anderson, Talan et al. 1990; Miyazaki, Yoshida et al. 2002; Gauvin, Tilley et al. 2006; Soloviev, Hamlin et al. 2006). However, Miyazaki et al demonstrated that the changes in HR and BP early in the morning are not related to physical activity (Miyazaki, Yoshida et al. 2002). In the study reported by Soloviev et al in 2006 heart rate showed 3 different peaks during a 24-hour recording: during dosing, and during daily observations of the dogs by the technical personnel both in the afternoon and in the morning (Soloviev, Hamlin et al. 2006).

In 2001, Matsunaga et al found that there are clear circadian rhythms in heart rate, absolute and normalized units of high frequency and low frequency powers, LF: HF ratio (Matsunaga, Harada et al. 2001)

The influence of time of day (daily variations of heart rate) can be divided into two different changes: short-term changes, long-term changes. Short-term (sudden and short-lived) changes are the result of exercise, excitement, and barking, and are not considered to be circadian, whereas long term-changes occur over 24 hours and are true circadian rhythms (Miyazaki, Yoshida et al. 2002; Gauvin, Tilley et al. 2006). There is evidence that circadian rhythms are not just determined by the changes from dark to light or vice
versa but they are also seasonal and are affected by different physiological events. In spring, heart rate is highest when compared to the other 3 seasons (Soloviev, Hamlin et al. 2006).

The patterns of diurnal variation of the heart rate reported in carnivores (especially in laboratory dogs) are different from those observed in monkeys, rodents and humans, in which there are two well-defined periods: One daily active period that alternates with one daily sleeping period. With these well-defined cycles it has been proven that during the sleeping period, in these species, there is no food or water consumption though the urine output is maintained. On the contrary, carnivores (including dogs) have short sleep-wake periods (Anderson, Talan et al. 1990; Miyazaki, Yoshida et al. 2002). Anderson et al reported that the laboratory dog sleeps 30% during daylight and 61% during dark (Anderson, Talan et al. 1990).

There are few studies on the influence of the circadian rhythms on heart rate variability in animals (Matsunaga, Harada et al. 2001). Matsunaga et al (2001) found that in dogs, the circadian rhythm of heart rate variability follows a similar pattern to that described in humans. The HF power found in this study was extremely high, resulting in a low LF: HF ratio, compared to that in rats, cats, horses and humans. They believe that this discrepancy can be due to the spontaneous sinus respiratory arrhythmia, since the variations in RR intervals reflects a vagal activity due to respiration, being reflected in the HF component (Matsunaga, Harada et al. 2001).
There are reports that indicate that there is a circadian variation in the incidence of cardiac sudden death in humans, with a greatly increased incidence during the morning (Willich, Levy et al. 1987; Willich 1990). In 1987, Willich et al, found that the data collected during the Framingham Heart Study, showed a meaningful circadian variation in the incidence of cardiac sudden death. They found that the incidence of cardiac sudden death was higher from 6AM to noon peaking between 7 AM and 9 AM. Additionally, they found a decrease in the incidence of cardiac sudden death between 9AM and 1 PM. Finally, they found that the incidence of cardiac sudden death during regular sleep hours (11 PM and 6 AM) was lower when compared to working hours. During this study people who were found dead in bed where considered to have died between midnight and 6 AM. (Willich, Levy et al. 1987).

It is believed that this circadian variation in cardiac sudden death is attributed to the increase in arterial pressure that occurs in the morning since it predisposes to the rupture of atherosclerotic plaques. Also, it is believed that the cardiac sudden death is the result of a primary arrhythmia, which is more likely to develop early in the morning due to the increased sympathetic tone (Willich, Levy et al. 1987; Willich 1990).

1.5 Electrocardiographic parameters (PQ, QRS, QT, QTcF, TQ, QT: TQ) and their relationship with Heart Rate

There is great variability among electrocardiographic parameters among different animal species and within the same species. Due to this variability, it is extremely important to
determine the physiologically normal ranges of electrocardiographic parameters for a specific animal species in order to reduce the sample size needed in a study to detect a significant change in these parameters due to a drug and not to a physiological status. Several studies have been performed in order to establish these normal physiological ranges in dogs.

1.5.1 PQ Interval

PQ interval is the time elapsing between the beginning of the P wave and the beginning of the QRS complex. It corresponds to the atrioventricular conduction time or the time taken for the electrical impulse to traverse the atria, to reach the AVN, and to begin to depolarize the ventricles.

AV conduction is regulated directly by the ANS, where the parasympathetic tone decreases the conduction velocity, and the sympathetic activity increases the conduction velocity through the head of the AVN. However, it is also influenced indirectly by changes in heart rate (Warner and Loeb 1986).

As previously mentioned, during night the parasympathetic tone increases and the sympathetic activity decreases leading to a decrease of the intra-atrial conduction velocity and in the conduction velocity from the atria to the ventricles, therefore, prolonging the PQ interval (Tzivoni and Stern 1973; Hanton and Rabemampianina 2006). However, sometimes a decrease of the PQ interval has been found in humans and animals (Soloviev,
Hamlin et al. 2006). Tzivoni and Stern, in 1973, found a slight increase in the PQ interval in healthy humans. However, this prolongation was not statistically significant (Tzivoni and Stern 1973).

On the other hand, Soloviev et al in 2006 observed a shortening of the PQ interval during night (Soloviev, Hamlin et al. 2006). This shortening of PQ interval associated with a slow heart rate is attributed to the fact that having a slower heart rate provides more time for the nodal cells to complete their relative refractory period, whereas during the day because of a faster heart rate, the cells have not completely recovered from the refractory period, thus prolonging the PQ interval (Hamlin 1972; Warner and Loeb 1986; Soloviev, Hamlin et al. 2006). These two different mechanisms have been proposed in order to explain two different results. The relationship between heart rate and PQ interval results from a balance of these two opposite mechanisms (Hanton and Rabemampianina 2006). Therefore, the AV conduction is regulated by a poorly-understood association between the autonomic nervous system (parasympathetic and sympathetic) and heart rate (Warner and Loeb 1986).

In another study, Gauvin et al, found a very stable PQ interval over 22 hours of recording with a variability of approximately 10ms (Gauvin, Tilley et al. 2006). In 1986, Warner and Loeb demonstrated that the AV conduction time changes with the respiratory cycle (tonic autonomic neural activity) and with changes in arterial blood pressure (baroreceptor reflex neural activity) (Warner and Loeb 1986).

There are few reports about the relationship between heart rate and the PQ interval. However, it has been reported that there is an inverse linear relationship between heart rate and PQ interval (Hanton and Rabemampianina 2006). Soloviev et al found that this
relationship is stronger in females than males, especially at slow heart rates (Soloviev, Hamlin et al. 2006).

1.5.2 QRS Complex

The QRS complex is the interval from the beginning of the Q wave to the termination of the S wave, representing the time of ventricular depolarization. The normal QRS complex in dogs lasts about 40 to 60 milliseconds and it does not seem to be affected by either heart rate (Gauvin, Tilley et al. 2006; Hanton and Rabemampianina 2006; Soloviev, Hamlin et al. 2006) or time of day.

1.5.3 QT interval and QT corrected (QTc) for heart rate or RR interval

QT interval is the time from the beginning of the Q wave to the end of the T wave, representing the duration of ventricular electrical activity from depolarization (QRS complex) to complete repolarization (ST-T segment) and it is known as electrical systole. Though the QRS complex is an important part of the QT interval, it is brief and it does not vary too much. On the other hand, the ST-T segment varies with time of day and with heart rate, and is affected by changes fluxes of specific ions (K⁺, Ca⁺⁺, Cl⁻) (Oguchi and Hamlin 1994; Hanton, Nahas et al. 2001; Hanton 2001).
In dogs, QT interval lengthens with different pathological states such as ion imbalances (e.g.; hypocalcemia, hypokalemia), autonomic imbalance, central nervous system disorders, interventricular conduction defects, and some poisonings (Hanton, Nahas et al. 2001).

QT interval is directly and curvilinearly related to RR interval and linearly and inversely related to heart rate (Hanton, Nahas et al. 2001). Due to this relationship between heart rate and QT interval, it is important to relate QT to the preceding RR interval or possibly to the history of RR intervals preceding it. Changes in QT interval are known to respond to changes in RR interval, which necessitates relating the two to exclude effects of heart rate. In dogs, the time constant for these changes has been reported to be approximately 2-3 seconds, which is translated to 3 to 5 heartbeats or a complete respiratory cycle. Therefore, during RSA (RR intervals shortened during inspiration and prolonged during expiration), the QT interval is somehow independent of the change in the preceding RR interval, and becomes more dependent on the mean RR interval for a group of beats preceding it—ST-T (Oguchi and Hamlin 1993; Oguchi and Hamlin 1994; Hanton, Nahas et al. 2001; Miyazaki and Tagawa 2002; Soloviev, Hamlin et al. 2006).

It has been proven that when the heart rate changes, there is a change in the rate of flux of ion responsible for repolarization. Some of these changes occur within seconds and some require more time (minutes or even hours) to be completed after the change in heart rate (Oguchi and Hamlin 1994; Hanton, Nahas et al. 2001). These slow modulations of repolarization and its prolonged retention or delays in repolarization are described as QT memory. Several theories have been proposed to explain the mechanism for this memory of the QT interval: 1- small ion currents can be developed between different regions of the
ventricular myocardium due to an asynchronous depolarization and/or repolarization, altering the repolarization of a specific area in the ventricular myocardium; 2- the presence of $I_{TO}$ channels in the subepicardial cells, driving $K^+$ more rapidly out of these cells when compared to subendocardial cells, creating an asynchronous repolarization of the ventricular myocardial wall (Oguchi and Hamlin 1993; Oguchi and Hamlin 1994; Hanton and Rabemampianina 2006; Kijtawornrat, Panyasing et al. 2010).

Also, it has been proven that the QT interval may be prolonged independently of heart rate during sleep hours, reflecting changes in the ANS activity (increased parasympathetic tone, decreased sympathetic activity or both) and or decreased concentration of plasma catecholamines (Bexton, Vallin et al. 1986; Smetana, Batchvarov et al. 2003). However, in Beagle dogs, Gauvin et al found limited changes in QT over 22 hours (Gauvin, Tilley et al. 2006). The influence of the ANS on the QT interval is still controversial. Although the influence of the sympathetic nervous system on the QT interval has been demonstrated, the influence of the parasympathetic nervous system is still not well understood. While a number of studies have suggested a strong influence of the parasympathetic activity on QT interval, others have made emphasis on the sympathetic tone suggesting a small influence of the parasympathetic activity on repolarization duration (Abildskov 1985; Murakawa, Yamashita et al. 2000; Can, Aytemir et al. 2002).

It is believed that under normal circumstances, the ventricular repolarization is mainly regulated by the sympathetic activity, while the effects of the parasympathetic tone are considered to be indirect and mainly secondary to changes in heart rate (Can, Aytemir et al. 2002).
In 1989, Lecocq et al, found that in humans the regulation of the QT interval is mainly due to parasympathetic activity during exercise, since they were able to mimic the effects of exercise (shortening of QT interval) by giving atropine while there was no effect on QT interval with β-adrenergic stimulation (Lecocq, Lecocq et al. 1989). QT interval is prolonged during sleep hours in humans and this effect was attributed to an increase in parasympathetic tone or a decrease in sympathetic activity (Lecocq, Lecocq et al. 1989).

On the contrary, Bexton et al (1986) found that, in humans, the QT interval tended to be prolonged during sleep, being the longest at 5 AM when the concentration of plasma catecholamines was the lowest. In this study, during the waking hours the QT interval decreased rapidly and the plasma concentrations of catecholamines rose and there was inhibition of the parasympathetic cardio-inhibitory center (Bexton, Vallin et al. 1986). However, in 1973, Tzovoni and Stern, found no differences between sleep hours and awake hours due to a high variability, though they reported a tendency to be prolonged (Tzivoni and Stern 1973).

During waking hours, QT was also influenced by other factor such as exercise and eating (Bexton, Vallin et al. 1986; Miyazaki and Tagawa 2002). In dogs, Miyazaki and Tagawa found that the diurnal rhythm of the QT interval behaved differently from that in humans. In their study the QT interval in Beagle dogs was shorter during feeding times, high activity and physical examinations, whereas it was longer after feeding and early in the morning. They believed that it is related to the short-term wake-sleep cycles during day and night, showing the dependence of the QT interval on the physical and emotional status of the animals (Miyazaki and Tagawa 2002).
Since it is well known that QT depends on the heart rate (HR), but it is usually compared to RR interval (60,000ms /HR). The QT interval should be evaluated based on the heart rate in order to determine if a drug has a deleterious effect on the QT interval that may or may not be related to its effect on heart rate (Hanton, Nahas et al. 2001). There are several formulae that are intended to correct QT for a specific RR interval (1000ms) or a heart rate (60bpm). It is believed that the correction of the QT interval for the heart rate reflects sympatho-vagal balance and it is the traditional method to evaluate the duration of the ventricular depolarization (Gauvin, Tilley et al. 2006). The best correction would produce a QT corrected (QTc) that is independent of RR interval, allowing a good comparison of the direct effect of the autonomic nervous system or the effect of a drug on the QT interval between the control group and treated groups (Smetana, Batchvarov et al. 2003). Several studies have been performed in order to determine which formula corrects best the QT interval for the heart rate in dogs. However, some of these formulae do not correct QT interval as well as others in dogs (Hanton, Nahas et al. 2001; Hanton and Rabemampianina 2006).

\textit{QTcB (Bazett):} It was the first quantification of the relationship between heart rate and QT interval in humans. Mathematically it is defined as: \( \text{QTcB} = \frac{\text{QT}}{\sqrt[2]{\text{RR}}} \text{(ms}^{1/2}) \). It has been thoroughly studied in research dogs, showing an overcorrection of the QT interval at high heart rates and an undercorrection at low heart rates. Bazett’s formula in dogs produces an inversion of the relationship between QT interval and RR interval (QTc decreases when RR increases), showing that QT depends more on RR interval (Hanton, Nahas et al. 2001). This leads to large errors at extremes of heart rate, and to incorrect conclusions of how drugs affect QT when drugs also change heart rate. Therefore, its use is not recommended in
safety pharmacology studies in Beagle dogs (Hanton, Nahas et al. 2001; Miyazaki and Tagawa 2002; Gauvin, Tilley et al. 2006; Soloviev, Hamlin et al. 2006). The difference in results when applying Bazett’s formula between dogs and humans can be explained by differences between species in cardiac electrophysiology due to complex interactions between regulators of cardiac function (autonomic nervous system). Additionally, physiological differences between dog breeds can impact the relationship between QT interval and RR interval or heart rate (Hanton, Nahas et al. 2001)

\[ QTcF \text{ (Fridericia): Is a formula, which takes into account the physiologic shortening of the QT interval, which occurs as the HR increases, allowing a comparison of the QT across a range of rates. It is mathematically defined as: } QTcF = \frac{QT}{RR^{1/3}} \text{ (seconds). It corrects the QT interval to that which would be observed at a heart rate of 1 cycle per second (60bpm) or a RR interval of 1,000ms. It has been reported that Fridericia’s correction formula has a tendency to “over-correct” the QT interval at high heart rates (between 60 and 160bpm). As well as Bazett’s formula, Fridericia can create false positives and negatives (Gauvin, Tilley et al. 2006; Soloviev, Hamlin et al. 2006). Hanton et al (2001), found that Fridericia’s formula fits better the correction of QT for heart rate than Bazett’s formula. However, they found also a negative correlation between QTcF and heart rate (Hanton, Nahas et al. 2001). Despite these, its use has been recommended in safety pharmacology studies performed in Beagle dogs with the understanding of this effect on the correction of the heart rate.}\]
QTcF is the widely used since it has been approved by the FDA as a method for evaluating the likelihood of developing arrhythmias as an undesired drug effect in safety pharmacology.

**QTcV (Van de Water):** Mathematically it is defined as: \( QTcV = QT - 0.087 \times [RR-1] \). As other formulas, it normalizes QT interval to an RR interval of 1,000ms or a heart rate of 60 beats per minute. However with this formula, QTc values are no longer dependent on heart rate. This formula is supposed to be more reliable than Bazett’s formula when detecting direct effects of drug on QT interval, showing a lower dependency of QT interval on heart rate (Hanton, Nahas et al. 2001). However, Hanton et al (2001) found a slight negative correlation between QTc and heart rate (Hanton, Nahas et al. 2001). In agreement with Hanton et al, Soloviev’s study (2006) they reported that the Van de Water’s correction showed to correct the QT interval for the heart rate in a more accurate form that the other two correction formulas (Soloviev, Hamlin et al. 2006).

Although, circadian variations of QTc could depend on an increased sympathetic activity during the day (Murakawa, Inoue et al. 1992), there is evidence that they depend more on systolic contractile function of the left ventricle than alterations in the ANS balance (Davey 2000; Whitsel, Boyko et al. 2000). Additionally, it has been shown that they also depend on sex, age, and on the correction formula used (Whitsel, Boyko et al. 2000). Smetana et al, 2003, found that by using the Bazett formula, circadian variations on QTc are abolished as a result of the overcorrection imposed by this formula. On the contrary, these variations are overestimated by formulas such as Fridericia, Framingham and Hodges as a result of their
undercorrection, therefore they copy the circadian pattern of the heart rate (Smetana, Batchvarov et al. 2003).

There are more formulas proposed in the literature that were created to correct QT, such as Matsunaga which corrects QT interval for a heart rate of 100bpm instead of 60bpm, Grauwiler, Todt, and Oguchi and Hamlin which correct QT interval based on linear relationship between heart rate (or RR interval) and QT interval in dogs (Hanton, Nahas et al. 2001).

Though the QT interval is dependent on the heart rate, it has been proven that in dogs, the QT interval is independent of the variations in heart rate due to a respiratory sinus arrhythmia. It rather depends on a mean heart rate (Oguchi and Hamlin 1993).

Oguchi and Hamlin studied and defined the relationship between QT interval and heart rate in dogs in 1993. They found that mean intercept (value of the dependent variable (y) when the independent variable (x) is zero), was 302 milliseconds, and the mean slope was -0.550 and more than 40% of the variability of QT could be explained by the heart rate alone (Oguchi and Hamlin 1993). Later, in 2001, Hanton et al found that just 47% of the variations in QT interval can be explained by changes in heart rate (Hanton, Nahas et al. 2001)

In 2002, Miyazaki and Tagawa described the distribution of the relationship between QT interval and RR interval. They demonstrated that the distribution of this relationship in ECGs obtained with telemetry units were different from the one obtained by traditional
ECG (restraint inducing stress). They described the telemetry relationship as a cloud of wide range of RR intervals, with a positive correlation curve and small variation. On the contrary, the traditional ECG relationship was described as an elliptical distribution, with a narrower range of RR intervals and a big variation. Additionally, they found that the mean QT interval was 15% longer and had a higher variability on the telemetry records when compared to the traditionally obtained ECGs. Finally, they found that the coefficient $\beta$ (slope) ranged from 0.153 to 0.495, with a big variability during the day showing unexpected changes that were related to physical activity, excitement, and stress (Miyazaki and Tagawa 2002).

The variability of the QT interval has been considered a non-invasive marker of cardiac repolarization, since a high QT variability (QT instability) and prolongation of QT interval duration have been associated with severe ventricular arrhythmias, TdP and sudden death (Lecocq, Lecocq et al. 1989; Hanton, Nahas et al. 2001; van der Linde, Van de Water et al. 2005; Gauvin, Tilley et al. 2006). While QT variability (instability) has been associated with enhanced risks of ventricular arrhythmias and cardiac sudden death, heart rate variability is related with a reduced risk of lethal arrhythmias. The most common measurements to evaluate QT variability are: total variability (TV), short-term variability (STV) and long-term variability (LTV) (van der Linde, Van de Water et al. 2005).
1.5.4 Ventricular Restitution (TQ and QT: TQ)

Electrical restitution is defined as the ability of the heart to recover form one beat to the
next one. It is the relationship between an action potential duration (APD) (QT interval in
the ECG) and its preceding diastolic interval (TQ interval in the ECG), and it is an
important determinant of the beat-to-beat dynamics at all different heart rates or RR
intervals (action potential duration shortens when heart rate increases). It has been used to
predict arrhythmia liability (Weiss, Chen et al. 2002; Berger 2004; Fossa, Wisialowski et
al. 2006; Wilson and Rosenbaum 2007).

The “restitution hypothesis” supposes that if during a specific diastolic interval (DI) the
kinetics of ion channels or intracellular calcium transients is not at its steady state, an APD
alternans (alternation of short-long diastolic intervals followed by a shot-long APD)
preserves stability at a given heart rate. When the degree of alternation is increased to the
point where there is no more diastolic interval available, there is a break of the wave,
leading to a triggered unstable reentry and arrhythmia develops (Fossa, Wisialowski et al.
2006; Wilson and Rosenbaum 2007).

Normally, wavelength oscillations because of the properties for the APD to decrease when
heart rate increases. This property is very important in cardiac physiology since shortening
systole duration at high heart rates ensures adequate time for diastole (Weiss, Chen et al.
2002). Around half of the time between heart beats; the hearts gets its nutrients and oxygen
through coronary blood flow, cardiac filling and for the ionic transients to return to a steady
state (DI or TQ) before the next beat. Usually, when heart rate increases, QT decreases in
order to keep constant the time in which the heart will be in diastole (RR-QT=TQ). When there is a prolongation of the QT interval (long QT syndrome or drug induced) and an increase in heart rate near the point in which TQ is disproportionally reduced or close to zero (RR=QT), is the perfect substrate for lethal ventricular tachycardia and or ventricular fibrillation to occur (Weiss, Chen et al. 2002; Fossa, Wisialowski et al. 2006). Therefore, drugs that prolong the APD, such as antiarrhythmic class III, affect the DI and could decrease the threshold heart rate for instability and the heart becomes more sensitive to abrupt changes in autonomic influences that originate rapid changes in heart rate to generate the arrhythmogenic liability (Fossa, Wisialowski et al. 2006).

The relationship between APD or conduction velocity (CV) and HR can be quantified by electrical restitution curves. These curves, describe the time course of recovery of the APD as a function of the DI or cycle length (Franz 2003). In these curves the APD or CV is plotted against DI (or QT against TQ) at a certain heart rate. The steepness of the restitution curve is considered to be of extreme importance for determining wavelength stability. If the slope is >1, a small change in DI leads to a larger change in the wavelength, originating APD alternans, and can lead to wavebreak (predisposing to fibrillatory rhythms). On the contrary, if the slope is <1, APD alternans is reduced and the oscillations are inhibited, therefore the wave remains intact (Gilmour and Chialvo 1999; Weiss, Chen et al. 2002; Franz 2003; Ng, Brack et al. 2007; Wilson and Rosenbaum 2007).

There are several physiological events that have been related to the development of T-wave alternans such as elevated heart rate, coronary arteries occlusion and reperfusion, and sympathetic nerve stimulation. On the contrary, parasympathetic stimulation, blockade of
beta-adrenergic receptor, and sympathetic denervation have shown to decrease T wave alternans, reducing the susceptibility to ventricular arrhythmias and fibrillation (Verrier, Kumar et al. 2009).

There is evidence that the ANS (corticosteroids and hormonal?) is closely related to ventricular arrhythmogenesis independently from heart rate, leading to sudden cardiac death (Ng, Brack et al. 2007; Verrier, Kumar et al. 2009).

In 2007, Ng, Bracker al, demonstrated that sympathetic stimulation of hearts, in Langendorff preparation, steepens the slope of the APD restitution curve and decreases the threshold to ventricular fibrillation, increasing the vulnerability of the heart to ventricular fibrillation. On the contrary, while vagal stimulation flattens the slope and raises the threshold to ventricular fibrillation, therefore offering a protective effect (Ng, Brack et al. 2007).

While the mechanisms by which the parasympathetic activity reduce the T wave alternans are not very well studied, the mechanisms involved in the sympathetic effect independent from the heart rate in T wave alternans have not been completely understood. However, it is believed that the increased metabolic demands can worsen the myocardial ischemia, decreasing the amounts of glucose and fatty acids. This energetic deficit creates an imbalance in intracellular adenosine triphosphate (ATP), which is important for the calcium reuptake by sarco/endoplasmic reticulum calcium ATPase (SERCA) (Verrier, Kumar et al. 2009).
Is more difficult to generate the scenario for arrhythmias in dogs than in humans in order to evaluate biomarkers of arrhythmic liability such as restitution. This could be explained by the pronounced RSA present in the normal cardiac physiology of the dog, in which ionic mechanisms may adjust faster to changes in heart rate (Fossa, Wisialowski et al. 2006). Therefore, unlike humans, RR intervals (heart rate) in dogs can fluctuate between 400 and 1600ms in each respiratory cycle (Fossa, Wisialowski et al. 2006).

Fossa et al (2006) found, that in dogs, potassium channel blockers (inhibition of both $I_{Kr}$ and $I_{Ks}$) produced an increment of around 70 to 120ms in the QT interval without incidence of arrhythmias. Contrary to this finding, in humans such prolongation of the QT interval couldn’t be tolerated without producing arrhythmia (Fossa, Wisialowski et al. 2006).

Additionally, Kijtawornrat et al (2010), found that in dogs the percentage of cardiac cycles that had a QT: TQ ratio greater than 1 was 5%, while it has been reported to be approximately of 20%. They suggest that this predisposition of humans to suffer ventricular arrhythmias compared to dogs may be associated to the incidence in humans of ischemia and infarction, morbidities quite rare in dogs (Kijtawornrat, Panyasing et al. 2010).

1.6 Heart failure model in dogs

Congestive heart failure is a common syndrome associated with a host of several alterations at multiple levels going from the subcellular level to the whole organ level. These changes
include structural and functional adjustments that may result in cellular death (Moe and Armstrong 1999). It is characterized by impairment of both systolic and diastolic left ventricular function, aberration of the neuroendocrine axis, and increase aortic impedance (Binkley, Nunziata et al. 1991; Komamura, Shannon et al. 1992; Eaton, Cody et al. 1993; Moe and Armstrong 1999).

The study of heart failure requires viable and reproducible animal models in which changes in myocardial structure and function can progress to heart failure, and the ventricular dysfunction can be understood and quantified, in order to evaluate the efficacy of the interventions needed to either reverse, stop or at least delay the process of the disease (Sabbah, Stein et al. 1991; Patten and Hall-Porter 2009).

A considerable amount of the understanding of the molecular and cellular basis of the cardiovascular biology has come from small animal models, mainly from mice. However, there are several significant differences in cardiac characteristics between mice and humans (e.g.; heart rate, oxygen consumption, adrenergic receptor ratios, contractile protein expression and phenotypic differences in stem cells from humans and mice) that make it difficult and problematic to extrapolate from rodents to humans (Shaffer, Singer et al. 2002; Haghighi, Kolokathis et al. 2003; Ginis, Luo et al. 2004; Dixon and Spinale 2009; Richter, Xie et al. 2011).

On the contrary, large animal models of heart failure mimic more closely human cardiac physiology, function, and anatomy. These similarities could make them more suitable to
evaluate the discoveries from rodents as possible therapies and interventions for heart failure (Dixon and Spinale 2009).

In order to be considered an ideal preparation, an animal model must fit some requirements: 1) availability, 2) it has to be as close to the clinical situation as possible 3) it has to be stable for adequate periods of time in such way that it will give reasonable flexibility for measurements and treatments, 4) it has to be standardized and reproducible. Additionally, there should be no need for induce trauma that could disturb compensatory mechanisms, and should produce predictable and quantifiable results (Smiseth and Mjos 1982; Armstrong, Stopps et al. 1986).

Though several animal models have been used to evaluate particular aspects of congestive heart failure, they may not entirely mimic the patterns that occur during heart failure in humans (Moe and Armstrong 1999). These models have involved different mechanisms such as pressure overload (aortic and/or pulmonary stenosis), volume overload (aortic insufficiency or arteriovenous shunts), tachycardia (pacing-induced), myocardial ischemia (ligation or embolisms), or myocardial toxins (doxorubicin or catecholamines) (Smith and Nuttall 1985; Toyoda, Okada et al. 1998; Moe and Armstrong 1999; Vanoli, Bacchini et al. 2004; Dixon and Spinale 2009; Patten and Hall-Porter 2009).
1.6.1 Tachycardia-induced cardiomyopathy (Pacing-induced heart failure)

Gossage et al. first described cardiomyopathy induced by tachycardia in 1913, in a patient with atrial fibrillation; it has also been documented in patients with hyperthyroidism, and with ANS and peripheral circulatory alterations (Coleman, Taylor et al. 1971; Umpierrez, Challapalli et al. 1995; Khasnis, Jongnarangsin et al. 2005). Then, in 1962, Whippel et al reported for the first time the experimental model for heart failure; subsequently, it was modified in order to provide a good model that can reproduce heart failure with fidelity (Armstrong, Stopps et al. 1986; Shinbane, Wood et al. 1997; Moe and Armstrong 1999; Khasnis, Jongnarangsin et al. 2005; Dixon and Spinale 2009).

In humans, this pathology can be described as a condition characterized by atrial or ventricular dysfunction as a result of and increased atrial or ventricular rate, such as atrial fibrillation, and atrial or ventricular tachycardia, without any underlying structural cardiac pathology (Khasnis, Jongnarangsin et al. 2005). Rate and duration of the tachycardia are the main factors of the beginning, development, and reversibility of the cardiomyopathy, in such a way that with higher rates the cardiomyopathy manifests earlier (Khasnis, Jongnarangsin et al. 2005).

The best-characterized and most commonly used large animal model of dilated cardiomyopathy (DCM) is the pacing induced tachycardia model (Vanoli, Bacchini et al. 2004; Dixon and Spinale 2009). In this experimental model, the dysfunction of the left ventricle is established early after pacing is started and is more noticeable in chronic ventricular tachycardia than in chronic supra-ventricular tachycardia (Khasnis,
It produces biventricular systolic and diastolic dysfunction that is time and rate dependent, meaning that the severity of the dysfunction can be controlled by increasing or decreasing pacing time and frequency (Shinbane, Wood et al. 1997).

There are several advantages that make this model very appropriate for studying congestive heart failure: 1) It avoids major surgical trauma (e.g. thoracotomy) 2) Heart failure is developed over a period of several weeks, allowing for sequential evaluation 3) the rate of the stimulus can be easily controlled by using a programmable pacemaker 4) it produces a simpler, more predictable, more stable and well defined clinical syndrome of biventricular failure with cardiomegaly, hypoperfusion, pulmonary congestion, cachexia and ascites, imitating very closely the human state, 5) neurohormonal alterations similar to those in humans, 6) generation of different and predictable degrees of left ventricular failure and dilation 7) heart failure induced by tachy-pacing is reversible after cessation of pacing (Wilson, Douglas et al. 1987; Armstrong, Howard et al. 1989; Moe and Armstrong 1999; Dixon and Spinale 2009).

Various pacing protocols have been proposed in different animal species such as canine, porcine and ovine (Dixon and Spinale 2009); the most commonly used uses 250 beats per minute (bpm) for 3-5 weeks (Armstrong, Stopps et al. 1986; Armstrong, Howard et al. 1989; Moe and Armstrong 1999).

Though the mechanism responsible for inducing cardiomyopathy is still not completely understood, myocardial energy depletion and impaired utilization of energy, myocardial ischemia, abnormalities of cardiac calcium regulation and myocyte and extracellular
remodeling have been proposed (Shinbane, Wood et al. 1997). However, in 1987, Wilson et al studied the contribution of myocardial ischemia to pace-induced heart failure and found that in the hearts studied there was no evidence ischemia since while myocardial lactate and oxygen extraction both remained stable, myocardial blood flow increased. Therefore, they concluded that myocardial ischemia is not responsible for pacing induced failure (Wilson, Douglas et al. 1987).

Several studies have been performed in order to evaluate the efficiency of the pace canine model of ventricular failure in mimicking all the pathophysiological components of the heart failure syndrome (Moe, Stopps et al. 1989; Binkley, Nunziata et al. 1991; Komamura, Shannon et al. 1992; Eaton, Cody et al. 1995).

**Fluid retention:** Pulmonary congestion and edema are two of the main manifestations of congestive heart failure. It has been proven that pacing dogs at a rate of 250 bpm for three weeks or more consistently produces severe radiographic evidence of pulmonary congestion, pulmonary edema, and/or ascites (Coleman, Taylor et al. 1971; Armstrong, Stopps et al. 1986; Moe, Stopps et al. 1989; Binkley, Nunziata et al. 1991; Moe and Armstrong 1999; Dixon and Spinale 2009). In radiographs, it is possible to observe increased cardiac size and pulmonary congestion (Armstrong, Stopps et al. 1986). Atrial natriuretic peptide (ANP) concentration increases early during pacing suggesting that it plays an important role during the early stages of heart failure (Moe, Stopps et al. 1989; Moe and Armstrong 1999), whereas hyponatremia develops during severe congestive heart failure, possibly due to activation of the renin-angiotensin system (RAS) in advanced stages (Moe and Armstrong 1999).
**Myocardial remodeling and dysfunction:** In all the species studied, chronic atrial and ventricular pacing produce marked dilation of all chambers getting a progressively spherical shape of the ventricles and atria, accompanied by little or no ventricular hypertrophy. On the contrary, there is evidence of a significant increase in left atrium size with an increase in left and right atrial appendage weights, indicating atrial hypertrophy (Wilson, Douglas et al. 1987; Armstrong, Howard et al. 1989; Shinbane, Wood et al. 1997; Moe and Armstrong 1999). In echocardiographic evaluation of cardiac function it has been demonstrated that there is a decrease in ventricular ejection fraction and fractional shortening (Binkley, Nunziata et al. 1991; Komamura, Shannon et al. 1992; Eaton, Cody et al. 1993; Eaton, Cody et al. 1995). Mitral regurgitation develops late in the model to a mild or moderate degree, which is correlated to the systolic cross-sectional area of the ventricle and opposed to the ejection fraction (EF), wall thickness and mitral annular size (Armstrong, Howard et al. 1989; Shinbane, Wood et al. 1997). Additionally, there is a reduction in cardiac output, stroke volume, left ventricular dP/dt, left ventricular systolic pressure, mean pulmonary arterial pressure, right atrial pressure, and mean arterial pressure (Armstrong, Stopps et al. 1986; Komamura, Shannon et al. 1992; Shinbane, Wood et al. 1997). On the contrary, there is an increase in left ventricular end diastolic pressure, left ventricular end systolic pressure, left ventricular end diastolic volume, pulmonary capillary wedge pressure, left ventricular filling pressure, end diastolic wall stress, impedance of the aorta and systemic vascular resistance (Armstrong, Howard et al. 1989; Komamura, Shannon et al. 1992; Eaton, Cody et al. 1993).

**Autonomic innervation:** It has been proven that in the pacing-induced heart failure model there is a decrease in the parasympathetic activity and an increase in the sympathetic tone
demonstrated by an increase in the low frequency band and a decrease of the high
frequency power when analyzing the heart rate variability by the spectral analysis. These
changes have been seen before there are evident changes in the arterial tone (Binkley,
Nunziata et al. 1991; Eaton, Cody et al. 1995). Baroreceptor desensitization occurs
(Armstrong, Howard et al. 1989) as well as a down-regulation of β1-adrenergic receptors
and post-receptor abnormalities of adenylate cyclase and calcium handling (Shinbane,
Wood et al. 1997)

*Neurohormones and cytokines:* Pacing-induced heart failure is the best model to study the
pathophysiologic role of neurohormonal activation in heart failure due to its facility to
produce a strong stimulation of almost all the neurohormonal system in a time-dependent
mode (Moe and Armstrong 1999; Dixon and Spinale 2009). Concentrations of
serum/plasma creatinine, urea, norepinephrine (NE), renin, angiotensin II, aldosterone,
corticosterone and antidiuretic hormone (ADH) increase progressively and significantly
(Riegger and Liebau 1982; Moe, Stopps et al. 1989). It has been demonstrated that in
pacing-induced heart failure there is an initial increase in catecholamines with a subsequent
steady state, while renin and endothelin rise later when the more advanced changes occur
(Dixon and Spinale 2009). In addition to the well-known changes in the sympathetic
nervous system, renin-angiotensin-aldosterone system and the natriuretic system, there is an
increase in endothelin-1 (ET-1), and proinflammatory cytokines such as tumor necrosis
factor alpha (TNF-α) (Moe and Armstrong 1999).
Some of the major limitations of this heart failure model include: 1) unlike human heart failure, failure produce by t achy-pacing, it is dependent upon continuous pacing. Therefore, once the pacemaker is turned off it is reversible. D ue to this limitation, physiological measurements must be performed during temporary interruptions of pacing. 2) There is no left ventricular hypertrophy. 3) The resultant myocardial structure is different from the one observed with other heart failure models such as volume overload and myocardial ischemia  4 ) This model fails to reflect the complete pathophysiology of heart failure (Moe and Armstrong 1999; Dixon and Spinale 2009).

1.6.2 Ischemic Cardiomyopathy

Myocardial ischemia, myocardial infarction or both commonly trigger heart failure in humans (Dixon and Spinale 2009). Different animal models have been created to produce myocardial ischemia by producing coronary occlusion (Smiseth and Mjos 1982; Smith and Nuttall 1985; Sabbah, Stein et al. 1991; Sakaguchi, Sakakibara et al. 2003; Schmitto, Ortmann et al. 2008; Patten and Hall-Porter 2009). T his coronary occlusion can be performed extravascularly (coronary ligation) or intravascularly by embolism (mercury, plastic cones, performed thrombus, microspheres, among others) (Smith and Nuttall 1985).

Though it is a very attractive model due to its clinical relevance in humans, the resultant infarcts in animal tend to be discrete, due to the different coronary anatomy, the lack of atherosclerotic lesions in animals and the impressive capability of animals to develop
collateral vessels to supply blood to the affected areas of the myocardium (Smith and Nuttall 1985).

**Coronary Microembolization:** Initially, this model implied a single injection in order to induce a big, extensive myocardial infarct. However, this method very often produced malignant arrhythmias and sudden death, therefore it was not an ideal model for chronic studies (Vanoli, Bacchini et al. 2004).

Sabbah et al proposed a modification of the model created by Smiseth et al in 1991. It consists in exposing the dogs to multiple graded and sequential coronary embolic procedures (either left anterior descending or circumflex coronary arteries) consecutively (once a week) over a period ranging from three weeks up to ten weeks, which allows partial recovery between ischemic insults, resulting in lower mortality (Sabbah, Stein et al. 1991; Vanoli, Bacchini et al. 2004; Dixon and Spinale 2009). Once a low left ventricular function is established, there is a progression of the left ventricular dysfunction in the following three months, accompanied by the consequent decreased ejection fraction, increased left ventricular filling pressure, systemic vascular resistance and atrial natriuretic peptide, activation of the sympathetic nervous system, and the development of heart failure (Sabbah, Stein et al. 1991; Vanoli, Bacchini et al. 2004; Dixon and Spinale 2009).

The chronic heart failure is the result of the loss of contractile myocardium and is characterized by decreased systolic and diastolic functions, left ventricular dilation and hypertrophy, reduced cardiac output, and increased systemic vascular resistance (Vanoli, Bacchini et al. 2004).
Advantages: The canine coronary microembolization model, reiterates the clinical phenotype of the ischemic cardiomyopathy. It is useful to investigate novel pharmacological and surgical treatments (Vanoli, Bacchini et al. 2004; Dixon and Spinale 2009).

Disadvantages: It is a model that requires serial surgical interventions making it complex to produce heart failure. Additionally, it produces heart failure by multiple sites of infarction and remodeling different from that in humans characterized by a single, large focal myocardial infarction. It can induce malignant dysrhythmias that can lead to sudden death after repetitive embolizations. The variety and heterogeneity of the myocardial reaction to the microembolizations makes difficult the interpretation of the biological responses (Dixon and Spinale 2009). Though some of the mortality in this preparation is due to myocardial arrhythmias, this model has not given any useful information about the mechanisms of arrhythmias responsible for sudden death in ischemic heart failure (Vanoli, Bacchini et al. 2004).

Though, in dogs, a partial recovery of the left ventricular function has been described possibly due to the presence of a good collateral circulation and/or recovery of cells that have been reversibly injured by the ischemic event (Sabbah, Stein et al. 1991), Sabbah et al reported no recovery of the left ventricular function when the coronary embolizations were discontinued. They claimed that multiple consecutive microembolizations may consume the compensatory mechanisms available to the myocardial cells over time in such way that the myocardium cannot respond to the loss of viable contractile tissue, leading to a permanent depressed cardiac function (Sabbah, Stein et al. 1991).
1.7 Heart Failure and Electrocardiographic Parameters

Changes in the normal variability of the heart rate (autonomic imbalances) have been associated with several diseases, such as heart failure (left ventricular systolic function), myocardial ischemia and diabetic autonomic neuropathy (Calvert and Wall 2001; Doxey and Boswood 2004; Pereira, Woolley et al. 2008). The degree of myocardial injury may be related to the degree of autonomic imbalance (increased sympathetic and decreased parasympathetic activity), baroreceptor function, and activation of the neuroendocrine system (Eaton, Cody et al. 1995; Haggstrom, Hamlin et al. 1996; Calvert and Wall 2001; Calvert and Wall 2001). Therapy improving the vagal stimulation is assumed to be valuable in patients with congestive heart failure (Haggstrom, Hamlin et al. 1996).

During heart disease, an increase in the heart rate and the loss of heart rate variability are mechanisms that occur in order to compensate the circulatory changes sensed and transmitted to the medulla oblongata by pressure and chemoreceptors. There is a parasympathetic impulse reduction at the SAN decreasing the automaticity at the pacemaker cells, and a reduction of the baroreceptor reflex sensitivity to variations in blood pressure (Eaton, Cody et al. 1995; Haggstrom, Hamlin et al. 1996).

There is evidence in humans that a high sympathetic nervous activity induces cardiac arrhythmias (ventricular tachycardia, ventricular fibrillation) and worsens the arrhythmias when they occur in an injured myocardium, by increasing the heterogeneity of refractoriness within the myocardium and predisposing to re-entrant ventricular arrhythmias (Calvert and Wall 2001; Calvert and Wall 2001; Ng, Brack et al. 2007).
However, the complete mechanism underlying the link between the autonomic nervous system and the development of arrhythmias is still poorly understood (Ng, Brack et al. 2007).

The increased sympathetic activity contributes to the increased peripheral vascular tone and elevation of ventricular afterload (characteristic in heart failure). On the other hand, the decreased parasympathetic activity creates an ideal environment for the development of vasoconstriction, increasing the afterload (Eaton, Cody et al. 1995).

Several studies in Doberman dogs (dilated cardiomyopathy), Cavalier King Charles Spaniel (myxomatous mitral valve disease), and Beagles (congestive heart failure) have shown decreased conventional heart rate variability measures (Pereira, Woolley et al. 2008).

Congestive heart failure has been related to reduced parasympathetic modulations reflected by decreased time domain measures such as SDNN and RMSSD and decreased frequency domain measures such as LF, HF and LF: HF (Calvert and Wall 2001).

Though exact origin of the LF band and the cause of its decreased in heart failure is still not very well understood, it is believed that it shows an imbalance in both sympathetic and parasympathetic outflows as well as a baroreceptor dysfunction (Calvert and Wall 2001).

Several contradictory studies have been done, searching for the correlation between heart rate variability, survival time, and prognostic value for predicting the risk of sudden death in dogs. Some studies have shown no prognostic value in the heart rate variability of dogs.
with cardiomyopathy, whereas some other have shown a very good prognostic value in the same category of patients (Pereira, Woolley et al. 2008)

In 2004, Doxey and Boswood reported that the VVTI in dogs with heart failure was less than 6.75, when the optimum cut-off in dogs is considered to be 7.0. They also reported that in dogs with heart disease but with absent heart failure the VVTI is not different from that in dogs without heart failure. However, heart failure was strongly associated to a low VVTI (Doxey and Boswood 2004).

Matinez Pereira et al, 2008, found that there is a significant correlation between VVTI and survival time in patients with dilated cardiomyopathy ISACHC classes 2 and 3. However, in class I patients, the differentiation between pathological and physiological status is difficult due to the high variability (Pereira, Woolley et al. 2008).

In 2001, Calvert and Wall reported that in dogs with mild and moderate heart failure had greater heart rate variability during night when compared to day time. They believe this occurs because the failure was not as severe to alter the factors that control heart rate variability or because the pronounced sinus respiratory arrhythmia makes heart rate variability an insensitive test for heart failure in dogs. However, in dogs with severe heart failure the heart rate variability did not differ from night to day. On the other hand the LF: HF ratio was not different between night and day in dogs with heart failure regardless the severity of the disease. They believe that there were no differences in this ratio since when spectral components are expressed in absolute values, variations in the total power affect both components of the frequency components (LF and HF) in the same direction and therefore, confound the appreciation of the fractional distribution (Calvert and Wall 2001).
In 2004, Spier and Meurs, found that Boxers with congestive heart failure have lower heart rate variability when compared to healthy Boxers, Boxers with ventricular premature contractions but without signs of heart failure or healthy non-Boxer dogs, showing a high sympathetic activity (Spier and Meurs 2004).

In 1973, Tzivoni and Stern, found that in humans with ischemic heart disease there was no change in PQ, and QT intervals when compared waking hours with sleep time (Tzivoni and Stern 1973). However, a prolongation of the QT and QTc intervals in heart failure has been reported in humans (Kramer, Brill et al. 1986; Davey, Barlow et al. 2000). Additionally, Davey et al., 2000, found that the QT prolongation during heart rate, is more evident at low HR when compared to high HR and suggests the importance of this prolongation in the pathophysiology underlying sudden cardiac death in people with left ventricular dysfunction (Davey, Barlow et al. 2000).

1.8 Rationale for Study

There are relatively few dogs with heart failure—class D of the AHA/ACC or functional class IV of the NYHA—for which there is a consensus to treat in an attempt to minimize or prevent morbidity and/or mortality. There is little argument that the few dogs in class D/IV were at one time in the orders of magnitude more dogs in class B/II—for which there is a consensus not to treat—but that no doubt have pathophysiology leading to morbidity and/or mortality. These pathophysiologies include reduction in EF (indicating decrease contractility and/or increase afterload), reduction in heart rate variability and increased heart rate (indicating altered autonomic balance), lengthening of QT and QTc and increased
in QT: TQ (indicating increased tendency to arrhythmia), and the increase in heart rate alters myocardial energetics stemming from increase myocardial oxygen demand but decreased myocardial oxygen delivery (Qcor). There is little difficulty identifying dogs with class D/IV heart failure; no new modalities are necessary. However if pathophysiologies leading to D/IV could be identified and remediated, the remediation might translate to vast benefits. Thus this study was designed to search for pathophysiologies that might be remediated with (for example) β-blockers, ACE inhibitors, and spironolactone. Furthermore, the ECG is used regularly in studies of Safety Pharmacology. However ECGs are seldom interpreted with respect to the time of day that they were recorded. Furthermore many persons dedicated to drugs development and testing of drugs, know that drugs act differently in patients with heart failure than in normal patients, therefore it is reasonable to conduct preclinical trials in animals with heart failure. Thus the results of this study may be important to the Safety Pharmacologist as well as to the veterinary cardiologist.
2.1 Introduction

Electrocardiography is no doubt the most effective means of interrogating the electrophysiological properties of the heart. This is documented by the ubiquitous use in clinical medicine, in physiology, and in toxicology and safety pharmacology. However electrocardiography has limited use unless the electrocardiographer knows the parameters of the “normal” electrocardiogram against which to compare electrocardiograms intended to interrogate a specific patient, physiological preparation, or experimental subject. Included in the understanding of the electrocardiogram from the “normal”, and to be considered in the interpretation of an electrocardiogram of a patient, physiological preparation, or experimental subject, are the species, age, sex, body temperature, and somatotype of the subject, the electrocardiograph used to obtain the electrocardiogram, and conditions under which the electrocardiogram is taken, including time of day.

The purpose of this study is to compare important electrocardiographic parameters obtained at three highly divergent times of the day: 6pm, 2am, and 6am. Comparisons were made
for all beats recorded during 1-hour periods, and for beats when heart rates recorded during those 1-hour periods were between 79 and 81 beats/minute, i.e., not different.

2.2 Hypotheses

(I) Electrocardiographic parameters are different depending upon from which 3 times during the day they are recorded in normal telemetered dogs.

(II) The differences in electrocardiographic parameters between the 3 times of the day are due to differences in heart rate.

2.3 Materials and Methods

For this study it was assumed that a difference in electrocardiographic parameters of less than 15% is not important in a clinical context. Therefore, this study was designed to produce a power of 0.8 at an alpha of 0.05 using 10 dogs per group to detect a 15% difference in parameters that have a coefficient of variation of 25%. Although a difference less than 15% may be “not obvious” or “not important”, it is clear that changes much less than that (e.g., increases in heart rate or systemic arterial pressure greater than 1%) may produce profound effects when they are sustained for long periods. They are dangerous because they produce an energetic imbalance. However, although an energetic imbalance is no doubt a cause of most or at least many instances of morbidity and/or mortality and impacts on orders of magnitude more lives than arrhythmia, it does so only after months of years, therefore studies documenting the risks from energetic imbalance are extremely
expensive and time-consuming (requiring years and many subjects), and results are less
dramatic (“glamorous”) than from say those due to acute sudden death. Therefore it may
be appropriate to discuss results in terms of tendencies rather than statistical validity,
although there is always a risk that a tendency may not translate to statistical significance
even if the number of subjects were increased.

2.3.1 Surgical and Experimental Protocol

All surgeries were performed in agreement with an Animal Care and Use Protocol
approved by the Institutional Animal Care and Use Committee. Nine, healthy, young-
mature, male mongrel dogs weighing between 14 and 28 kg were instrumented to telemeter
(DSI) a modified lead II ECG as previously described. Once they recovered from the
surgical procedure, dogs underwent clinical evaluation that consisted of physical
examination, echocardiographic evaluation for assessing ejection fraction and fractional
shortening, and blood samples for NTproBNP evaluation were taken. After all dogs were
considered to be clinically normal and with normal hearts (physical examination,
echocardiography and NTproBNP), dogs were in a room without windows, and a timer
turned lights on at 6 AM and off at 6 PM. ECGs were recorded (EMKA IOX) continuously
from 5 PM until 9 AM. Dogs were fed between 7:00AM and 8AM. Cages were cleaned
between 9AM and 11AM. Mean heart rates for all 9 dogs were graphed and the times
when heart rates were slowest and fastest were recorded. Analysis of heart rate was made
during the 1 hour when the heart rates were slowest (2-3 AM), and at the time lights were
turned off (6-7 PM) and were turned (6-7 AM) just before cleaning of the kennels and
feeding. During the recording period, the recording tracing of one dog was lost from 9pm until the end of the recording period (9am), therefore just an n of 9 dogs was used in this portion of the study.

2.3.2 Data Analysis

ECG parameters, measured or computed, were: HR (beats/minute), an estimate of HR variability (SD, SDNN, low-frequency power, high frequency power and the ratio of low power to high frequency power), PQ interval (ms), QRS duration (ms), QT interval (ms), an estimate of QT instability: Standard deviation (SD) of QT interval and short-term variability (STV) of QT, QT interval corrected by the Fridericia formula (ms\(^{2/3}\)), TQ (ms) interval, and an estimate of ventricular restitution (QT: TQ). Validated EMKA ECG AUTO was used to make measurements of ECG parameters. The LF and HF powers were calculated by EMKA ECG AUTO spectral density in specific and well defined frequency bands: 0.04 to 0.15 Hz for LF and 0.15 to 0.6 Hz for HF. Means of each parameter at the three different times were compared by One way ANOVA with repeated measures design, and when differences were indicated by a significant F-statistic, specific means were compared by Bonferroni multiple comparison test requiring a P<0.05 for significance. Data that were not normally distributed were normalized by using the natural logarithmic transformation before being compared by the One Way ANOVA design. All figures were color coded as red for 6 PM, green for 2 AM and blue for 6 AM.
2.4 Results and Discussion

Table 1 shows echocardiographic and NTproBNP values for all nine dogs considered as clinically normal and having normal hearts. Figure 4 shows an echocardiographic ejection fraction measurement in m-mode.

<table>
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<tr>
<th></th>
<th>EF</th>
<th>FS</th>
<th>NTproBNP</th>
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<tbody>
<tr>
<td>Mean ± SD</td>
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<tr>
<td>Max</td>
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<td>Min</td>
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</tbody>
</table>

Table 1. EF, FS and NTproBNP in clinically normal dogs.

Figure 4. M-mode echocardiography in a normal dog.
With only few, brief interruptions, ECGs satisfactory for interpretation (Figure 5) were recorded from all dogs from 5 PM on one day to 9 AM the next day.

![Telemetry signal (ECG and BP).](image)

Figure 5. Telemetry signal (ECG and BP).

Mean values for HR for all normal dogs are shown (Figure 6). All data are presented in tables and figures as means with standard deviations. Means and SDs for each parameter at each time are shown in Table 2 and in Figures 7 through 13. In the plots, means that did not differ (P>0.05) among recordings at 6 PM, 2 AM and 6 AM are followed by the same letter. No parameter was different when comparing all three times, however it is clear that more parameters differed between 6 PM and 2 AM than between either 2 AM and 6 AM or between 6 PM and 6 AM.
Figure 6. Average heart rates in normal dogs during the recording period.

This table containing means and SDs, and demonstrates values of all-important ECG parameters for normal dogs at each time of day. These parameters represent all-important electrophysiological properties: chronotrope (heart rate), dromotrope (PQ and QRS durations), and potential for arrhythmia (QT, QTcF, QT: TQ, QT SD, QT STV). These values are to be used when comparing dogs in heart failure to normal dogs.

Figure 7 shows the inverse relationship between heart rate and RR interval. It can be observed that HR was slowest at 2 AM, was most rapid at 6 PM (P<0.001), and at 6 AM differed only slightly (P=0.08) from that at 2 AM. The relationship between heart rate and time of day is similar to what has been reported (Ashkar 1979; Matsunaga, Harada et al. 2001; Soloviev, Hamlin et al. 2006). It was expected that heart rate would be slower at night when dogs were quiet or sleeping. It was also expected that heart rate would accelerate in the early morning just before lights go on, and dogs anticipate being fed to interact with caretakers.
It was found that there was more variability of the heart rate on the time domain measurements (SD and SDNN) at 2AM. For SD (Figure 8-A) specifically, 2 AM was different from 6 PM (P=0.026). For SDNN (Figure 8-B), 2 AM was different from both 6 PM and 6 AM (P=<0.001 and P=0.007). These results could be attributed to the accentuation of the respiratory sinus arrhythmia during night (2 AM), which is exacerbated by an increase in the parasympathetic tone.
It was observed (Figure 9-B) that, as expected, the high frequency power of the heart rate variability was higher at 2 AM when compared to 6 PM (P=0.006) and 6 AM (P=0.033), which is attributed to the high parasympathetic activity during night. On the contrary, it was found that the low frequency power (Figure 9-A) is higher at 2 AM when compared with 6 PM (P<0.001) and 6 AM (P<0.001). Additionally, low frequency power is higher at 6 AM when compared to 6 PM (P=0.006). These findings agree with some researchers (Eaton, Cody et al. 1995; Haggstrom, Hamlin et al. 1996; Calvert and Wall 2001) that claim that the low frequency power is determined by both sympathetic and parasympathetic activities rather than just sympathetic influence.

Figure 8. Heart rate variability in time-domain in normal dogs.
In telemetered normal dogs, LF: HF ratio (Figure 9-C) was higher at 2 AM when compared to 6 PM (P=0.017). This finding is completely unexpected. The fact that at 2 AM the LF: HF ratio was higher when compared to both 6 AM and 6 PM, means that either there was a stronger influence of the low frequency power, a weaker influence of the high frequency or both of them together. All these three possibilities contradict the fact that high frequency power is supposed to be greater than the low frequency component during night, showing the high influence of the parasympathetic activity during night. It is evident that 3 out of 9 dogs are pulling the LF: HF ratio up. It is well known that the patterns of diurnal variation
of the heart rate in laboratory dogs and carnivores in general are characterized by short sleep-wake periods rather than one wake period followed by one sleep period (humans) (Anderson, Talan et al. 1990; Miyazaki, Yoshida et al. 2002). Additionally, it is known that during these short sleep-wake periods, dogs eat, drink water and are active inside their cages. It is possible that these 3 dogs were in one of the wake short periods during the measurements at 2 AM, which could decrease the high frequency power influence (parasympathetic activity of the autonomic nervous system).

It was found that the atrio-ventricular conduction in clinically normal dogs, was prolonged at 2 AM (P=0.002) and 6 AM (P=0.024) when compared to 6 PM (Figure 10-A). This finding is in agreement with some others researchers’ findings (Tzivoni and Stern 1973; Hanton and Rabemampianina 2006). Though this difference was statistically significant it is probable that it does not have any clinical relevance when considering this increase as a biomarker for arrhythmogenic liability. It is considered that this slight prolongation of the PQ interval reflecting slower atrio-ventricular conduction as a result of an increased parasympathetic activity during night. Though at 6 AM the atrio-ventricular conduction seems to be slightly faster than that at 2 AM (less parasympathetic activity) it is evident that at 6 AM in clinically normal telemetered dogs, there is still some parasympathetic influence early in the morning when compared to that at 6 PM.
On the contrary, ventricular conduction time (QRS) did not change at any time (Figure 10-B), replicating what has been reported in the literature (Oguchi and Hamlin 1994; Gauvin, Tilley et al. 2006; Hanton and Rabemampianina 2006; Soloviev, Hamlin et al. 2006). According to our results it seems that ventricular conduction in clinically normal telemetered dogs is completely independent of heart rate and autonomic nervous system influences as well as short and long-term changes in diurnal circadian rhythm. It is interesting that duration of QRS is unaffected by changes in heart rate or time of day despite well-known changes in autonomic traffic, temperature, and neuro-endocrines. Thus, although many other ECG parameters may be influenced by both disease and circadian physiology, QRS duration appears to be affected only by pathology (e.g., cardiomegaly, myocardial fibrosis), and since there are no differences in pathology between day and night, there are no differences in duration of QRS.

It was found that QT interval (ventricular repolarization) did not differ statistically from time to time (Figure 11-A). We believe that the lack of statistical significance is due to the high variability at each time point. However, there is a tendency of the mean at 2AM to be
longer when compared to 6 AM and 6 PM, when the heart rates are very similar (6 AM)
higher (6 PM). This could be explained by an increased parasympathetic activity. These
changes are in agreement with what has been reported by other authors in dogs and humans
(Tzivoni and Stern 1973; Gauvin, Tilley et al. 2006).
There are more differences between 6 PM and 2AM (P corresponds to the general P value for the ANOVA test; * <0.05; ** <0.01; *** <0.001; NS not significant after the Bonferroni post-hoc test.)
It was observed that although there was a P value equal to 0.028 for QTcF (Figure 11-B), when running the Bonferroni post-hoc test, there was a “do not test” result, meaning that there was no significant difference when correcting the P value for the repeated measures. Therefore, QTcF was treated as if there was no significant difference between the means, even though it may appear to exist. Though there was no statistical difference, it can be observed that there was a tendency of QTcF for being longer at 6PM when compared to 2 AM and 6 AM. This could mean that normal telemetered dogs could have a slight increased risk of ventricular arrhythmias at 6 PM. However, care should be taken when interpreting these results since they are influenced by the overcorrection of QT produce by Friedericia’s formula at high heart rate and underestimates QT prolongation at low heart rates.
Figure 12. QT instability in normal dogs.

It was found that QT SD did not change from time to time (Figure 12-A) as well as for QT STV (Figure 12-B). Although QT instability (as a biomarker for arrhythmia liability) in clinically normal telemetered dogs did not differed between time points, there is a tendency for it to be slightly increased at 6 PM, which could be translated as a slight increased tendency to proarrhythmic liability in the evening. However, we believe that this slight increase does not have any clinical relevance in normal telemetered dogs. Additionally, this finding contradicts what has been found in humans during the Framingham study that claims that human beings are more prone to suffer ventricular arrhythmias and cardiac sudden death early in the morning, mainly between 6 AM and 9 AM (Willich, Levy et al. 1987). This must be interpreted in terms of why humans die in the early morning! These deaths are most often due to tachycardia evolving into ventricular fibrillation; this usually arises without lengthening of QTc. Sudden deaths due to drugs, however are usually preceded by increased QTc or WQT instability. Thus that these makers did not lengthen at 6AM is not surprising.
Regarding ventricular restitution, we found that TQ interval was shorter at 6 PM when compared to 2 AM (P<0.001) and to 6 AM (P=0.005) (Figure 13-A). Though TQ interval was not statistically significant between 2 AM and 6 AM (P=0.061) it was very close to the significant margin. These findings were expected and correlate very well to those obtained for heart rate and RR interval, due to the direct and strong relationship between heart rate/RR interval and TQ interval. Additionally, it was found that the QT: TQ ratio (biomarker for arrhythmic liability) it was higher at 6 PM when compared to 2 AM (P<0.001) and 6 AM (<0.001), while there was no difference between 2 AM and 6 AM (Figure 13-B). Though there is an increased likelihood for arrhythmia in the evening in normal telemetered dogs, which also contradicts the findings of the Framingham study in humans, we believe that it does not have a profound clinical impact in these dogs.
Means and SDs for each parameter recorded when heart rate was 80/minute (actually between 79 and 81 beats/minute) are shown in Table 3 and figures 14 to 16. It can be observed that PQ (p=0.79) and QRS (p=0.37) were not different among the 3 times, that QT (p=0.052) and QTcF (p=-0.052) were close to being different (2 AM and 6 PM), but that TQ (p=0.007) and the ratio, QT: TQ (p=0.022), differed significantly between 6 PM and 2 AM. These results address hypothesis II that claims differences in all parameters observed among the 3 times of day may be attributable to differences in heart rate. It may be concluded that differences in TQ and QT: TQ occurred despite no change in heart rate (rejecting hypothesis II). Since there were no differences in QRS among the times, then the hypothesis has no meaning for QRS. Since PQ was longer at 2 AM and 6 AM than at 6 PM when heart rates were different, but did not differ at comparable instantaneous heart rates (i.e., 80/minute), then hypothesis may be accepted. It is an important finding of this study that QRS duration is not affected by the time of day or to changes in heart rate. It is interesting that duration of QRS is unaffected by changes in heart rate or time of day despite well-known changes in autonomic traffic, temperature, and neuro-endocrines. Thus, although many other ECG parameters may be influenced by both disease and circadian physiology, QRS duration appears to be affected only by pathology (e.g., cardiomegaly, and myocardial fibrosis).
Figure 14. PQ interval and QRS complex at 80bpm in normal dogs.

Figure 15. Ventricular repolarization at 80bpm in normal dogs.
Figure 16. Ventricular restitution at 80bpm in normal dogs.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>6PM</th>
<th>2AM</th>
<th>6AM</th>
<th>P</th>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<td>106 ± 7</td>
<td>105 ± 9</td>
<td>0.790</td>
<td>NS</td>
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<tr>
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<td>51 ± 8</td>
<td>52 ± 6</td>
<td>0.366</td>
<td>NS</td>
</tr>
<tr>
<td>QT</td>
<td>209 ± 15</td>
<td>223 ± 11</td>
<td>214 ± 11</td>
<td>0.052</td>
<td>NS</td>
</tr>
<tr>
<td>QTcF</td>
<td>231 ± 16</td>
<td>245 ± 12</td>
<td>236 ± 12</td>
<td>0.052</td>
<td>NS</td>
</tr>
<tr>
<td>TQ</td>
<td>543 ± 8</td>
<td>524 ± 11</td>
<td>536 ± 12</td>
<td>0.007</td>
<td>**</td>
</tr>
<tr>
<td>QT/TQ</td>
<td>0.39 ± 0.03</td>
<td>0.42 ± 0.03</td>
<td>0.4 ± 0.02</td>
<td>0.022</td>
<td>*</td>
</tr>
</tbody>
</table>

Table 3. Descriptive statistics for each parameter at each time point at a heart rate of 80bpm in normal dogs.

There are differences between 6 PM and 2AM for ventricular restitution (TQ and QT: TQ) (P corresponds to the general P value for the ANOVA test; * <0.05; ** <0.01; ***<0.001; NS not significant after the Bonferroni post-hoc test).
2.5 Conclusions and Limitations

Some of the limitations of this study include:

- The sample size (n) was small but was adequate to achieve sufficient power to identify changes in heart rate and most of the parameters. However, parameters such as QTcF (P=0.028), and QT (P=0.052) and QTcF (P=0.052) at a heart rate of 80bpm were very close to being significantly different. This should be considered in future studies in normal telemetered dogs.

- In order to have recordings clear of noise and artifacts due to animal handling and dog excitement, we did not obtain data for 24 hours. Therefore this study just shows the fluctuations of these electrographic parameters during 1 hour epochs at evening, night and early morning. Due to the obvious fluctuation hour-by-hour in the electrocardiographic parameters, care should be taken when extrapolating these results to other times different than 6 PM, 2 AM and 6 AM to other studies in normal telemetered dogs or patients.

- This study was performed in laboratory male mongrel dogs, located in a rigorously controlled environment (light cycle, feeding time, humidity, etc). Therefore, care must be taken when extrapolating these data to animals with different physical or environmental characteristics.

We can conclude that these results address hypothesis I that claims that there are electrocardiographic differences among different times of the day in normal telemetered dogs. However, we can reject the hypothesis for electrocardiographic parameters such as
QRS (ventricular depolarization), QT interval (ventricular repolarization), QTcF, and QT instability (QT SD and QT STV). QRS seems to be completely independent from heart rate and autonomic influence. On the contrary, it is well known that ventricular repolarization is influenced by both heart rate and autonomic nervous system. However, in this study we can conclude that most of the electrocardiographic biomarkers commonly used to detect likelihood of arrhythmia (QT, QTcF, QT SD and QT STV) do not change among the three time points studied (6 PM, 2 AM and 6 AM) in normal telemetered dogs. This can be translated to indicate that when, during the diurnal periods ECGs are obtained, may be inconsequential in predicting risk for arrhythmia. Additionally, the results obtained also address hypothesis II that claims differences in all parameters observed among the 3 times of day may be attributable to differences in heart rate. It may be concluded that differences in TQ and QT:TQ occurred despite no change in heart rate (rejecting hypothesis II).
CHAPTER 3. RELATIONSHIPS AMONG IMPORTANT ECG PARAMETERS TO HEART RATE BETWEEN DAY AND NIGHT IN NORMAL MONGREL DOGS.

3.1 Introduction

It is obvious that values for the measurements mentioned above are important to identify effects of drugs and/or disease, but there is every reason to believe that the relationships between each parameter and heart rate are also important, in fact they may be even more relevant. For example QT is known to be dependent on heart rate, and only when heart rate is considered, can it be known that a change in QT is relevant. Thus this portion of the study was conducted to determine if the effects on heart rate differ during the different times of day when recordings are made. It is common to regress QT for heart rate, however the relationship may very according to the time of day.

3.2. Hypothesis

In normal dogs the relationships between PQ, QRS, QT, and QTcF, to heart rate (HR) do not differ between the 3 different times (6PM, 2AM, 6AM).
3.3 Materials and Methods

This data was obtained from the same normal dogs used for the initial study. Thus the animals were the same, the methods of recording were the same, but the method of analysis differed since parameters were regressed to heart rate but at 3 different times of day. All intervals (e.g., PQ, QRS, QT, QT: TQ) were then plotted against HR for the 3, 1-hour periods. “Clouds” of plots of each ECG parameter recorded at 6 PM, 2 AM and 6AM versus HR were made by EMKA ECG AUTO for each dog and graphed so that recordings at 6 PM were in green, 2AM in red and 6AM in blue. “Clouds” were displayed and assessed by inspection to determine if they were superimposed or if one cloud appeared to be separate from another. However $R^2$’s and slopes of linear regressions of each dog for each cloud (6 PM, 2 AM and 6 AM) were compared as for each parameter. By using a one-way ANOVA with repeated measurements design, slopes for each dog at each time point were compared. Data that was not normally-distributed but was normalized by its natural log and then was analyzed by one-way ANOVA with repeated measurements design for statistical purposes. However, all raw data will be presented in tables and figures. The slope (ms⋅min) refers to the change in each parameter (ms for each parameter) obtained for changes in heart rate (min^-1). Of course $R^2$ refers to the percentage of change in each parameter that can be attributable to change in heart rate.
3.4. Results and Discussion

It is important to know these relationships, because if adjustments must be made according to heart rate (i.e., normalizing for heart rate) it may be relevant as to when the adjustments were calculated. For example, if the relationship between QT and heart rate may have been calculated from data recorded over 24 hours or during the day or night, and one is seeking that knowledge for 2 AM, the normalization may be incorrect.

Means and standard deviations for the slopes of each parameter vs. heart rate at each time point are shown in Table 4 and figures 17 to 22. Means and standard deviations for the $R^2$ of each parameter vs. heart rate at each time point are shown in Table 5. Clouds obtained by plotting all parameters versus HR are shown for all 9 dogs (Appendix 1). To achieve the same X and Y-axes, clouds for each time were superimposed, and clouds for 1 time often obfuscated clouds for another time. However it became obvious that deleting 6 AM (blue) from 2 AM and 6 PM permitted comparing 2 AM (green) to 6 PM (red).
Table 4. Descriptive statistics for the slope of each relationship at each time point for normal dogs.

<0.05; ** <0.01; *** <0.001; NS not significant.

Table 5. Descriptive statistics for the R² of each relationship at each time point for normal dogs.
Average PQ intervals were 103 (SD 9), 111 (SD 8), and 108 (SD 9) for 6 PM, 2 AM and 6 AM, respectively, such that \( (2\ AM = 6\ AM) > 6\ PM \) \( (p=0.002) \). These slight differences should be of neither toxicological nor clinical significance. Average heart rates were 91 (SD 12), 66 (SD 6), and 76 (SD 11) for 6 PM, 2 AM, and 6 AM, respectively, such that \( (2\ AM = 6\ AM) < 6\ PM \). These differences could translate to both toxicological and clinical significance, since heart rate is a prime determinant of myocardial oxygen consumption and diastole (the reciprocal of heart rate) is a prime determinant of myocardial oxygen delivery. Thus change in heart rate may produce an energetic imbalance. The slopes of the relationships between PQ interval and heart rate (beats/ minute) are shown in Table 3, all slopes are negative, but none of the slopes differed among times. It was expected that PQ would lengthen when heart rate slowed, since reduction in heart rate is most often a consequence of increased vagal tone and that increase in tone should lengthen PQ as well.

![Figure 17. Slopes of PQ vs. HR for normal dogs.](image)

However when heart rate slows, the AV node is bombarded fewer times per minute and has more time for recovery, therefore PQ interval may actually shorten when heart rate slows.
This did not occur, however. It must be emphasized that the relationships were calculated based upon means recorded from recordings of 1 hour. Within that hour when respiratory sinus arrhythmia occurs, change in intervals may be different because heart rate changes are rather sudden during inspiration and expiration.

Average QRS durations were 50 (SD 5), 49 (SD 4) and 50 (SD 4) at 6 PM, 2 AM and 6 AM respectively, and none differed among times. Average (as seen above) heart rates were 91 (SD 12), 66 (SD 6), and 76 (SD 11) for 6 PM, 2 AM, and 6 AM respectively such that (2 AM=6 AM)<6 PM. The slopes of the relationships between QRS duration and heart rate (beats/ minute) are shown in Table 3, and none differ among times. This was an expected lack of relationship between QRS duration and heart rate, since heart rate changes because of waxing and waning of vagal efferent activity to the SA node, and there is little vagal innervation to the ventricular syncytium.
Average QT intervals were 208 (SD 14), 219 (SD 9), and 211 (SD 10) for 6 PM, 2 AM and 6 AM respectively. None were different. This is contrary to results of Soleviev et al who showed longer QTs at night. In addition, since heart rates were slower at night and there is a well-known relationship between heart rate and QT, QTs at night should have been longer. That they were not may be explained the fact that ion channels for ventricular repolarization may manifest different physiology at night than during the day. However a more plausible explanation is that the p value was 0.09 (close to significant) and, had a bigger number of dogs been studied, QT would have been, longer a night. Average heart rates were 91 (SD 12), 66 (SD 6), and 76 (SD 11) for 6 PM, 2 AM, and 6 AM respectively such that (2 AM=6 AM)<6 PM. The slopes of the relationships between QT interval and heart rate (beats/ minute) are shown in Table 3, all slopes are negative, but none of the slopes differed among times.
Average QTc(F)s were 243 (SD 14), 234 (SD 11), and 233 (SD 6) for 6 PM, 2 AM and 6 AM, respectively. All slopes relating QTc(F) to heart rate are positive. Slopes at 6 PM and 2 AM differed (P<0.001), and slopes between 2 AM and 6 AM differed (P<0.05), but there was no difference between slopes at 6 PM and 6 AM. This data supports the contention that correcting QT for heart rate is inadequate, i.e., slopes should be 0, but that the adequacy or inadequacy varies according to time of day. It is interesting to speculate if correcting QT using individual data for each dog would be more appropriate. It should be remembered that the individual correction is based upon heart rates obtained during 24 hours, because slow heart rates occur at night and high heart rates during the day. Thus it is not known if such individual corrections would apply at 2 AM, 6 AM, or 6 PM.
Average TQs were 468 (SD 92), 696 (SD 73), and 604 (SD 104) for 6 PM, 2 AM and 6 AM respectively. Average TQs differed between 6 PM and 2 AM (P<0.001) and between 6 PM and 6 AM (P<0.01). Heart rates were 91 (SD 12), 66 (SD 6), and 76 (SD 11) for 6 PM, 2 AM, and 6 AM respectively such that (2 AM=6 AM)<6 PM. The slopes of the relationships between TQ and heart rate (beats/minute) are shown in Table 3. All slopes are negative, and all differed from each other (Ps< 0.001, <0.05). Since when heart rate (1/RR interval) changes, QT changes slightly, but TQ changes dramatically, a clear relationship between heart rate and TQ would be expected.

Figure 20. Slopes of QTcF vs. HR for normal dogs.
Because the slopes of the relationships between QT: TQ versus heart rate were so small, there are no differences in this slope among times.

The percentage of change in the dependent variables (e.g., PQ, QRS, QT, QTcF, QT: TQ) attributable to the change in heart rate was assessed by $R^2$, however comparing $R^2$s is not statistically valid. None-the-less it appears that $R^2$s for PQ versus HR appeared to differ between 2 AM and 6 AM. $R^2$s for QTc(F) appeared to differ between 6 PM and 2 AM. $R^2$s did not appear to differ for QRS, QT, TQ, or for QT: TQ. This challenges the correctness of expressing changes in intervals versus changes in HR unless the relationships are sought at the specific times of ECG recordings.
Figure 22. Slopes of QT: TQ vs. HR for normal dogs.
CHAPTER 4. TIME (DIURNAL) DIFFERENCES IN ELECTROCARDIOGRAPHIC PARAMETERS OF ARRHYTHMIC LIABILITY IN TELEMETERED DOGS WITH FAILING HEARTS

4.1 Introduction

The purpose of this study was to evaluate important electrocardiographic parameters obtained at three highly divergent times of the day (6 PM, 2 AM, and 6 AM) for dogs with failing hearts—and mild heart failure classified as stage II of the NYHA, stage B2 of the AHA/ACC, or class I-B of the International Small Animal Cardiac Health Council (ISACHC) (Atkins, Bonagura et al. 2009). Classification was documented by reduced EF and elevated NTproBNP but asymptomatic. Comparisons were made for all cardiac cycles recorded during 1-hour periods, and for cycles when heart rates recorded during those 1-hour periods were between 79 and 81 beats/minute, i.e., considered to be not different.

4.2 Hypotheses

(I) Electrocardiographic parameters are not different in asymptomatic dogs but with induced failing hearts, among 3 divergent times of day.
(II) If there are electrocardiographic differences among the 3 times, these differences are not due to differences in heart rate.

4.3 Materials and Methods

4.3.1 Surgical and Experimental Protocol

All surgeries were performed in agreement with an Animal Care and Use Protocol approved by the Institutional Animal Care and Use Committee. Ten, healthy, young-mature, male mongrel dogs weighing between 14 and 28 Kg (average, SD) were instrumented to telemeter (DSI) a modified lead II ECG as previously described. Five of them were implanted with a pacemaker with a single lead that was placed in the right ventricular apex for rapid pacing to induce failing hearts. The pacing protocol consisted in pacing the heart at 140 ppm for one week. Then it was increased to 160 ppm for one week and finally it was increased to 175 ppm and kept at that rate for 6 more weeks. After 8 weeks of high-rate pacing it was expected to find an increased NTproBNP (>1800pM/L) and a decrease in ejection fraction of at least 25% from baseline but no clinical signs of heart failure. Dogs that met all criteria were included in the study. In a second group (n=5), a previously described approach from the femoral artery (Smiseth and Mjos 1982; Smiseth, Lindal et al. 1983) was used to induce heart failure by serial coronary microembolizations as described (Sabbah, Stein et al. 1991) given one week apart by injecting 90 µm polystyrene microspheres into the left main coronary artery. Starting with a dose of 1000 microspheres/Kg and doubling the dose in every following embolization. Between 4 and 10
embolizations one week apart were required to get a reduction of the ejection fraction of at least 25% from baseline and an increased NTproBNP (>1800pM/L) but no symptoms of clinical disease. Ejection fractions differed insignificantly (P=0.17) between the ischemic (42%, SD 3.6) and the rapid-paced (38, SD 5) dogs; NTproBNPs differed insignificantly (P=0.59) between the ischemic (2685, SD 377) and the rapid-paced (2882, SD, 264) therefore these 2 groups were taken together as having documented failing hearts but no symptoms, and were compared to normal dogs.

Blood samples and echocardiograms were collected in both groups at baseline and once a week during initiation of the failure hearts in order to measure NTproBNP and EF, and to determine if a dog qualified to be considered to have a failing heart.

M-mode echocardiography was obtained using the 2-D right parasternal short axis view. Measurements of parameters of the left ventricle were obtained to evaluate left ventricular function. Measurements collected from echocardiography were left ventricular ejection fraction (LVEF), and left ventricular fractional shortening (LVFS). Parameters were measured over 5 beats and average for analysis.

Once included in the study, dogs were in a room without windows, and a timer turned lights on at 6 AM and off at 6 PM. ECGs were recorded (EMKA IOX) continuously from 5 PM until 9 AM. Dogs were fed between 7:00AM and 8AM. Cages were cleaned between 9AM and 11AM. Mean heart rates, measured over 1 hour for all 10 dogs were graphed and the times when heart rates were slowest and fastest were recorded. Analysis of heart rate was made during the 1 hour when the heart rates were slowest (2-3 AM), and at the time
lights were turned off (6-7 PM) and were turned on (6-7 AM) just before cleaning of the kennels and feeding, when heart rates were fastest.

4.3.2 Data Analysis

ECG parameters, measured or computed, were: HR (beats/minute), estimates of HR variability (SD, SDNN, low-frequency power, high frequency power and the ratio of low power to high frequency power), PQ interval (ms), QRS duration (ms), QT interval (ms), an estimate of QT instability [SD of QT interval and short-term variability (STV) of QT], QTc (ms^{2/3} interval), TQ (ms) interval, an estimate of ventricular restitution (QT: TQ). Validated EMKA ECG AUTO made measurements of ECG parameters.

NTproBNP values equal or above 3000 were considered as 3000 for statistical purposes. Values for EF, FS and NTproBNP were compared at baseline and after induction of failing hearts by a Paired t-test design, considering statistically significant differences at P<0.05. Descriptive statistics performed include mean, standard deviation, maximum and minimum values for each parameter evaluated. Shapiro-Wilk normality test was performed for each parameter evaluated. By using the natural log transformation data that was not considered to be normally distributed was normalized. Means of each electrocardiographic parameter at the three different times were compared by One Way ANOVA with repeated measures design, and when differences were indicated by a significant F-statistic, specific means were compared by Bonferroni multiple comparison test requiring a P<0.05 for significance.
4.4 Results and Discussion

Ten dogs with failing hearts—half due to ischemia and half due to rapid-pacing—were used to broaden the applicability of the results to include the failing heart in general, and not just the failing heart due to either etiology by itself.

This study was not intended to be conducted on dogs in frank (symptomatic) heart failure that constitute but a small percentage of patients with heart disease and that are known to manifest obvious abnormal cardiac electrophysiology, but rather in animals with documented failing hearts but without heart failure that constitute the vast majority of patients with heart disease.

In table 6 and figures 23 and 24 it can be observed that dogs identified as being in heart failure stage II of the NYHA, stage B2 of the AHA/ACC, or class I-B of the International Small Animal Cardiac Health Council (ISACHC) (Atkins, Bonagura et al. 2009) had, in fact, failing hearts since their EF’s were precipitously lower from baseline (40.1 versus 62.2, P<0.001) and their NTproBP’s dramatically higher when compared to baseline (2640 versus 717, P<0.001). However, none of them was showing clinical symptoms (e.g., ascites, shortness-of-breath, tachypnea, crackles, cyanosis, exercise intolerance) of heart failure.
Table 6. EF, FS and NTproBNP before (BL) and after inducing failing hearts (FH).

<table>
<thead>
<tr>
<th></th>
<th>EF</th>
<th>FS</th>
<th>NTproBNP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Max</td>
<td>Min</td>
</tr>
<tr>
<td>BL</td>
<td>62.2 ± 7</td>
<td>71</td>
<td>49</td>
</tr>
<tr>
<td>FH</td>
<td>40.1 ± 5</td>
<td>48</td>
<td>30</td>
</tr>
</tbody>
</table>

Figure 23. EF, FS and NTproBNP before (BL) and after inducing failing hearts (FH).
Figure 24. M-mode before and after inducing a failing heart.

Figure 25. Telemetry signal (ECG and BP).
Mean values for HR for all dogs with failing hearts are shown (Figure 26). It can be observed that HR was slowest at around 3 AM, and was fastest around 6 PM and 6 AM. There were no differences in HR between 6 AM and 6 PM (P=1.0). However there were highly significant differences between for 2 AM versus 6 PM (P=0.02) and for 2 AM versus 6 AM (P=0.02) (Figure 27). The relationship between heart rate and time of day is similar to what has been reported in normal telemetered dogs (Ashkar 1979; Matsunaga, Harada et al. 2001; Soloviev, Hamlin et al. 2006). It was expected that heart rate would be similar to that at 6 PM or 6 AM when dogs were active due to the increase in heart rate associated to cardiac disease. However, telemetered dogs with failing hearts showed a normal behavior of the heart rate, showing a significant decrease of heart rate during night when they were quiet and sleeping.
Means and SDs for each parameter at each time are shown in Table 7 and in Figures 27 through 33. In the plots, means that did not differ (P>0.05) among recordings at 6 PM, 2 AM and 6 AM are followed by the same letter. No parameter was different between 6 PM and 6 AM, but many parameters (e.g., heart rate, HRV, PQ, QT) differed between 6 PM and 2 AM and between 2 AM and 6 AM. This table containing means, SDs, maxima, and minima demonstrates values of all-important ECG parameters for dogs the dogs with failing hearts at each time of day. These parameters represent all-important electrophysiological properties: chronotrope (heart rate), dromotrope (PQ and QRS durations), and potential for arrhythmia (QT, QTcF, QT: TQ, QT SD, QT STV). These values are to be used when comparing dogs in heart failure to normal dogs (Chapter VI).

Figure 27. Heart rate and RR interval in dogs with failing hearts.
It was found that there was more variability of the heart rate on the time domain measurements (SD and SDNN) at 2AM. For SD (Figure 28-A) specifically, 2 AM was greater than 6 PM (P=0.002) and was greater than 6 AM (P=0.001). On the contrary, 6 PM was not different from 6 AM (P=1.0). For SDNN (Figure 28-B), 2 AM was also greater from both 6 PM and 6 AM (P=<0.046 and P=0.048), and there was no difference between 6PM and 6 AM (P=1.0). Although these results were not expected, they could be associated to a more pronounced respiratory sinus arrhythmia during night (2 AM), as a result of the increased parasympathetic activity at night. It was expected to see a decrease of the heart rate variability at 2AM due to the lack of presence of respiratory sinus arrhythmia in dogs with heart disease or heart failure, however the dogs were not in heart failure despite having failing hearts. This loss of respiratory sinus arrhythmia is associated to the imbalance in the autonomic nervous system in which there is an increase of the sympathetic tone and a decrease of the parasympathetic activity.
It was observed (Figure 29-B) that, the high frequency power of the heart rate variability was higher at 2 AM when compared to 6 PM (P=0.021) and 6 AM (P<0.001), and there were no differences between 6 PM and 6 AM (P=0.332). This result could be attributed to the high parasympathetic activity during night. Contrary to what was observed, a decreased high frequency power at 2 AM was expected due to the pathophysiological decrease of the parasympathetic activity evident in cardiac disease. This suggests that telemetered dogs with failing hearts but without heart failure do not have a strong imbalance in the autonomic activity. On the other hand, it was found that the low frequency power (Figure 29-A) is also higher at 2 AM when compared with 6 PM (P=0.021) and 6 AM (P=0.041) but did not differ between 6 PM and 6 AM (P=1.0). These findings agree with some researchers (Eaton, Cody et al. 1995; Haggstrom, Hamlin et al. 1996; Calvert and Wall 2001) that claim that the low frequency power is determined by both sympathetic and parasympathetic activities rather than just sympathetic influence. If this is true, changes in low frequency component are difficult to analyze during disease, since this change could depend on the degree of influence of both the sympathetic and parasympathetic activities, the degree of imbalance of the autonomic nervous system in general. In telemetered dogs with failing hearts, but no clinical disease low frequency power of the heart rate variability was higher at 2 AM, suggesting a strong parasympathetic component during night. In telemetered dogs with failing hearts, LF: HF ratio (Figure 29-C) was lower at 2 AM when compared to 6 AM (P=0.004). Though there was no statistical difference, there is a tendency of 6 AM to be also greater than 6 PM (P=0.079). This finding supports the fact that at 2 AM the high frequency power was greater than the low frequency component at 2 AM when compared to 6 AM and 6 PM, showing the great influence of the parasympathetic activity during night. There was a stronger influence of the low frequency
power of the heart rate variability at 6 PM and 6 AM. Though, there were no differences between 6 PM and 6 AM, the ratio of LF: HF, there is a tendency for either stronger influences of the low frequency component of the heart rate variability at 6 AM, weaker influences of the high frequency power (less parasympathetic activity), or both. This could potentially increase the risk of ventricular arrhythmias at both 6 PM and 6 AM (mainly 6 AM) due to an increased heterogeneity of refractoriness within the myocardial cells.
**Table 7.** Descriptive statistics for each parameter at each time point for dogs with failing hearts.

None of the parameters were different between 6 PM and 6 AM. However, heart rate, heart rate variability, PQ, QT and TQ differed between 6 PM and 2 AM and between 2 AM and 6 AM. (P corresponds to the general P value for the ANOVA test; * <0.05; ** <0.01; *** <0.001; NS not significant after the Bonferroni post-hoc test).
Figure 29. Heart rate variability in frequency domain in dogs with failing hearts.

A) Shows low frequency component of the heart rate variability. B) High frequency component of the heart rate variability. C) Low frequency and high frequency ratio.
It was found that the atrio-ventricular conduction in dogs, was prolonged at 2 AM when compared to 6 PM (P=0.044), while 6 AM was not different from 2 AM (P=0.175) or 6 PM (P=1.0) (Figure 30-A). Though this difference was statistically significant it is probable that it does not have any clinical relevance when considering this increase as a biomarker for proarrhythmic risk. It is considered that this slight prolongation of the PQ interval reflecting slower atrio-ventricular conduction is a result of an increased parasympathetic activity during night.

When evaluating ventricular conduction time (QRS) it was found that QRS did not change at any time (Figure 30-B), replicating what has been reported in the literature (Oguchi and Hamlin 1994; Gauvin, Tilley et al. 2006; Hanton and Rabemampianina 2006; Soloviev, Hamlin et al. 2006). According to our results it seems that ventricular conduction in telemetered dogs with failing hearts is completely independent of changes in heart rate and autonomic nervous system, temperature, and neuro-endocrines influences as well as short
and long-term changes in diurnal circadian rhythm. QRS duration appears to be affected only by pathology (e.g., cardiomegaly, myocardial fibrosis) and of course on drugs. Since there are no differences in pathology between day and night, there are no differences in duration of QRS.

It was found that QT interval (ventricular repolarization) was longer at 2 AM when compared to 6 PM (P=0.012) and 6 AM (P<0.001) (Figure 31-A), and it was also observed that there was no difference for QTcF (Figure 31-B), at any of the three time points. The prolongation of the QT at 2 AM could be associated to the increased parasympathetic activity that the group of dogs with failing hearts has shown with other electrocardiographic parameters. The fact that there were no differences in QTcF at any time point show that this group of dogs is not predisposed to ventricular arrhythmias at any of the three time points.

---

Figure 31. Ventricular repolarization in dogs with failing hearts.
It was found that QT SD did not change from time to time (Figure 32-A) as well as for QT STV (Figure 32-B). In telemetered dogs with failing hearts there are no changes in QT instability from time to time. This is of extreme importance since it means that there are no differences in the likelihood of arrhythmia at the three time points in this particular group of dogs. These findings coincide with those for QTcF in dogs with failing hearts.

Figure 32. Measures of QT instability in dogs with failing hearts.
Regarding ventricular restitution, we found that TQ interval was longer at 2 AM when compared to 6 PM (P<0.002) and to 6 AM (P=0.005) (Figure 33-A). These findings were expected and correlate very well to those obtained for heart rate and RR interval, due to the direct and strong relationship between heart rate/RR interval and TQ interval. Additionally, it was found that the QT: TQ ratio (biomarker for arrhythmic liability) was no different at any time point, showing, once again, that in dogs with failing hearts there is no difference in of arrhythmic liability between 6 PM, 2 AM and 6 AM (Figure 33-B). However, there is a slight tendency for it to be higher at 6 PM and 6 AM when compared to 2 AM.

Means and SDs for each parameter recorded when heart rate was 80/minute (actually between 79 and 81 beats/minute) are shown (Table 8) and figures 34 to 36. It can be observed that PQ (p=0.087), QRS (p=0.16) and TQ (p=0.851) were not different among the
3 times, whereas QT \((p=0.002)\), QTc(F)\((p=-0.002)\) and QT/TQ \((p=0.02)\) differed significantly between 2 AM and 6 AM. These results address hypothesis II that claims, for dogs with failing hearts, differences in all parameters observed among the 3 times of day may be attributable to differences in heart rate. It may be concluded that differences in TQ and QT: TQ occurred despite no change in heart rate (rejecting hypothesis II). Since there were no differences in QRS among the times, then the hypothesis has no meaning for QRS. Since PQ was longer at 2 AM and 6 AM than at 6 PM when heart rates were different, but did not differ at comparable instantaneous heart rates (i.e., 80/minute), then hypothesis II may be accepted. This is an important finding, namely that TQ, and QT: TQ were different between day and night, but that the differences were not due to differences in heart rate. It is also an important finding of this study that QRS duration is not affected by the time of day or to changes in heart rate.

Figure 34 PQ interval and QRS complex at 80bpm in dogs with failing hearts.
Figure 35. Ventricular repolarization at 80bpm in dogs with failing hearts.

Figure 36. Ventricular restitution at 80bpm in dogs with failing hearts.
### Table 8. Descriptive statistics for each parameter at 80bpm in dogs with failing hearts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>6PM</th>
<th>2AM</th>
<th>6AM</th>
<th>P</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6PM vs 2AM</td>
<td>2AM vs 6AM</td>
<td>6PM vs 6AM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PQ</td>
<td>111</td>
<td>116</td>
<td>112</td>
<td>0.087</td>
<td>NS</td>
</tr>
<tr>
<td>QRS</td>
<td>52</td>
<td>52</td>
<td>51</td>
<td>0.16</td>
<td>NS</td>
</tr>
<tr>
<td>QT</td>
<td>225</td>
<td>237</td>
<td>219</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>QTcF</td>
<td>248</td>
<td>261</td>
<td>241</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>TQ</td>
<td>481</td>
<td>473</td>
<td>472</td>
<td>0.851</td>
<td>NS</td>
</tr>
<tr>
<td>QT/TQ</td>
<td>0.40</td>
<td>0.43</td>
<td>0.37</td>
<td>0.02</td>
<td>*</td>
</tr>
</tbody>
</table>

(* <0.05; ** <0.01; ***<0.001; NS not significant).

### 4.5 Conclusions and Limitations

Some of the limitations of this study include:

- The sample size (n) was small but was adequate to achieve sufficient power to identify changes in heart rate and most of the parameters.

- In order to have recordings clear of noise and artifacts due to animal handling and dog excitement, we did not obtained data for 24 hours. Therefore this study just shows the fluctuations of these electrographic parameters during evening, night and early morning. Due to the obvious fluctuation hour by hour in the electrocardiographic parameters, care should be taken when extrapolating these results to other times different than 6 PM, 2 AM and 6 AM to other studies in telemetered dogs with failing hearts or patients.

- This study was performed in laboratory male mongrel dogs with failing hearts, located in a complete controlled environment (light cycle, feeding time, humidity,
etc). Therefore, care must be taken when extrapolating these data to animals with different physical or environmental characteristics.

- Two different canine models of heart failure were used for this study. The idea was to keep the heterogeneity of the cardiac disease in general instead of studying a specific etiology of heart disease. However, we cannot assure if both groups contributed equally to the results. Therefore, care must be taken when extrapolating these results to a group of patients with a specific etiology of heart disease.

We can conclude that these results address hypothesis I that claims that there are no electrocardiographic differences among different times of the day in telemetered dogs with failing hearts for ventricular depolarization (QRS), and parameters for arrhythmic liability such as QTcF, QT instability (QTSD and QT STV) and ventricular restitution (QT: TQ). However, we can reject the hypothesis for every other electrocardiographic parameter such as heart rate, heart rate variability (time and frequency domain), ventricular repolarization (QT interval), and ventricular restitution (TQ). As in normal dogs, in dogs with failing hearts, QRS seems to be completely independent from heart rate and autonomic influence. In this study we can conclude that none of the electrocardiographic biomarkers commonly used to detect likelihood of arrhythmia (QTcF, QT SD and QT STV, and QT: TQ) change among the three time points studied (6 PM, 2 AM and 6 AM) in telemetered dogs with failing hearts. This can be translated to a low clinical risk of ventricular arrhythmias and cardiac sudden death in dogs with failing hearts during these times. Additionally, these results address hypothesis II that claims, for dogs with failing hearts, differences in all parameters observed among the 3 times of day may be attributable to differences in heart
rate. It may be concluded that differences in TQ and QT: TQ occurred despite no change in
heart rate (rejecting hypothesis II).
CHAPTER 5. RELATIONSHIPS AMONG IMPORTANT ECG PARAMETERS AND HEART RATE BETWEEN DAY AND NIGHT IN MONGREL DOGS IN HEART FAILURE.

5.1 Introduction

It is reasonable that values for ECG parameters are important to identify effects of drugs and/or disease, but there is every reason to believe that the relationships between each parameter and heart rate are also important, in fact may be even more so.

5.2 Hypothesis

In dogs with mild failing hearts the relationships between PQ, QRS, QT, and QTcF, to heart rate (HR) do not differ between the 3 different times (6PM, 2AM, 6AM).
5.3 Materials and Methods

This data were obtained from the same heart failure dogs used for the initial study. Thus the animals were the same, the methods of recording were the same, but the method of analysis differed since parameters were regressed to heart rate but at 3 different times of day. All intervals (e.g., PQ, QRS, QT, QT: TQ) were then plotted against HR for the 3, 1-hour periods. “Clouds” of plots of each ECG parameter recorded at 6 PM, 2 AM and 6AM versus HR were made by EMKA ECG AUTO for each dog and graphed so that recordings at 6 PM are in green, 2AM are in red and 6AM are in blue. “Clouds” were displayed and assessed by inspection to determine if they were superimposed or if one cloud appeared to be separate from another. However R²’s and slopes of linear regressions of each dog for each cloud (6 PM, 2 AM and 6 AM) were compared as for each parameter. The slope (ms·min) refers to the change (ms) in each parameter obtained for change in heart rate (min^-1). Of course R^2 refers to the percentage of change in each parameter that can be attributable to change in heart rate.

5.4 Results and Discussion

Means and standard deviations for the slopes of each parameter vs. heart rate at each time point are shown in Table 9 and figures 37 through 5.6 for dogs in heart failure. Means and standard deviations for the R2 of each parameter vs. heart rate at each time point are shown in Table 10. Clouds obtained by plotting all parameters versus HR are shown for all 9 dogs (Appendix 2). To achieve the same X and Y-axes, clouds for each time were super
imposed, and clouds for 1 time often obfuscated clouds for another time. However it became obvious that deleting 6 AM (blue) from 2 AM and 6 PM permitted comparing 2 AM (green) to 6 PM (red). It is important to note that the slope of the relationship between QT and heart rate (used to calculate QTc) varied between 2 AM and 6 AM. Thus if a mathematical correction of QT for heart rate was made at one time, it might be incorrect for times when heart rate differs. Of course it is well known that all linear or even curvilinear mathematical corrections are incorrect, and that QT must be expressed as clouds versus heart rate or RR interval.
### Table 9. Descriptive statistics for the slopes for dogs with failing hearts.

(* <0.05; ** <0.01; *** <0.001; NS not significant).

<table>
<thead>
<tr>
<th>SLOPES</th>
<th>6PM</th>
<th>2AM</th>
<th>6AM</th>
<th>P</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td>6PM vs 2AM</td>
</tr>
<tr>
<td>PQ vs HR</td>
<td>-0.16 ± 0.11</td>
<td>-0.14 ± 0.099</td>
<td>-0.15 ± 0.11</td>
<td>0.575</td>
<td>NS</td>
</tr>
<tr>
<td>QRS vs HR</td>
<td>-0.014 ± 0.04</td>
<td>-0.004 ± 0.028</td>
<td>-0.010 ± 0.03</td>
<td>0.31</td>
<td>NS</td>
</tr>
<tr>
<td>QT vs HR</td>
<td>-0.21 ± 0.14</td>
<td>-0.13 ± 0.07</td>
<td>-0.29 ± 0.19</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>QTcF vs HR</td>
<td>0.71 ± 0.32</td>
<td>0.99 ± 0.28</td>
<td>0.64 ± 0.25</td>
<td>0.01</td>
<td>*</td>
</tr>
<tr>
<td>TQ vs HR</td>
<td>-8.73 ± 3.64</td>
<td>-12.21 ± 3.81</td>
<td>-9.47 ± 3.52</td>
<td>0.01</td>
<td>**</td>
</tr>
<tr>
<td>QT:QT vs HR</td>
<td>0.008 ± 0.001</td>
<td>0.009 ± 0.001</td>
<td>0.007 ± 0.001</td>
<td>0.006</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Table 10. Descriptive statistics for the R^2 for dogs with failing heart.

<table>
<thead>
<tr>
<th>R2</th>
<th>6PM</th>
<th>2AM</th>
<th>6AM</th>
<th>P</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td>6PM vs 2AM</td>
</tr>
<tr>
<td>PQ vs HR</td>
<td>0.54 ± 0.12</td>
<td>0.46 ± 0.19</td>
<td>0.50 ± 0.11</td>
<td>0.229</td>
<td>NS</td>
</tr>
<tr>
<td>QRS vs HR</td>
<td>0.21 ± 0.17</td>
<td>0.18 ± 0.12</td>
<td>0.21 ± 0.16</td>
<td>0.822</td>
<td>NS</td>
</tr>
<tr>
<td>QT vs HR</td>
<td>0.60 ± 0.19</td>
<td>0.46 ± 0.26</td>
<td>0.64 ± 0.08</td>
<td>0.0560</td>
<td>NS</td>
</tr>
<tr>
<td>QTcF vs HR</td>
<td>0.85 ± 0.11</td>
<td>0.94 ± 0.041</td>
<td>0.80 ± 0.16</td>
<td>0.0610</td>
<td>NS</td>
</tr>
<tr>
<td>TQ vs HR</td>
<td>0.95 ± 0.013</td>
<td>0.95 ± 0.013</td>
<td>0.94 ± 0.016</td>
<td>0.322</td>
<td>NS</td>
</tr>
<tr>
<td>QT:QT vs HR</td>
<td>0.98 ± 0.01</td>
<td>0.98 ± 0.007</td>
<td>0.98 ± 0.02</td>
<td>0.127</td>
<td>NS</td>
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</tbody>
</table>
Average heart rates were 88 (SD 18), 70 (SD 15), and 89 (SD 22) for 6 PM, 2 AM, and 6 AM, respectively, such that (6 PM=6 AM)>2 AM (p<0.001). These differences could translate to both toxicological and clinical significance, since heart rate is a prime determinant of myocardial oxygen consumption and diastole (the reciprocal of heart rate) is a prime determinant of myocardial oxygen delivery. Thus change in heart rate may produce an energetic imbalance leading to ischemia and possibly to infarction and fibrosis.

Average PQ intervals were 109 (SD14), 115 (SD 18), and 111 (SD 16) for 6 PM, 2 AM and 6 AM, respectively, such that (2 AM = 6 AM), 6 PM= 6 AM, but 2 AM>6 PM, p=0.038. These slight differences should be of neither toxicological nor clinically significant ion dogs with failing hearts, that is they should not lead to significant resynchronization between atria and ventricles or to atrioventricular block. It is not known from this study whether or not dogs with more severe heart failure would behave similarly.

The slopes of the relationships between PQ interval (ms) and heart rate (beats/ minute) are shown in Table 9 and figure 37. All slopes are negative, but none of the slopes differed among times. As with the shams, it was expected that PQ would lengthen when heart rate slowed, since reduction in heart rate is most often a consequence of increased vagal tone and that increase in tone should lengthen PQ as well.
However when heart rate slows, the AV node is bombarded fewer times per minute and has more time for recovery, therefore PQ interval may actually shorten when heart rate slows. This did not occur, however. It must be emphasized that the relationships were calculated based upon means recorded from recordings of 1 hour. Within that hour when respiratory sinus arrhythmia occurs, change in intervals may be different because heart rate changes are rather sudden during inspiration and expiration.

Figure 37. Slopes of PQ vs. HR for dogs with failing hearts.
Average QRS durations were 52 (SD 6), 52 (SD 6) and 51 (SD 6) at 6 PM, 2 AM and 6 AM respectively, and none differed among times. Average (as seen above) heart rates were 88 (SD 18), 70 (SD 15), and 89 (SD 22) for 6 PM, 2 AM, and 6 AM, respectively, such that (6 PM=6 AM)>2 AM (p<0.001). The slopes of the relationships between QRS duration and heart rate (beats/minute) are shown in Table 9 and figure 38, and none differ among times. This was an expected lack of relationship between QRS duration and heart rate, since heart rate changes because of waxing and waning of vagal efferent activity to the SA node, and there is little vagal innervation to the ventricular syncytium.

Average QT intervals were 222 (SD 32), 238 (SD 27), and 216 (SD 26) for 6 PM, 2 AM and 6 AM respectively, such that (6 PM = 6 AM)< 2 AM (p<0.001). This is in agreement with Soleviev et al who showed longer QTs at night. In addition, since heart rates were slower at night and there is a well-known relationship between heart rate and QT, QTs at night should have been longer (Soloviev, Hamlin et al. 2006). Average heart rates were 91
(SD 12), 66 (SD 6), and 76 (SD 11) for 6 PM, 2 AM, and 6 AM respectively such that (2 AM=6 AM)<6 PM. The slopes of the relationships between QT interval and heart rate (beats/ minute) are shown in Table 9 and figure 39. All slopes are negative, but they differed by time of day. Slopes at 6 PM (-0.21) and 2 AM (-0.13) did not differ (P=0.4); slopes between 6 PM (-0.21) and 6 AM (-0.29) did not differ (P=0.6); but slopes between 2 AM (-0.13) and 6 AM (-0.29) differed (0.04) with the slope at 6 AM being steeper than at 2 AM.

![Figure 39. Slopes of QT vs. HR for dogs with failing hearts.](image)

This indicates that for any change in heart rate, QT lengthens more at 6 AM and than at 2 AM. Thus unless the mathematical correction of QT for heart rate was performed at 2 AM, using the value obtained at other times may be correct inappropriately. This fascinating observation may explain, in part, why patients tend to die from arrhythmias around 6 AM when the relationship between QT and heart rate is the steepest. The QT, however, was longest at 2 AM, however patients are not more likely to die at this time. This is consistent
with the knowledge that QT duration is not as important to arrhythmogenesis as is QT instability.

Average QTc(F)s were 254 (SD 21), 255 (SD 19), and 247 (SD 16) for 6 PM, 2 AM and 6 AM, respectively. All slopes relating QTc(F) to heart rate are positive (Figure 40). Slopes at 6 PM (0.71) and 2 AM (0.99) differed (P<0.001), and slopes between 2 AM (0.99) and 6 AM (0.64) differed (P<0.05), but there was no difference between slopes at 6 PM (0.71) and 6 AM (0.64).
As for the shams, this data supports the contention that correcting QT for heart rate is inadequate, i.e., slopes should be 0, but that the adequacy or inadequacy varies according to time of day. It is interesting to speculate if correcting QT using individual data for each dog would be more appropriate. It should be remembered that the individual correction is based upon heart rates obtained during 24 hours, because slow heart rates occur at night and high heart rates during the day. Thus it is not known if such individual corrections would apply at 2 AM, 6 AM, or 6 PM.

Average TQs were 501 (SD 149), 667 (SD 162), and 521 (SD 163) for 6 PM, 2 AM and 6 AM respectively. There were no differences in TQ for different times, however the P value for the F-statistic in the ANOVA was 0.052, close to being significant indicating a trend that TQ at 2 AM (667) was longer than at 6 PM (501) or at 6 AM (521). This is consistent with the differences in heart rates, since TQ—the period of electrical diastole—changes more for change in heart rate than does QT—electrical systole.
Figure 41. Slopes of TQ vs. HR for dogs with failing hearts.

Although the slopes of the relationships between QT: TQ versus heart rate were small (Figure 42), there are differences in this slopes among times. Slope for 6 PM (0.008) did not differ (P=0.54) from that at 2 AM (0.009); slopes between 6 PM (0.008) did not differ (P=0.098) from slope at 6 AM (0.007); but slope between 2 AM (0.009) was steeper (P=0.005) than for 6 AM (0.007). Although statistical differences did occur, as mentioned the slopes are so trivial that the differences, too, were no doubt trivial.
Figure 42. Slopes of QT: TQ vs. HR for all dogs with failing hearts.

The percentage of change in the dependent variables (e.g., PQ, QRS, QT, QTc, QT: TQ) attributable to the change in heart rate was assessed by R^2, however comparing R^2s is not statistically valid. None-the-less it appears that R^2s for PQ appeared to differ between 2 AM and 6 AM. R^2s for QTc(F) appeared to differ between 6 PM and 2 AM. R^2s did not appear to differ for QRS, QT, TQ, or for QT: TQ
CHAPTER 6. COMPARISON OF TIME DIFFERENCES IN
ELECTROCARDIOGRAPHIC PARAMETERS OF ARRHYTHMIC LIABILITY
IN TELEMETERED NORMAL DOGS AND DOGS WITH FAILING HEARTS

6.1 Hypotheses

(I) Electrocardiographic parameters are different in dogs with induced failing hearts when compared to normal healthy dogs, depending upon from which 3 times during the day records were obtained.

(II) There are no differences in electrocardiographic parameters between the 3 times of the day due to differences in heart rate when comparing normal healthy dogs with induced heart failure.

6.2 Materials and Methods

6.2.1 Surgical and Experimental Protocol

All dogs (normal and with failing hearts) were surgically prepared as mentioned previously in chapters II and IV. Blood samples and echocardiograms were collected in both groups
before starting ECG data collection. M-mode echocardiography was obtained as explained previously by using the 2-D right parasternal short axis view. ECG recording methods were already explained previously in chapters II and IV.

6.2.2 Data Analysis

ECG parameters were measured or computed as mentioned previously in chapters II and IV by using a validated EMKA ECG AUTO.

In the group of dogs with failing hearts, NTproBNP values equal or above 3000 were considered as 3000 for statistical purposes. Values for EF, FS and NTproBNP were compared between both groups (normal dogs and dogs with failing hearts) by a t-test design, considering statistically significant differences at P<0.05. Descriptive statistics performed include mean, standard deviation, maximum and minimum values for each parameter evaluated. Coefficients of variation were calculated for each parameter for each group. By using the natural log transformation, all data that was normalized, in order to decrease the variation of each parameter. Transformed means of each electrocardiographic parameter at the three different times were compared by Two Way ANOVA with repeated measures design, and when differences were indicated by a significant F-statistic, specific means were compared by Bonferroni multiple comparison post-hoc test requiring a P<0.05 for significance.
6.3 Results and Discussion

Table 11 and figures 43 and 44 show left ventricular function for both groups of dogs (normal and with failing hearts). It can be observed that dogs identified as having failing hearts did in fact have failing hearts since their EF’s were precipitously lower (40.1 versus 58.48, P<0.001) and their NTproBP’s dramatically higher (2716 versus 491, P<0.001) than shams. These 2 parameters are considered to be “gold standards” for identifying failing hearts, whereas symptoms (e.g., respiratory distress, exercise intolerance or limitation) identify patients with either heart failure or congestive heart failure.

<table>
<thead>
<tr>
<th></th>
<th>EF</th>
<th>FS</th>
<th>NTproBNP</th>
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</thead>
<tbody>
<tr>
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<td>Mean ± SD</td>
<td>Max</td>
<td>Min</td>
</tr>
<tr>
<td>Normal</td>
<td>58 ± 7</td>
<td>69</td>
<td>49</td>
</tr>
<tr>
<td>FH</td>
<td>40 ± 5</td>
<td>48</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 11. EF, FS and NTproBNP for normal dogs (Normal) and dogs with failing hearts (FH).
Figure 43. EF, FS and NTproBNP levels for all normal dogs and dogs with failing hearts.

Figure 44. M-mode in a normal dog and in a dog with failing heart.
Figure 45. Average heart rates for normal dogs and dogs with failing hearts during the recording period.

In general, during the recording period, heart rate for both groups (normal and failing heart) behaved in the same pattern. It started decreasing at 6 PM (when lights were turned off), remained low during night and started increasing again at 6 AM (when lights were turned on). It is noticed that in the failing heart group, heart rate falls faster group, and increases before than in the normal group. Additionally, in the failing heart group the heart rate during night did not fall as much as in the normal group and then it rises faster and higher when compared with the normal group. This finding was expected since it is well known that in heart disease there is an increase of the heart rate due to an imbalance of the autonomic nervous system (increased sympathetic tone, decreased parasympathetic activity), baroreceptor dysfunction and activation of the neuroendocrine system in order to compensate the circulatory changes. Though dogs with failing hearts still have some
parasympathetic influence evidenced by the profound decrease in heart rate during night, a slight autonomic imbalance can be suspected since in general heart rate does not decrease as much as normal dogs during night. Around midnight, the failing heart group showed a slight increase in heart rate that could be attributed to the short sleep-wake periods, in which dogs eat, drink water and are active inside their cages.

Table 12 and figures 46 through 59 show values for important ECG parameters obtained at 3 times of day for both normal dogs and for dogs with failing hearts. Table 13 shows the coefficient of variations for each parameter at each time point (6 PM, 2 AM, 6 AM) for each group (normal and failing heart). These tables show how in general dogs with failing hearts have increased variability in most of the parameters when compared to normal dogs. This increased variability, could be attributed to a high heterogeneity in the group due to either different degrees of severity or different etiologies of cardiac disease.

Figure 46. Heart rate for normal dogs and dogs with failing hearts.
Mean values and standard deviations for HR for all normal dogs and dogs with failing hearts are shown in figure 46. There were no differences in HR between both groups normal dogs and dogs with failing hearts (P=0.549). However, there is a tendency for the failing heart group to have higher heart rates than normal dogs at 6 AM, probably related to the increased sympathetic tone characteristic in heart disease. On the contrary, by time of day there were differences just between 2 AM and 6 PM (P<0.001), mainly in normal dogs (P=0.005). This can be the result of the high variability found in the failing heart group mainly at 6 PM and 6 AM.
<table>
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<tr>
<th>Parameter</th>
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<th>SHAM 6:00 a.m.</th>
<th>HF 6:00 p.m.</th>
<th>HF 6:00 a.m.</th>
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<tr>
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Table 12. Descriptive statistics for each parameter for normal dogs and dogs with failing hearts.
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<td>0.18</td>
<td>0.16</td>
<td>0.14</td>
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Table 13. Coefficients of variation for normal dogs and dogs with failing hearts.

The failing heart group has bigger coefficients of variation in most of the parameters.
There were no differences in HR SD (Figure 47) between both groups normal dogs and dogs with failing hearts (P=0.426). On the contrary, by time of day there were differences between 2 AM and 6 PM (P<0.001), and between 2 AM and 6 AM (P=0.002). For SDNN (Figure 48), there were no differences among groups either (P=0.309). By time of day, there were differences between 2 AM and 6 PM (P=<0.001) and between 2 AM and 6 AM (P<0.001). There is a tendency for SDNN to have more variability in dogs with failing hearts when compared to normal dogs. These results suggest that dogs with failing hearts but no clinical signs of cardiac disease have a normal behavior of the heart rate variability in the time domain. At this stage of disease is probable that there is no imbalance of the autonomic nervous system, activation of the neuroendocrine system or dysfunction of the baroreceptors. The higher variability for SDNN in dogs with failing hearts may be the result of different degrees of cardiac disease in this group of dogs.
It was found that low frequency component of HRV by time of the day was greater at 2 AM when compared to 6 PM (P<0.001) and 6 AM (P<0.001) (Figure 49). However, for dogs with failing hearts, was greatest at 2 AM (during sleep) when compared to 6 PM (P=0.042), and there were no differences of significance between 2 AM and 6 AM (P=0.121) or 6 AM and 6 PM (P=1.0) when heart rates were high (chapter IV). On the contrary, for normal dogs, by time of day, all low frequency components (LFC) of HRV differed. LFC were greater at 2 AM than 6 PM (P<0.001), and were also greater than at 6 AM (P<0.001), while 6 PM was lower than 6 AM (P=0.009) (chapter II). Since LF power is influenced equally by sympathetic and parasympathetic efferent activities, it appears that parasympathetic activity dominates sympathetic activity. Though, there were no differences of significance in LF power at any time of day between shams and dogs with failing hearts (P=0.503), at 2 AM dogs with failing hearts appeared to have lower (5055 for dogs with failing hearts and 13,739 for shams) LF power than for shams. Since dogs with failing heart would be
expected to have higher sympathetic efferent activity than shams, this result is unexpected, but may be explained by the domination of the vagus.

Figure 49. Low frequency component in normal dogs and dogs with failing hearts.

Figure 50. High frequency component in normal dogs and dogs with failing hearts.
It was found that high frequency component of HRV by time of the day was greater at 2 AM when compared to 6 PM (P<0.001) and 6 AM (P<0.001) (Figure 50). For dogs with failing hearts, by time of day, high frequency component (HFC) to HRV were greatest between 2 AM and 6 PM (P=0.019) and between 2 AM and 6 AM (P<0.001), and there were no differences of significance between 6 AM and 6 PM (P=0.918). As expected, HFC to HRV—dominated by parasympathetic efferent activity—was greatest while dogs slept. For normal dogs, differences in HFC to HRV occurred between 6 PM and 2 AM and 2 AM and 6 AM (P=0.033 and 0.006 respectively). This finding is expected since parasympathetic efferent activity normally dominates while animals sleep. There were no differences in HFC to HRV at any time of day when comparing shams with dogs with failing hearts (P=0.066). It was found that low frequency to high frequency ratio (LF: HF) (Figure 6.10) for dogs with failing hearts was lower at 2 AM when compared to 6 AM (0.039), and there were no differences between 6 PM and 2 AM (P=1.0) and between 6 PM and 6 AM (0.693). This finding is expected due to the high parasympathetic influence during night. For normal dogs, differences in LF: HF (Figure 51) were between 6 PM and 2 AM (P=0.013). Additionally, there were no differences in HFC to HRV at any time of day when comparing shams with dogs with failing hearts (P=0.315). This is accountable by the fact that dogs with failing heart were not in heart failure (i.e., were not symptomatic), and addresses a major goal of this study, which is to determine what changes in parameters may serve as prodromata to symptoms. Thus changes in various components to HRV do not appear to be useful for identifying dogs with failing hearts that are at risk for developing symptoms and therefore requiring therapeutic interventions.
Figure 51. LF: HF in normal dogs and dogs with failing hearts.

Figure 52. PQ interval in normal dogs and dogs with failing hearts.
It was found that the atrio-ventricular conduction time (PQ interval) by time of the day was longer at 2 AM when compared to 6 PM ($P<0.001$) (Figure 52). For normal dogs, by time of day, PQ interval was longest at 2 AM and 6 PM ($P=0.005$), and there were no differences of significance between 2 AM and 6 PM ($P=1.0$) or between 6 AM and 6 PM ($P=0.121$). Additionally, there were no differences in PQ interval at any time of day when comparing shams with dogs with failing hearts ($P=0.491$). Results show an influence of the parasympathetic activity over the atrio-ventricular conduction time in both normal dogs and dogs with failing hearts. This suggests that the degree of cardiac disease in dogs with failing hearts is not severe enough to induce changes in the atrioventricular conduction. Though, there are no differences between normal dogs and dogs with failing hearts, the second group has a greater variability of the PQ interval, that may result from the heterogeneity of the cardiac disease in this group.

![QRS](image)

Figure 53. QRS for dogs with failing hearts at each time point.

It was found that ventricular depolarization time (QRS) by time of the day did not change (Figure 53) ($P=0.906$). Additionally, there were no differences in PQ interval at any time of
day when comparing shams with dogs with failing hearts (P=0.491). As previously found in chapters II and IV, QRS (ventricular conduction) seems to be completely independent of heart rate, autonomic influences, and temperature. Though there are no differences between normal dogs and dogs with failing hearts there is a tendency for the group of dogs with failing hearts to have a QRS complex longer at all the three time points when compared to normal dogs. This result suggests that in dogs with failing hearts the cardiac disease is not severe enough to induce all the remodeling changes that may contribute with the prolongation of the ventricular depolarization. However, it tends to move in the expected direction since all means for failing hearts were slightly longer than those for normal dogs. Additionally to this tendency there is an increased variability of the QRS complex in dogs with failing hearts, suggesting the heterogeneity of the cardiac disease in this group of dogs.

![Figure 54. QT interval for normal dogs and dogs with failing hearts.](image)

It was found that ventricular repolarization time (QT) by time of the day was longer at 2 AM when compared to 6 PM (P<0.001) and 6 AM (P<0.001) (Figure 6.13). Within the group of dogs with failing hearts, by time of the day the QT interval was longer at 2 AM when compared to 6 PM (P<0.001) and 6 AM (P<0.001), while for the normal dogs, there
were no differences. Additionally, there were no differences in PQ interval at any time of day when comparing shams with dogs with failing hearts (P=0.302). As previously observed in chapters II and IV, QT interval is slightly longer at 2 AM probably due to the influence of the parasympathetic activity during night. Though there are no differences between normal dogs and dogs with failing hearts, there is a tendency in the second group to have longer QT intervals. This tendency can be related to the initiation of cardiac disease, and can be considered as a marker of arrhythmic liability. Additional to the tendency to have longer QT interval, dogs with failing hearts showed greater variability of the QT interval, which could be explained by the heterogeneity of the disease in the group.

![Figure 55. QTSD for normal dogs and dogs with failing hearts.](image)

Figure 55. QTSD for normal dogs and dogs with failing hearts.
It was found that QTSD (a marker of QT instability) by time of the day did not change at any time point (P=0.683) (Figure 55). On the other hand, there were no differences in QTSD at any time of day when comparing shams with dogs with failing hearts (P=0.065). Additionally, it was observed that QT STV (marker of QT instability) by time of the day did not change at any time point. On the other hand, dogs with failing hearts have bigger QT STV when compared to normal dogs (P=0.024) (Figure 6.15). Finally, QTcF by time of the day was greater at 6 PM than 6 AM (P=0.005), and dogs with failing hearts have longer QTcF compared to that for normal dogs (P=0.034) (Figure 56). Though there were no differences in QTSD between both groups, it tended to be higher at 2 AM and 6 AM and in dogs with failing hearts and there is a tendency for this group of dogs to have bigger QTSD variability, especially at 2 AM and 6 AM. On the other hand, QT STV also was greater and with a bigger variability in dogs with failing hearts at 2 AM and 6 AM when compared to normal dogs. Finally, at all three time points, dogs with failing hearts have longer QTcF intervals than normal dogs, specially at 2 AM and 6 AM. All three markers of arrhythmogenic liability are greater in dogs with failing hearts than in normal dogs at both 2 AM and 6 AM. These results suggest an increased likelihood for arrhythmias in dogs with failing hearts when compared to normal dogs at 2 AM and 6 AM.
Figure 56. QT STV for normal dogs and dogs with failing hearts.

Figure 57. QTcF for normal dogs and dogs with failing hearts.
Figure 58. TQ interval for normal dogs and dogs with failing hearts.

Figure 59. QT: TQ ratio for normal dogs and dogs with failing hearts.
It was found that TQ (a marker of ventricular restitution) by time of the day TQ was longer at 2 AM when compared to 6 PM (P<0.001) and 6 AM (P<0.001) (Figure 58). On the other hand, there were no differences in TQ interval at any time of day when comparing shams with dogs with failing hearts (P=0.474). Additionally, it was observed that QT: TQ (marker of ventricular restitution) in normal dogs was greater at 6 PM when compared to 2 AM (P<0.001) and 6 AM (P=0.005). On the contrary, in dogs with failing hearts, by time of the day QT: TQ did not change (Figure 59). On the other hand, there were no differences in QT: TQ interval at any time of day when comparing shams with dogs with failing hearts (P=0.125). Though there are no statistical differences between normal dogs and dogs with failing hearts, there is a tendency of the dogs with failing hearts to have greater QT: TQ ratios, with a greater variability especially at 6 AM, suggesting an increase likelihood of arrhythmias in this group of dogs at the three time points evaluated. Despite this tendency, it is possible that that tendency does not have any clinical relevance, by itself, in dogs with failing hearts.

Table 14 and figures 60 through 63 show all parameters at a heart rate of 80, to determine if there were differences between shams and dogs with failing hearts that were due to differences in heart. Since there were no differences in parameters monitored between normal dogs and dogs with failing hearts at the same heart rates, this treatment is unnecessary. However TQ (electrical diastole when ventricular restitution occurs) was shorter (P<0.001) for dogs with failing hearts than for shams. This indicates that dogs with failing hearts had shorter periods of diastole compared to systole, and this is a known electrophysiological substrate for ventricular ectopia. This supports the potential need for either Holter monitoring or occasional clinical monitoring of rhythm. Unfortunately it is not
known whether or not dogs ill with natural clinical heart disease and with this electrophysiological substrate are at risk for arrhythmia and sudden death. None-the-less this finding may be an indication to determine heart size and/or NTproBNP in dogs with heart disease that have (asymptomatic) failing hearts. Why were there no difference in TQ between shams and dogs with failing hearts when TQ was compared at various times of day but without hearts of 80? This can be explained by wide variability of TQ at varying heart rates. For example mean TQs/SDs for shams and for dogs with failing hearts at 2 AM were 696/73 and 667/162. TQs/SDs for shams and for dogs with failing heart but at heart rates of 80 were 524/11 and 473/46. Clearly this phenomenon should be explored in dogs with natural-occurring asymptomatic heart disease.

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<td>QT</td>
<td>231 ± 16</td>
<td>248 ± 32</td>
<td>245 ± 12</td>
<td>236 ± 12</td>
</tr>
<tr>
<td>QTcF</td>
<td>543 ± 8</td>
<td>481 ± 46</td>
<td>524 ± 11</td>
<td>536 ± 12</td>
</tr>
<tr>
<td>TQ</td>
<td>0.39 ± 0.03</td>
<td>0.40 ± 0.10</td>
<td>0.42 ± 0.03</td>
<td>0.40 ± 0.03</td>
</tr>
</tbody>
</table>

Table 14. Descriptive statistics at 80bpm in normal dogs and dogs with failing hearts.
Figure 60. PQ interval for normal dogs and dogs with failing hearts at 80 bpm.

Figure 61. QRS for normal dogs and dogs with failing hearts at 80 bpm.
Figure 62. QT interval for normal dogs and dogs with failing hearts at 80bpm.

Figure 63. QT interval for normal dogs and dogs with failing hearts at 80bpm.
Figure 64. TQ interval for normal dogs and dogs with failing hearts at 80bpm.

Figure 65. QT: TQ interval for normal dogs and dogs with failing hearts at 80bpm.
6.4 Conclusions and Limitations

Some of the limitations of this study include:

- The sample size (n) was small but was adequate to achieve sufficient power to identify changes in heart rate and most of the parameters by time. However, there were no differences among groups probably due to small sample size with big variability.

- Two different canine models of heart failure were used for this study. The idea was to keep the heterogeneity of the cardiac disease in general instead of studying a specific etiology of heart disease. However, we cannot assure if both groups contributed equally to the results. Therefore, care must be taken when extrapolating these results to a group of patients with a specific etiology of heart disease. Additionally, this heterogeneity of the cardiac disease could contribute to the big variability, making the study less sensitive to potential differences between groups.

- In order to have recordings clear of noise and artifacts due to animal handling and dog excitement, we did not obtained data for 24 hours. Therefore this study just shows the fluctuations of these electrographic parameters during evening, night and early morning. Due to the obvious fluctuation hour by hour in the electrocardiographic parameters, care should be taken when extrapolating these results to other times different than 6 PM, 2 AM and 6 AM to other studies in telemetered dogs with failing hearts or patients.
• This study was performed in laboratory male mongrel normal dogs and mongrel dogs with failing hearts, located in a complete controlled environment (light cycle, feeding time, humidity, etc). Therefore, care must be taken when extrapolating these data to animals with different physical or environmental characteristics.

All parameters were compared by 2-way ANOVA with repeated measures design on both time and group. The only differences of statistical significance between groups was that QTc(F) for dogs with failing hearts was greater than for normal dogs. However it appeared that SDs and Cs for most other parameters (heart rate, RR, PQ, QT, QTc, TQ, SDNN) differed, therefore, we sought to compare these expressions of increased variance after natural logarithmic transformation. After this transformation the following differences were noted: QTc(F) was longer for dogs with FH than normal dogs, suggesting increased likelihood of arrhythmia, short-term QT variability was longer for dogs with FH than for normal dogs suggesting increased likelihood of arrhythmia, SD of QT (i.e., QT instability) tended to be greater in dogs with FH than normal dogs, suggesting increased likelihood for arrhythmia, and the high frequency power of heart rate variability tended to be lower in dogs with FH than in normal dogs, suggesting decrease in vagal restraint—a known pro-arrhythmic substrate—or increased sympathetic activity—a known proarrhythmic physiology.


Moe, G. W., T. P. Stopps, et al. (1989). "Alterations in serum sodium in relation to atrial natriuretic factor and other neuroendocrine variables in experimental pacing-


APPENDIX A

Clouds of PQ vs. HR for each one of the normal dogs (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of QRS vs. HR for each one of the normal dogs (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of QT vs. HR for each one of the normal dogs (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of QTcF vs. HR for each one of the normal dogs (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of TQ vs. HR for each one of the normal dogs (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of QT: TQ vs. HR for each one of the normal dogs (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of PQ vs. HR for each one of the dogs with failing hearts (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of QRS vs. HR for each one of the dogs with failing hearts (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of QT vs. HR for each one of the dogs with failing hearts (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of QTcF vs. HR for each one of the dogs with failing hearts (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of TQ vs. HR for each one of the dogs with failing hearts (6 PM=red; 2 AM=green; 6 AM=blue).
Clouds of TQ: TQ vs. HR for each one of the dogs with failing hearts (6 PM=red; 2 AM=green; 6 AM=blue).