FACULTY OF HEALTH AND MEDICAL SCIENCES
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Master's Thesis

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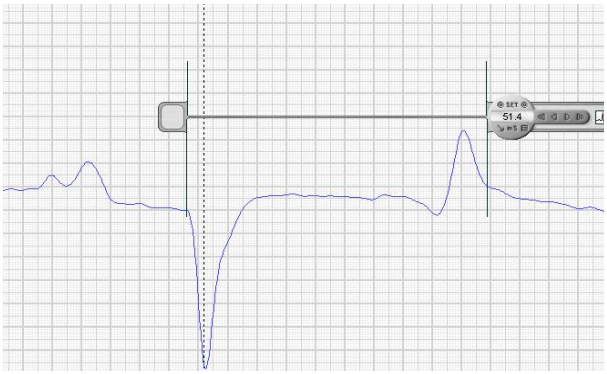
kln203

Characterisation of the QT interval in horses

- Is there a difference in the length of the QT interval between breeds?



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Preface

The master thesis "Characterisation of the QT interval in horses- is there a difference in the length of the QT interval between breeds?" has been conducted as a part of the veterinary education. The thesis has been conducted within the Equine Cardiac Group, which is a collaboration between the Department of Large Animal Science, the Department of Basic Animal and Veterinary Sciences, the Department of Biomedical Sciences and the Laboratory of Experimental Cardiology at the University of Copenhagen. The thesis corresponds to 30 ECTS points and has been written in the period from February to July 2013 with some preliminary fieldwork in November 2012. The fieldwork was performed at two racing yards near Copenhagen Racecourse in Klampenborg, Denmark.

The result of this thesis will be of interest for anyone interesting in equine cardiac physiology and especially the ventricular repolarisation process.

I would like to thank everyone who has contributed to the work of this master thesis:

- My supervisor, Rikke Buhl for helping me with the practical work, providing of ECG recordings, enthusiasm and support throughout the study period.
- My co- supervisor, PhD student Philip Juul Pedersen, for help with the QT interval measurements, data management, providing of ECG recordings and answering of many questions.
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- At last Jesper Torbøl Pedersen for patience, proof reading, help with setup and support throughout the process.

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Malin Karlsson

Abstract

The QT- and T_{peak} to T_{end} (T_pT_e) interval on the electrocardiogram (ECG) represent the cardiac repolarisation. In humans, irregularities of these intervals due to genetic mutations, drugs or electrolyte disturbance can lead to fatal arrhythmias, syncope and sudden cardiac death (SCD). The QT- or T_pT_e interval in horses has so far not been used clinically due to a lack of reference values and correction formulas. Horses have though also spontaneous arrhythmias, suffer from SCD and are treated with pharmaceuticals that are known to prolong the QT interval in humans.

This study compared the length of the QT interval in different breeds and tested whether a piecewise linear regression model can describe the relationship between the QT- and R_{peak} to R_{peak} (RR) interval. It was further tested whether the T_pTe interval is independent of heart rate (HR) or breed.

ECG tracings from 58 geldings from seven different breeds with a mean age of 7.3 years were used. 40 QT and T_pT_e intervals were measured from ECG tracings of each horse during rest, exercise and recovery and were plotted against RR. Data from the QT measurements were analysed as a non- linear regression model and T_pT_e measurements as a linear regression model.

The relationship between QT- and RR intervals showed to be best described by a piecewise linear regression model ($0.92<r^2<0,98$), consistent with the results of Pedersen *et al* (2013), which is different from the linear regression model described in humans (Rajappan *et al* 2003). There was a significant effect of breed on slopes ($p < 0.0001$) and RR_{bend} (p 0.0071), and a tendency of higher inter- breed variation at lower HR than at higher HR. The reason for the inter-breed variation may be inexperience of the observer, differences in the cardiac electrophysiology or in the parasympathetic tone between breeds. The relationship between T_pT_e- and RR intervals had a significant deviation from zero in six out of seven breeds and the regression model for each breed varied significantly. The result indicates that analyses of the T_pT_e interval in a mixed group of horses as a single model gives a false reference value but further studies including more than 10 horses in each breed and more than one ECG observer are needed to validate the results.

This study indicates that future studies testing the relationship between QT- and RR interval in horses should use the piecewise linear regression model. These studies should include older horses, stallions and mares and pony breeds to get a better representation of the natural horse population. There is though also a need for further investigations of the biophysical explanation behind the piecewise linear regression model.

Keywords: QT interval; ECG; Horse/Equine; LQTS; Cardiac repolarisation

Resumé

QT- og T_{peak} til T_{end} (T_pT_e) intervallerne på elektrokardiogrammet (EKG) repræsenterer hjertets repolarisering. Uregelmæssigheder i intervallerne hos mennesker, som følge af genetiske mutationer, lægemidler eller elektrolyt forstyrrelser, kan forårsage fatale arytmier, synkope og pludselig hjertedød. QT eller T_pT_e intervallet hos heste bruges ikke klinisk endnu, da der mangler referenceværdier og korrektions formler. Hos heste ses der dog også spontane arytmier, tilfælde af pludselig hjertedød, og heste behandles med lægemidler, der er kendte for at forlænge QT intervallet hos mennesker.

Studiet havde til formål at jævnføre QT intervallet hos forskellige hesteracer, og teste hvorvidt en todelt lineær regressions model kan beskrive forholdet mellem QT- og R_{peak} til R_{peak} (RR) intervallerne. Desuden havde studiet til formal at teste hvis T_pT_e intervallet er uafhængigt af hjerte rytme (HR) og race.

EKG optagelser, fra 58 vallakker fra syv forskellige racer med en middelalder på 7,3 år, blev brugt i studiet. På hver EKG optagelse blev der målt 40 QT- og T_pT_e- intervaller fordelt på henholdsvis hvile, arbejde og restitution, og disse intervaller blev plottet imod RR. Data fra QT målingerne blev analyseret som en ikke lineær regressions model og T_pT_e målingerne som en lineær regressions model.

Studiet viste at forholdet mellem QT- og RR intervallerne beskrives bedst af en todelt lineær regressions model ($0.92<r^2<0,98$), i overensstemmelse med resultaterne fra Pedersen *et al* (2013), hvilket er forskelligt fra den lineære regressions modellen der er beskrevet i mennesker (Rajappan *et al* 2003). Studiet viste at race havde en signifikant effekt på hældningen ($p < 0,0001$) og RR_{bend} (p 0,0071), samt en tendens til større variation mellem racer ved lav HR end ved høj HR. Årsagen til denne inter- race variation kan vare uerfarenhed hos måleren, uligheder i hjertets elektrofysiology eller i styrken af det parasympatiske nervesystem mellem racer. Forholdet mellem T_pT_e- og RR intervallet viste signifikant afvigelse fra nul hos seks ud af de syv racer, og regressionsmodellen var signifikant forskellig mellem racerne.

Resultatet indikerer at analyse af T_pT_e intervalet i en gruppe heste af blandet race i en samlet analyse, giver en forkert referenceværdi. Flere studier der inkluderer mere end 10 heste pr. race samt mere end en observant er dog nødvendig for at validere denne påstand.

Studiet indikerede også at fremtidige studier af forholdet mellem QT- og RR intervallerne bør bruge den todelte lineære regressionsmodellen. Fremtidlige studier bør også inkluder ælde heste, hopper og hingste samt ponyracer for at opnå en bedre repræsentation af den naturlige heste

population. Der er dog samtidig brug for mere forskning på den biofysiske baggrunden til den todelte lineære regressions modellen.

Nøgleord: QT interval; EKG; Heste/Equine; LQTS; Kardiologisk repolarisering

List of Abbreviations

AP	Action potential
APD	Action potential duration
AV	Atrioventricular node
bpm	Beats per minute
BWT	Body Weight
EAD	Early afterdepolarisation
ECG	Electrocardiogram
HR	Heart rate
HVA	High voltage- gated activated calcium channels
LQTS	Long QT Syndrome- prolongation of the QT interval on a surface ECG
LVA	Low voltage- gated activated calcium channels
LVH	Left Ventricular Hypertrophy
MEA	Mean electrical axis
MiRP	minK related peptide
QTc	QT interval corrected for HR
RR_{bend}	Bending point of the piecewise linear regression model
RR interval	Interval from R_{peak} to next R_{peak}
SA	Sinus atrial node
SCD	Sudden cardiac death
T_{end}	Offset of the T- wave
T_pT_e	Interval between T_{peak} to T_{end}

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1. Introduction

Spontaneous arrhythmias (Barbesgaard *et al* 2010) and SCD are well known in horses, but it is shown to be difficult to definitely make an association between SCD and arrhythmias (Gelberg *et al* 1985; Kiryu *et al* 1999). In the study by Lyle *et al* (2011) 54% of the horses suffering SCD did not have significant gross or histological cardiac lesions that could rule out a diagnosis of fatal arrhythmias. This is a common problem because primary conduction abnormalities and accessory conduction pathways are difficult to diagnose in post mortem findings and there is a need for finding arrhythmias before the case of SCD (Lyle *et al* 2011). In the study of Kiryu *et al* (1999) they were able to uptake an ECG from a horse immediately before it suffered SCD, and an arrhythmic death was confirmed. Therefore it can be speculated that more of the cases of sudden death in horses are due to arrhythmias, as seen in humans (Al-Khatib *et al* 2003; Moss 2003; Goldenberg *et al* 2006). In human medicine, acquired or inherited prolonged QT interval on a surface ECG (LQTS) is associated with an increased risk for fatal ventricular arrhythmias (known as Torsades de Points), syncope and SCD (Al-Khatib *et al* 2003; Moss 2003; Goldenberg *et al* 2006). So far, the inherited form is only described in humans, whereas the acquired form is described in both humans and companion animals (Finley *et al* 2003; Lyle *et al* 2011; Buhl *et al* 2013). Horses treated with pharmaceuticals, such as quinidine and erythromycin that are known to prolong QT interval in humans, have not been monitored for LQTS, due to the lack of correction formulas and reference values of the QT interval in horses. Instead the practitioner needs to rely on clinical signs of intoxications (Blissitt 1999; Pedersen *et al* 2013).

Since the fifties studies measuring the QT interval in horses have been published. These measurements are however made on a specific race and/or within a limited HR range (Lannek *et al* 1951; Fregin 1982; Ayala *et al* 1995; Pasławska *et al* 2012a; Pasławska *et al* 2012b; Schwarzwald *et al* 2012; Pedersen *et al* 2013). The recent study of Pedersen *et al* (2013) shows that the relation between QT and RR is best described by a piecewise linear regression model. This model performed better than commonly used human correction models. The same study does also provide a table with reference values of the QT interval in horses (Pedersen *et al* 2013). These values are divided into mares, stallions and geldings but they are still derived from a single breed only- the race fit Standardbred trotter.

The first part of this master thesis consists of a literature study necessary for understanding the analysis, discussion and conclusion of the relationship between the QT or T_pT_e- and the RR interval in horses of different breeds.

2. Background

Knowledge of the electrophysiology and the different ion currents and ion channels in the heart is a precondition for understanding the physiology of prolonged QT interval and LQTS. The electrophysiology of the heart and the main receptors involved will therefore be outlined in this background section. The connection between the electrophysiology and LQTS respective the ECG will also be described. Further will a description of ECG and ECG recordings in horses and techniques for measuring and correction of the QT interval be outlined.

2.1 The electrophysiology of the action potential

The primary receptors, or ion channels, involved in the contraction mechanism of the myocardium are voltage gated Na^+-, Ca^{2+}- and K^+ channels. The ion channels are integral membrane proteins with three important properties which all together controls the electrochemical potential (Cunningham 2002; Finley *et al* 2003; Ganong 2005):

1. Allow membrane passage of ions along their electrochemical gradient through a pore in the cell membrane
2. Selective filter that controls which ion can cross and enter the pore
3. Voltage gated mechanism that changes the confirmation from open to closed (Finley *et al* 2003).

The ventricular action potential (AP) can be described in five phases as illustrated in Figure 1.

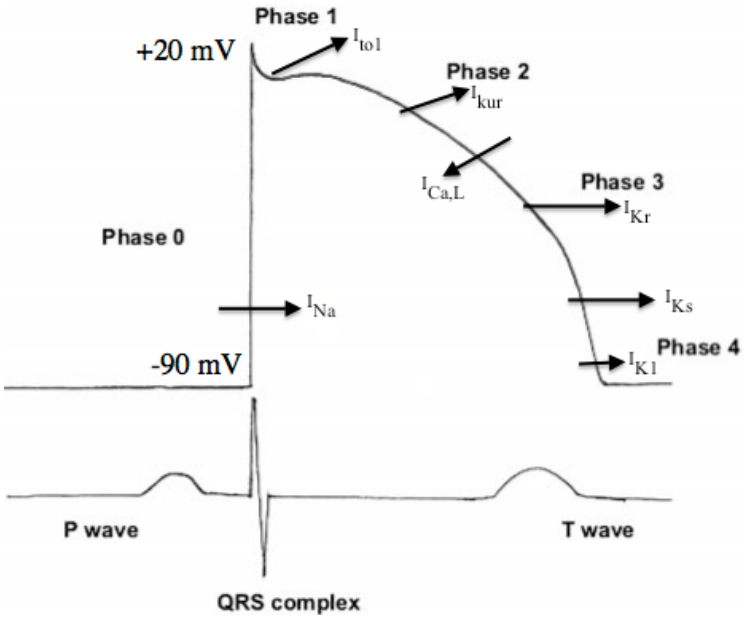

Figure 1 The ventricular action potential with the responsible ion currents and the corresponding ECG curve under. The QT interval corresponds to the repolarisation phase of the action potential. Modified from (Finley *et al* 2003)

Phase 0: Is the depolarisation phase. It starts as soon as a cell has reached the threshold. In fast conducting cells (those not situated in the sinus-/atrioventricular node) the rapid influx of sodium (the Na^+ current- I_{Na}) is responsible for the depolarisation. The influx quickly brings the membrane potential from its resting potential of -90mV to around +20mV (Patteson 1996; Finley *et al* 2003; Ganong 2005).

Phase 1: In this phase the sodium channels get inactivated which causes a short, sharp drop in membrane potential. This drop in membrane potential is also due to rapidly activating and inactivating potassium channels leading to transient outward potassium current (I_{to}) (Patteson 1996; Finley *et al* 2003; Ganong 2005).

Phase 2: This is the plateau phase were a system of balanced ion movements of sodium, potassium, calcium, magnesium and chloride results in a slowly decrease of the intracellular potential towards zero (Patteson 1996; Finley *et al* 2003). The inward current is largely due to Ca^{2+} (I_{CaL}) influx through L-type Ca^{2+} channels (Finley *et al* 2002; Finley *et al* 2003; Ganong 2005). The outward current primary consists of K^+ efflux (I_K) associated with activation of various delayed rectifier channels. Because I_K is the primary current responsible for

termination of AP, the duration is dependent on the relative densities of these three components (Dumaine *et al* 2002; Finley *et al* 2002; Finley *et al* 2003).

Phase 3: This is the repolarisation phase and it is due to closure of the Ca^{2+} channels and an opposed outward current, I_K. I_K subsides at the end of the repolarisation due to voltage dependent deactivation giving a decreased driving force for the outward current. The non-voltage gated inward rectifier K^+ current (I_{K1}) contributes to the terminal repolarisation during phase 3 (Finley *et al* 2003; Ganong 2005).

Phase 4: Is the resting phase with a membrane potential of -90 mV. The inward K^+ current, I_{K1}, maintains the resting potential (Patteson 1996; Finley *et al* 2003; Nerbonne *et al* 2005).

The action potential of the pacemaker cells in the sinus atrial- (SA) and atrioventricular (AV) node is kind of different from the one in the ventricles. The resting membrane potential is less negative (around -60mV) and the depolarisation is largely due to an influx of Ca^{2+} ions. The pacemaker cells are therefore more sensitive for changes in calcium concentration and for drugs affecting calcium channels (Patteson 1996; Ganong 2005). The cells in the SA and AV node lack the rapid sharp raise of phase 0 in the depolarisation (see Figure 2) because of a lack of the fast voltage- gated Na^+ channels seen in the ventricles. Instead there is "funny channels" (I_f), which is permeable for both Na^+ and K^+ (Cunningham *et al* 2007; Silverthorn *et al* 2007). These channels are closed during an AP and opens spontaneously as soon as one AP is finished. When they open, Na^+ influx exceeds K^+ efflux and slowly depolarise the cell towards threshold. At more positive membrane potentials the channels responsible for I_f close and the slow Ca^{2+} channels begin opening, which speeds up the final approach to threshold. The spontaneous depolarisation is called the "pacemaker potential" because the membrane potential never rests at a constant value (Cunningham *et al* 2007; Silverthorn *et al* 2007).

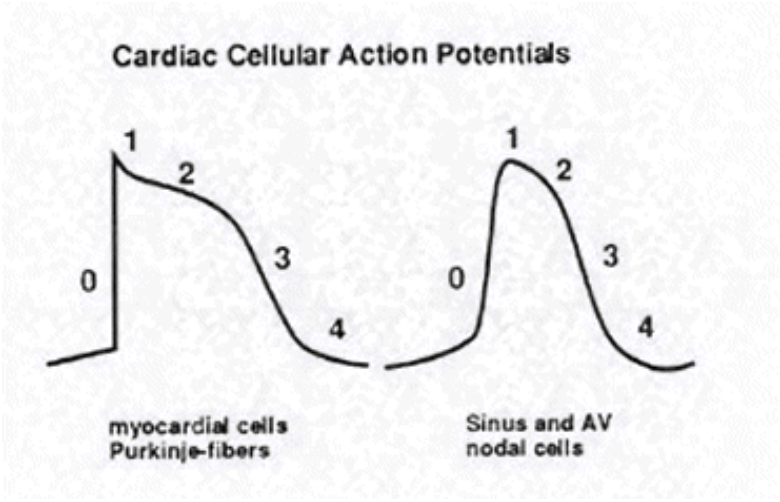

Figure 2: Comparison between the action potential in ventricular cells (left) and the pacemaker cells (right). From: http://cpharm.vetmed.vt.edu/vm8784/CARDIOVASCULAR/antidys.htm

2.2 The ventricular ion currents and channels

2.2.1 Na+ current and channels:

The voltage gated sodium channels open rapidly and give raise to the inward Na^+ current I_{Na} responsible for the repolarisation during phase 0 of the AP (Nerbonne *et al* 2005). These channels are among others with similar structure members of the S4 superfamily of voltage gated ion channels (Catterall 2000a; Nerbonne *et al* 2005). The sodium channels consist of a central pore formed by a single α subunit constituted of four linked homologous domains (I-IV) (see Figure 3) and possibly interaction with auxiliary β subunits that are auxiliary interacting and regulating proteins (Nerbonne *et al* 2005). The β subunits are necessary for normal kinetic voltage dependence but do also appear to be multifunctional cell adhesion molecules that target the channels to the plasma membrane (Catterall 2000a; Nerbonne *et al* 2005). Each domain of the α subunit has six transmembrane α helical regions named S1-S6 (see Figure 3) (Catterall 2000a; Finley *et al* 2003; Nerbonne *et al* 2005). The pore-loop is placed between S5 and S6 and determines to the ion selectivity whereas the voltage-gated properties are based between S3 and S4. Both the N- (amino-) and C- (carboxy-) terminal are placed intracellular. (Yellen 1998; Catterall 2000b; Catterall 2000a; Finley *et al* 2003; Nerbonne *et al* 2005).

Both the rapid activation and the inactivation of few milliseconds duration are voltage dependent (Nerbonne *et al* 2005). The S4 segments form the "voltage sensor" which moves outwards in a rotational movement generating a confirmation change and opening of the pore

(Catterall 2000a). Between S3 and S4 is there a cytoplasmatic "linker" which plays a pivotal role in inactivation (Nerbonne *et al* 2005).

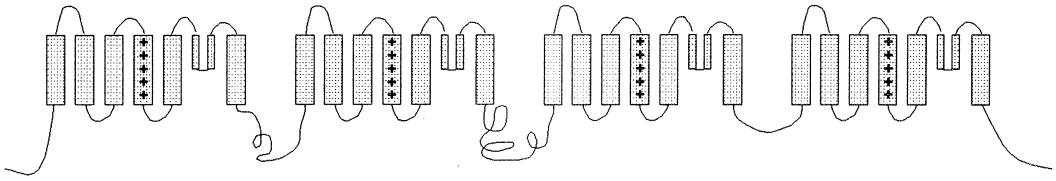

Figure 3: Four homologue domains each with six transmembrane spanning segments assembles to create one α subunit of voltage- gated Na+ or Ca^{2+} channels. Modified from (Yellen 1998).

2.2.2 Ca^{2+} currents and channels:

The cardiac calcium channels giving raise to the inward calcium current I_{CaL} open delayed compared to sodium channels and thereby give little contribution to phase 0 of the ventricular AP but more contribution to the plateau phase (phase 2) (Ganong 2005; Nerbonne *et al* 2005). Two types of calcium channels are described. The low voltage activated channels (LVA) also called transient or T-type channels, and the high voltage activated (HVA) channels (Catterall 2000b; Ganong 2005; Nerbonne *et al* 2005). LVA channels inactivate and activate rapidly and are insensitive to calcium antagonist drugs. The HVA channels are more long lasting and are activated at a more positive membrane potential and inactivate over a longer period. The HVA group is heterogeneous and divided into L, N, P, Q or R- type (Catterall 2000b; Nerbonne *et al* 2005). The L-type is expressed ubiquitous in mammalian hearts with similar densities and properties (Nerbonne *et al* 2005).

The calcium channels are members of the same S4 superfamily as sodium channels. They have a similar construction of a single pore forming α subunit with two types of accessory subunits (see Figure 3). The accessory β subunits are cytosolic proteins that assemble with the α subunits and regulate the expression of the functional cell surface HVA Ca^{2+} channels, hereunder the cardiac L- type channels. The co-expression in heterologous system also increase the amplitude of the current and modifies the kinetic and voltage dependence of the channels (Catterall 2000b; Nerbonne *et al* 2005).

Inactivation of the channels is both voltage dependent and Ca^{2+} dependent. The accumulation of entering Ca^{2+} inactivates the current in milliseconds. This mechanism responds faster than the voltage dependent inactivation and is the primary determinant of the length of the current and thereby the length of the plateau- and repolarisation phase (Catterall 2000b).

2.2.4 K^+ currents and channels:

The voltage gated potassium channels include to two broad classes of outward K^+ currents- transient outward K^+ current, $I_{to,}$ and delayed outwardly rectifying K^+ current, I_K- these are the primary determinants of the repolarisation phases of the AP in mammals (Nerbonne 2000; Nerbonne *et al* 2005).

Also non- voltage gated potassium channels are expressed in the myocardium. These provide inwardly rectifying K^+ currents (Kir) that also contribute to the repolarisation phases of the AP primary through the I_{K1} currents. The I_{K1} currents are so far neither proven to be expressed nor proven to be absent in the equine myocardium (Finley *et al* 2003). There are also other currents (I_{KATP}, $I_{K(ACh)}$) among the Kir currents group that contribute to the normal function of the heart. These seems though not to contribute with important properties during normal physiological conditions in humans and will therefore not be dealt with in this thesis (Nerbonne *et al* 2005).

2.2.4.1 Voltage gated potassium currents:

I_{to}: The channels responsible for I_{to} will give transient outward potassium current and are described in many cardiac cell types. They activate and inactivate rapidly at membrane potentials more positive than -30 mV and contribute to the early phase of repolarisation (phase 1) in ventricular and atrial cells (Nerbonne 2000; Nerbonne *et al* 2005). I_{to} is expressed in the myocardium of, among others, humans, dogs, cats and rabbits but is, interestingly, not expressed in horses (Finley *et al* 2003; Nerbonne *et al* 2005).

I_K: Channels responsible for I_K will activate at similar membrane potential as I_{to} but in contrast to this current, I_K contributes to the latter phase of repolarisation (phase 2 and 3), the phases that brings the membrane potential back to its resting potential (Dumaine *et al* 2002; Finley *et al* 2002; Finley *et al* 2003; Nerbonne *et al* 2005). I_K can in horses be divided into three different components namely **I_{Ks}**, which activates slowly, **I_{Kr}**, which have a marked inward rectification, activates rapidly, and inactivates very rapidly and **I_{Kur}**, which activates very rapidly and inactivates very slowly (Nerbonne 2000; Finley *et al* 2002; Finley *et al* 2003; Nerbonne *et al* 2005). I_{Kur} is described in both atrial and ventricular equine cardiac tissue but is rarely described in ventricular myocytes in humans and other species (Finley *et al* 2003; Nerbonne *et al* 2005). In other animals (dogs, mice's, guinea pigs) it has been shown that the densities of the three components vary in different part of the heart. I_K is the primary current responsible for termination of the AP and the duration of phase 3 therefore largely depends on

the densities of these three different components (Dumaine *et al* 2002; Finley *et al* 2002; Finley *et al* 2003).

2.2.4.2 Molecular structure:

The voltage gated potassium channels do also belong to the same S4 superfamily as voltage gated sodium and potassium channels (Nerbonne 2000; Nerbonne *et al* 2005). In contrast to sodium or calcium channels, the α subunit of potassium channels only consists of one domain (with six transmembrane spanning segments with same properties as in sodium or calcium channels, see Figure 4A) and four α subunit assembles to create the pore (see Figure 4B) (Nerbonne 2000; Finley *et al* 2003; Nerbonne *et al* 2005).

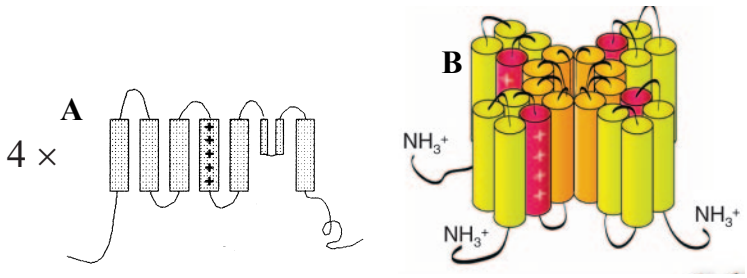

Figure 4: A: One α subunit with a domain constituted of six transmembrane spanning segments Modified rom Yellen (1998) **B:** Four α subunits assembled to create the pore of the voltage gated K^+ channels. Modified from Nerbonne *et al* (2005)

The potassium channels do also have various accessory subunits; they will be described more thoroughly than the ones in sodium and calcium channels because mutations in some of them lead to LQTS or other diseases (Nerbonne 2000; Nerbonne *et al* 2005; Pongs *et al* 2010). In addition some of the accessory subunits are described in horses (Finley *et al* 2002).

The first accessory subunit described in humans was KCNE1/minK ("minimal K^+ channel"). It is a small protein with a single transmembrane spanning segment that by itself not is capable of forming voltage- gated potassium channels (Nerbonne 2000; Nerbonne *et al* 2005). KCNE1 do assembly with the pore forming α subunit in I_{Ks}, encoded for by KCNQ1. Together they create the molecular basis of the delayed rectifying current, I_{Ks}, in the heart of horses as well as in humans (Finley *et al* 2002; Pongs *et al* 2010). The properties of I_{Ks} are markedly different when KCNE1 are absent (Pongs *et al* 2010). Finley *et al* (2002) has shown that KCNE1 interacts with the pore forming α subunits of I_{Kr}, encoded for by ERG1/KCHN2 in native equine cardiac membrane. This interaction has been reported to not change the properties as much as the interaction of KCNE1 and KCNQ1, but it still has a potentially

significant effect in cardiac ion channels physiology (Pongs *et al* 2010). KCNE2/MiRP1 and KCNE3/MiRP2, also small proteins with a single transmembrane spanning segment (Pongs *et al* 2010), are two other members of the MiRP (minK related peptide) subfamily that are expressed in the equine heart (Finley *et al* 2002; Finley *et al* 2003). KCNE2 is shown to assembly with ERG1/KCHN2 in in vitro system but it has so far not been able to show this assembly in mammalian expression system (Pongs *et al* 2010). KCNE3 are not yet shown to interact but neither to not interact with KCNQ1 in horses (Finley *et al* 2002). In heterologous expression system this interaction leads though to voltage independent K^+ channels (Finley *et al* 2002; Pongs *et al* 2010).

2.2.4.3 Non voltage- gated potassium currents:

Kir channels are so far neither shown to be expressed nor shown not to be expressed in equine cardiac tissue, but is described in for example, dogs, cats and humans (Finley *et al* 2003). These channels carry inward K^+ currents better than outward. It is though never an opportunity for an inward movement of K^+ ions through Kir or other channels because the myocardial membrane potential never gets more negative than the K^+ reversal potential (Nerbonne *et al* 2005). The channels contribute to an outward K^+ current, I_{K1}, during late phase 3 and phase 4 of the repolarisation (Finley *et al* 2003; Nerbonne *et al* 2005).

Kir channels are also built up by an assembling of four α subunits. These α subunits though have only two transmembrane spanning segments, which makes them smaller than the voltage- gated channels (see Figure 5) (Nerbonne *et al* 2005).

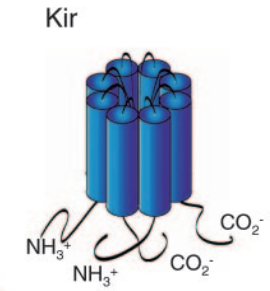

Figure 5: Four α subunits constituted of two transmembrane spanning segment assembled to create a Kir channel. Modified from (Nerbonne *et al* 2005).

2.3 Long QT Syndrome (LQTS)

In order do characterise and discuss whether LQTS is present in horses as well as in humans the theory and facts of this disorder in humans need to be known. The following two sections will describe the inherited- respectively the acquired form of LQTS.

2.3.1 The genetically inherited form:

The inherited form of LQTS was first discovered in a human family in 1957. Currently mutations in 13 different genes encoding for different proteins in ion channels responsible for the ventricular repolarisation have been linked to LQTS (Hedley *et al* 2009; Yang *et al* 2010). These mutations leads to changes in ion currents, which can cause an increased refractoriness, a prolongation of the action potential duration (APD) during phase 2 or 3 and favours induction of early after depolarisations (EADs) (Hedley *et al* 2009). EADs are pro-arrhythmic oscillations in membrane potential that occurs as a result of different refractory periods and/or when APD is prolonged enough to allow calcium or sodium channels to recover from inactivation and reactivate during the plateau- or repolarising phases (Anderson *et al* 2002; Finley *et al* 2003).

As an example of mutated genes, mutations in SCN5A (known as LQT3) encoding for the sodium channels, affect the "linker" between S3 and S4 in the pore forming α subunit. This disrupts the channels normally inactivated state during the plateau phase and gives rise to a gain of function effect. This effect leaves a small percentage of the sodium channels open which counter the K^+ efflux, leading to a slower and delayed repolarisation, an increased late I_{Na} current and a prolonged AP (Catterall 2000a; Dumaine *et al* 2002; Finley *et al* 2003; Moss 2003; Nerbonne *et al* 2005).

As another example, mutations in potassium channels lead to an alteration of potassium currents and thereby a prolongation of the QT interval (Nerbonne 2000; Dumaine *et al* 2002; Finley *et al* 2003; Nerbonne *et al* 2005; Hedley *et al* 2009). LQT1 are linked to the gene KCNQ1 and mutations are identified throughout the protein sequence and results in a loss of function mutation and leads to a reduction in functional cell surface channel expression of channels responsible for I_{Ks} and a decreased amplitude of the current (Nerbonne 2000; Dumaine *et al* 2002; Finley *et al* 2003; Nerbonne *et al* 2005). LQT2 describes mutations in KCNH2/ERG1 protein sequence, which also results in a loss of function mutation with reduced expression of channels responsible for I_{Kr} and reduced amplitude of the current (Nerbonne 2000; Dumaine *et al* 2002; Finley *et al* 2003; Nerbonne *et al* 2005). LQT5 proves

the physiological significance of the auxiliary subunit encoded for by KCNE1/minK because mutations in this gene also reduce the amplitude of I_{Ks} (Nerbonne 2000; Dumaine *et al* 2002; Finley *et al* 2003; Nerbonne *et al* 2005).

As a final example, mutations in the gene CACNA1C, that encodes the α subunit in cardiac calcium channels cells, results in an increased I_{CaL} current known as the Timothy syndrome or LQT8. It is a multisystem disorder including profound cardiac arrhythmias in humans (Nerbonne *et al* 2005; Hedley *et al* 2009).

Due to the well described inherited mutations in human families it can be speculated that some of the mutations also exist in specific horse breeds or families.

2.3.2 The acquired form:

The acquired form of LQTS is more common in humans and also of more importance in veterinary practice (Finley *et al* 2003). Both class I or class III antiarrhythmic drugs (such as quinidine and ibutilide) and a variety of non-cardioactive drugs (such as antihistamine and erythromycin) can induce LQTS (Boyle *et al* 2001; Anderson *et al* 2002; Finley *et al* 2003). Pharmaceuticals responsible for acquired LQTS do often either have a blocking effect on potassium channels or are interacting with other medicals (Al-Khatib *et al* 2003). The majority of the human patients with acquired LQTSs do though never experience Torsades de Pointes (Al-Khatib *et al* 2003; Finley *et al* 2003) and it is shown that silent mutations can predispose individuals for acquired LQTS (Kramer *et al* 2011). Acquired QT prolongation can also be induced by other factors such as hypokalemia, hypomagnesemia or hypocalcemia, left ventricular hypertrophy (LVH), female gender and bradycardia (Al-Khatib *et al* 2003; Finley *et al* 2003).

2.4 The QT interval on a surface ECG

The QT interval is currently the only routinely used measurement of the ventricular repolarisation (Haarmark *et al* 2010). The QT interval is negatively correlated with HR, leading to a decrease in the interval when the HR increases (Davey 2002; Al-Khatib *et al* 2003; Moss 2003; Rajappan *et al* 2003). To get a value that is independent of HR the QT interval is corrected (QTc) for HR before evaluation.

2.4.1 Measuring the QT interval:

In order to check patients for QT prolongation, QT duration, on the ECG needs to be measured. The measurements are preferably done in lead II on the 12 lead ECG because this lead provides the highest amplitude of the T-wave (Lepeschkin *et al* 1952; Couderc *et al* 2005; Goldenberg *et al* 2006). QT measurements can be performed manually or automatically where the manually methods are preferred and recommended by human core laboratories (Couderc *et al* 2005; Goldenberg *et al* 2006).

Couderc *et al* (2005) defines the QT interval as *"the interval between the earliest beginning of the QRS complex and the latest end of the T-wave from all measurable leads"*. The main difficulty is to determine the termination of the T-wave because abnormal T-wave morphology and U-waves change the normal morphology of the T-wave and thereby make the offset of the T-wave less visible (Lepeschkin *et al* 1952; Couderc *et al* 2005; Goldenberg *et al* 2006). The U- wave is a small deflection occurring immediately after the T-wave in some leads in both humans and horses. It is an inconstant finding assumed to originate from slow depolarisation of the papillary muscles or Purkinje fibers (Corley *et al* 2003; Ganong 2005). Because there are no guidelines for determination of the offset of the T-wave it is so far in human medicine still to a great extent individually preferences of which method to use (Al-Khatib *et al* 2003; Panicker *et al* 2009; Salvi *et al* 2011).

One of the most commonly used methods in human medicine are the threshold method (Salvi *et al* 2011). The offset of the T-wave is here determined as the point where it reaches the isoelectric baseline (see Figure 6) (Couderc *et al* 2005; Panicker *et al* 2009; Salvi *et al* 2011).

Figure 6: The vertical line represent the offset of the T-wave according to the threshold method. Recordings from a Standardbred gelding with paper speed of 200 mm/s with a gain of 40 mm/mV.

Another used method is the tangent method. In this method a tangent is drawn along the steepest part of the descending slope of the T-wave and the offset point is where this tangent intersects the isoelectric baseline (see Figure 7) (Lepeschkin *et al* 1952; Panicker *et al* 2009; Salvi *et al* 2011).

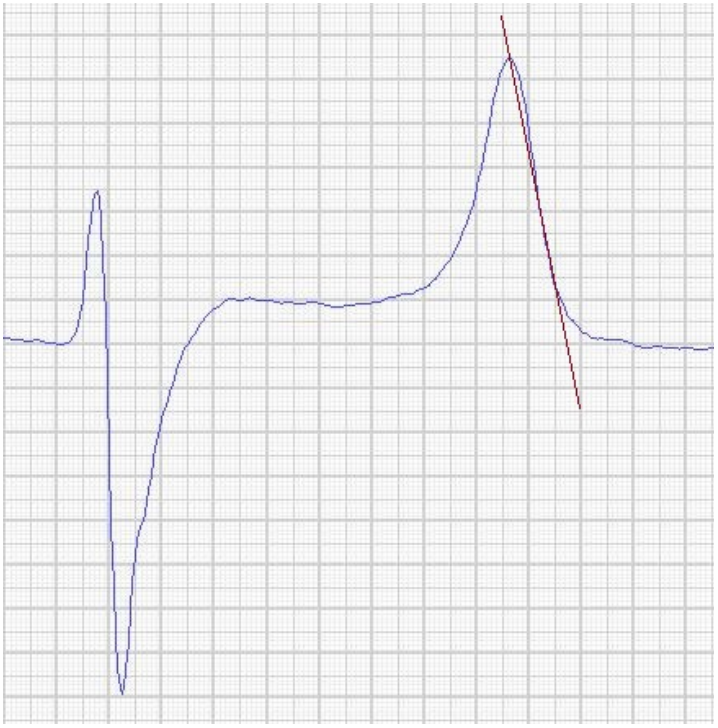

Figure 7: The line represent the "tangent" used in the tangent method. The T-wave offset is where this line intersects the isoelectrical baseline. Recordings from a Standardbred gelding with paper speed of 200 mm/s and a gain of 40 mm/mV.

The best method to measure the QT interval would be the one with least inter- and intra-observer variability (Panicker *et al* 2009). Studies have concluded that the difference between the inter- and intra-observer values of each above mentioned method is small when experienced readers read the ECG (Panicker *et al* 2009; Salvi *et al* 2011). It is important to remember that most reference values are based on the threshold method and that measurements with the tangent method may be up to 10 ms shorter than the same measurements made with the threshold method (Panicker *et al* 2009).

Core laboratories recommend to measure QT interval on computer screen with on screen calibers (Goldenberg *et al* 2006). The QT interval should be determined as a mean value of at least 3-5 heartbeats and the onset is the first deflection of the QRS complex and the offset is where the T-wave merges with the isoelectric baseline (Goldenberg *et al* 2006).

There are currently no guidelines published of how to measure or which method to use in horses.

2.4.2 QT correction:

After measuring the QT interval it needs to be corrected (QTc) for the inversely correlation with HR. The QTc corresponds to the expected QT interval at a HR of 60 bpm (beats per minute) (Luo *et al* 2004). In human medicine this correction has been done with various formulas whereof the one described by Bazett[1] (1997) is the most frequent used (Davey 2002; Luo *et al* 2004; Goldenberg *et al* 2006). Other formulas used in the field are the Fridericia[2] cube root correction, and Framingham's[3] linear regression equation (Al-Khatib *et al* 2003; Goldenberg *et al* 2006). Despite the popularity of Bazett's formula it is shown to overcorrect the QT interval at faster HR, undercorrect them at lower HR and relative to other formulas it has the strongest dependency on HR (Luo *et al* 2004; Goldenberg *et al* 2006; Haarmark *et al* 2010). The formula of Fridericia perform better at higher HR but undercorrects as well at low HR and is less dependent on HR relative to Bazett (Luo *et al* 2004; Goldenberg *et al* 2006). The linear Framingham formula tends to give too low values at low HR, have a slightly higher HR correlation than Fridericia and is not supported at higher HR (Davey 2002; Luo *et al* 2004; Goldenberg *et al* 2006; Haarmark *et al* 2010). In the study by Luo *et al* (2004) they conclude that another formula- Hodges[4]- is the best choice globally because it is least

[1] QTc = √(RRinterval
[2] QTc = ^{3}√(RRinterval
[3] QTc = QT + 0.154(1-RR)
[4] QTc = QT + 1.75(HR-60)

dependent on HR (compared with the others) and gave the best fit of their data base with more than 10,000 recorded ECG.

When measuring the QT interval of horses different human correction formulas have been used (Lannek *et al* 1951; Fregin 1982; Ayala *et al* 1995; Pasławska *et al* 2012a; Pasławska *et al* 2012b; Schwarzwald *et al* 2012; Pedersen *et al* 2013). The recent study of Pedersen *et al* (2013) does though present a piecewise linear regression correction model for horses. This consist of two straight half lines ($Slope_1$ vs $Slope_2$) which joins at a bending point (RR_{Bend}) and this model was shown to perform better than the two human formulas of Bazett and Fridericia at a comparison (Pedersen *et al* 2013).

2.5 T_{peak} to T_{end} (T_pT_e) interval

T_pT_e reflects the dispersion of repolarisation in the ventricles and it is in humans shown to be independent of HR, age and sex (Haarmark *et al* 2010). In human medicine prolongation of this interval is, as prolongation of the QT interval, associated with an increased risk of SCD (Panikkath *et al* 2011). T_pT_e analysis seems to be an alternative prognostic tool in evaluation of cardiac repolarisation, particularly in cases of disturbance where QTc is normal or not valid (Haarmark *et al* 2010; Panikkath *et al* 2011).

It is shown that the T_pT_e interval in horses is significantly prolonged after sedation with detomidine prior to euthanasia (Buhl *et al* 2013). In the study by Pedersen *et al* (2013) it was shown that the T_pT_e interval in mares was nearly independent of HR whereas in geldings and stallions an increase in T_pT_e interval correlated with a decrease in the RR interval.

The T_pT_e interval is measured from the highest top of the T-wave ($T_{peak/p}$) until the offset of the T- wave where the downward leg of the T-wave reaches the baseline ($T_{end/e}$).

2.6 ECG recordings in horses

In order to be able to use an ECG recording as a diagnostic tool for QT prolongation/evaluation and other miscellaneous dysrhythmias of the heart it is necessary to understand the origin and meaning of the different deflections on the ECG curve. The following section will combine the electrophysiology described in the above paragraph with the different waves of the ECG curve and additionally describe how to obtain an ECG of a horse.

2.6.1 How ECG works and what the different deflections symbolise:

When a cardiac cell de- or repolarise different currents (as described in the paragraph above) will flow across the cell membrane and give a battery effect where the cell acts as a dipole. When sufficient cardiac cells de- or repolarise at the same time the resulting electrical field can be detected from the body surface as the potential difference between two electrodes. This link between a negative and a positive electrode is called a (bipolar) lead (Patteson 1996; Verheyen *et al* 2010a). If the electrical activity is directed towards the positive electrode it gives an upward deflection and if directed away from the positive electrode the deflection on the ECG will be downward (Patteson 1996; Verheyen *et al* 2010a). The depolarisation of such a small bunch of cells as the pacemaker cells in the SA or AV node does not affect the electrical field sufficient enough to be visible on the ECG (Patteson 1996). The following spreading of the impulse through the atria is though shown as the ***P-wave***. This can be biphasic, bifid or simple positive and changes often with HR (Menzies-Gow 2001; Verheyen *et al* 2010a). At slower HR the P-wave is often bifid with the first peak representing the depolarisation of the right atrium and the second peak originating from the left atrium. The duration should be < 0.16 seconds and it is normal that the morphology can be different in successive heartbeats (Patteson 1996; Menzies-Gow 2001; Verheyen *et al* 2010a). The following flat PQ or PR interval (see Figure 8) with a duration of up to 0.5 seconds represents the slow conduction through the AV-node (Patteson 1996; Verheyen *et al* 2010a). The next detectable change is the depolarisation of the ventricles which gives rise to the ***QRS complex (***see Figure 8B) that shall have duration of < 0.14 seconds. The ***Q-wave*** is the first negative deflection, the ***R-wave*** is the first positive deflection and the ***S-wave*** is first negative deflection following the R-wave (Cunningham 2002; Verheyen *et al* 2010b, a). Horses often not have the typical "QRS" complex morphology but rather a RS morphology (see Figure 8A) (Verheyen *et al* 2010b).

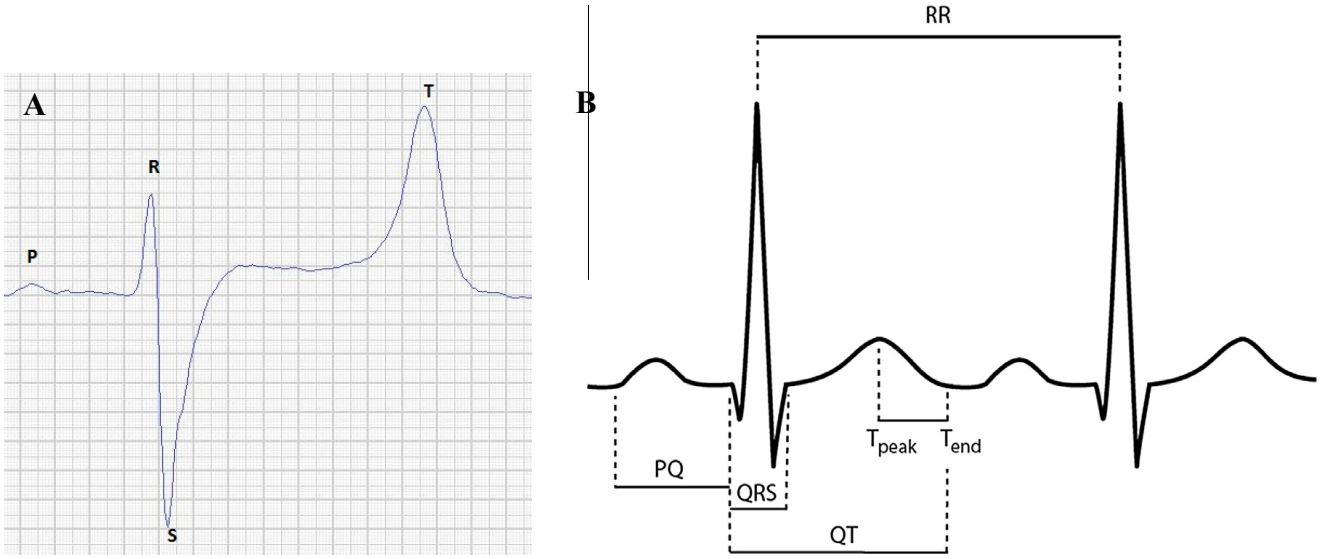

Figure 8: A: The RS morphology common seen in ECG from horses. Recordings from a Standardbred gelding with the paper speed of 200 mm/s and a gain of 40 mm/mV. **B:** ECG recording with a QRS morphology, showing the different intervals, from (Buhl *et al* 2013).

All of the different waves in the QRS complex describe parts of the depolarisation in the ventricles:

Q-wave: A depolarisation that spreads from left to right through the interventricular septum, causing a small voltage difference and a little negative deflection (Cunningham 2002).

R-wave: Is due to the massive depolarisation of the two ventricles. The action potential is spread by the left and right bundle branches to apex where the purkinje network continuous to rapidly spread the action potential up the inside of the walls of the ventricles. The action potential then continuous its' spread outwards from cell to cell through the walls of the ventricles. Because of the larger mass of the left ventricle the electrical changes therein dominates over the changes in the right ventricle and gives a net electrical effect to the left and thereby a positive deflection (Cunningham 2002).

S-wave: The net electrical effects returns to zero and there can be seen a negative deflection on the ECG. The physical reason for this is, however, not yet clearly understood (Cunningham 2002).

The depolarisation is followed by a repolarisation of the ventricles and it is seen as the ***T-wave*** on the ECG. The direction of the repolarisation (same or opposite the way of repolarisation) decides if the deflection is negative or positive. The morphology of the T-wave can be very different in horses and is particularly dependent on HR. This makes the morphology unreliable as a diagnostic tool of cardiac diseases, electrolyte disturbance or systemic diseases (Patteson 1996; Menzies-Gow 2001; Cunningham 2002; Verheyen *et al* 2010a). The same is true for the equine QRS complex, which compared to humans and other animals, provides little or no information about the size of the chambers of the heart. This is

due to the particularly widespread and rapidly conducting network of Purkinje fibers in the ventricles of horses and other ungulates, which activates the adjacent cells of heart muscle and gives a rapid depolarisation and a simultaneous contraction (Patteson 1996; Menzies-Gow 2001; Verheyen *et al* 2010a; Pasławska *et al* 2012a). Therefore ECG recordings in horses are primarily used for detecting and diagnosis of arrhythmias (Reef *et al* 2009; Verheyen *et al* 2010a).

2.6.2 Obtaining an ECG:

Einthovens triangle is a system of limb leads recording electrical activity with the heart assumed to be located approximately in the centre of an equilateral triangle formed by the two forelimbs and the left hind limb (see Figure 9). This system can be used in horses and is commonly used in humans and small animals (Patteson 1996).

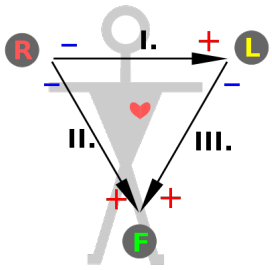

Figure 9: Einthovens equilateral triangle with the heat located in the centre, illustrating lead I, II and III from http://www.indiegogo.com/projects/phonologic

However other systems have been designated to adjust for the fact that in horses the heart is not a point source located in the centre of an equilateral triangle formed by the limbs (Patteson 1996). Usually a system with four electrode is used in horses but there is though no single universally accepted ECG lead system (Reef *et al* 2009; Verheyen *et al* 2010a). The European colouring standard of this four lead system gives that the black electrode act as the right leg and serves as a reference electrode and can be put anywhere on the body surface of the horse, the red electrode is the right arm, the yellow the left arm and the green the left foot. Lead I is constructed between the red electrode (-) and the yellow (+) electrode. Lead II between the red (-) and the green (+) electrode and lead III between the yellow (-) and the green (+) electrode (see Figure 9) (Patteson 1996; Menzies-Gow 2001; Verheyen *et al* 2010a). But the exact placement of the electrodes is not critical in horses and can be adapted to the circumstances. A thumb rule is that electrodes need to be placed along the mean electrical axis (MEA). MEA represents the sum of potential difference of all individual cells, which is directed from the apex of the heart towards the base and slightly cranial to the right

(Verheyen *et al* 2010a). This means that one electrode (e.g. green in lead II) shall be placed on the lower thorax near the cardiac apex between the elbow and xiphoid area. The second electrode (e.g. red in lead II) should be positioned more dorsal in the region between the lower neck and the withers, at the cardiac base (Patteson 1996; Menzies-Gow 2001; Verheyen *et al* 2010a). The base-apex system is most commonly used for resting ECG and corresponds best with the MEA whereas an adapted version is conventionally used for exercise ECG to avoid electrodes from falling of and reduce movement artefacts (Menzies-Gow 2001; Verheyen *et al* 2010a).

3. Hypotheses and Aims of the project

This study is a continuation of the work and findings in the study by Pedersen *et al* (2013) in order to achieve better understanding of the characteristics of QT interval in horses. Due to a limited study period the thesis will only include geldings. The following hypotheses will be investigated:

3.1 Hypotheses

- There will be no differences in the length of the QT interval between different breeds
- The QT/RR relationship of all breeds will follow a piecewise linear regression model
- The T_pT_e interval is independent of HR/RR and breed

3.2 Aims of the project

1. Obtain electrocardiographic recordings in rest and during exercise of 10 Thoroughbred geldings.
2. Measure the QT interval at different HR of they in paragraph 1 mentioned horses and of stored electrocardiographic recordings from geldings of Arabian Horses, Icelandic Horses, Standardbreds, North- Swedish Trotting Horses, Dressage horses and Show-Jumping horses in order to study the relationship between the QT and RR intervals in the different breed. There will be created a breed-differentiated model (a piecewise linear regression) in order to compare the results of the breed to each other and to determine if this model is the best description of the QT/RR relationship in all breeds.
3. Measure the T_pT_e interval in all horses mentioned in paragraph 2 to study its relation to RR interval in each breed.

4. Animals, Materials and Methods

4.1 Horses

ECG was obtained from 10 Thoroughbred geldings. The horses were between 3 and 8 years old at time of recording and they were a mix from two different trainers with a shared stable area situated near Copenhagen Racecourse at Klampenborg, Denmark. Nine horses were in pre-training for the race season of 2013 and run their first race of the season a few days to a couple of weeks after the ECG recording. One horse had raced once this season before the recording. The horses did not undergo a physical examination prior to the recordings but the trainer did not complain of any health issues and found the horses healthy and ready to perform a proper fast work.

In addition to the ECG recordings obtained by the author ECG recordings from 48 geldings: 10 Standardbred aged 3-6 years, 10 Icelandic Horses aged 5-21 years, 6 Dressage horses (Danish Warmbloods) aged 7-9 years (one horse with unknown age), 8 Show- Jumping horses (Danish Warmbloods and other similar Warmblood-breeds) aged 8-13 years, 4 North Swedish Trotting Horses aged 5-8 years and 9 Arabian Horses aged 7-19 years plus 1 Shagya-Arabian Horse aged 11 years were included in the study. These data have been used in other studies before and were provided by the supervisors Rikke Buhl, co-supervisor and PhD student Philip Juul Pedersen and by PhD student Mette Flethøj.

For a full overview of the study- group see Appendix I.

4.2 ECG recordings

The ECGs obtained by the author were recorded with a digitally telemetric ECG system[a]. Surface electrodes[b] were placed in an adapted version of the base-apex system as described by Verheyen *et al* (2010). The red electrode was placed at the scapula around 10-20 cm ventral the withers on the right side of the horse and the black electrode was placed just beneath it, as seen in Figure 10.

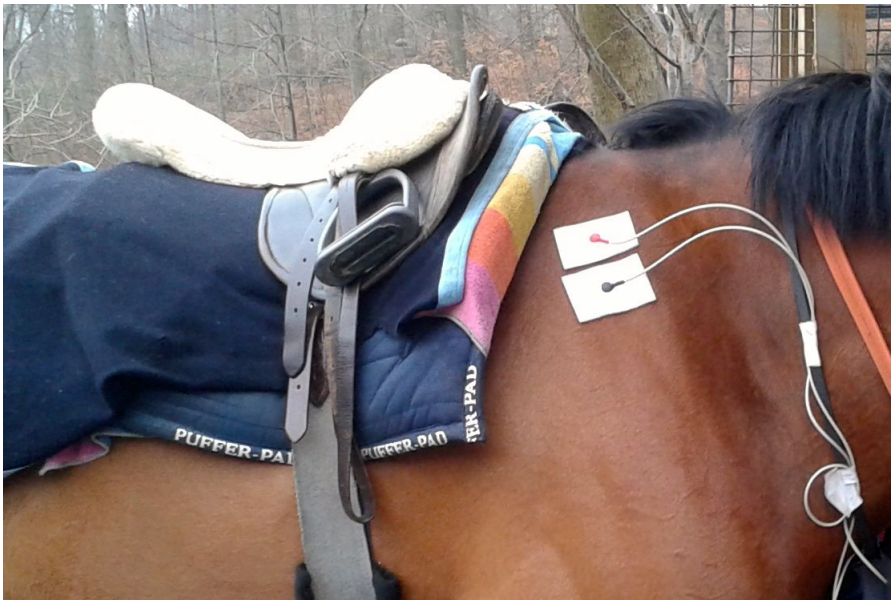

Figure 10: Showing the placement of the red and black electrode at the scapula at the right side of the horse.

The yellow electrode was placed 5-15 cm behind the girth around the level of the olecranon on the left side of the horse and the green electrode was placed just beneath the yellow one, as seen in Figure 11.

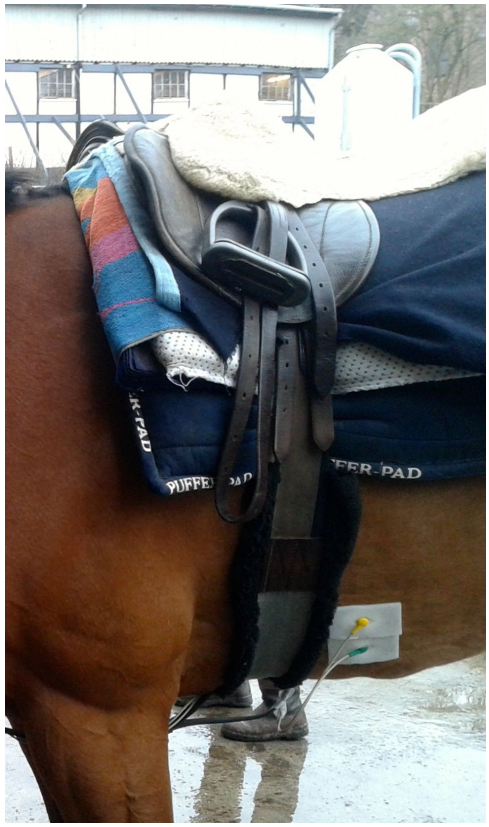

Figure 11: Showing placement of the yellow and green electrodes behind the girth at the level of the olecranon.

The surface electrodes[b] were further secured to the skin with a patch of sticky foam[c] ("Snögg") before the electrode cables were fastened. The recording device[a] were fastened to a

martingale or a breastplate and the recordings were saved on a memory card before transfer to storage on a laptop computer.

The recordings of the Thoroughbred geldings were continuous and started from rest with the horse in the box during the approximately 10-20 minutes it took for the rider to saddle up. The system was left on the horse for the full workout period of more or less an hour of duration and was removed when the horse returned to the stable. The workout period consisted of a period of walking, a period of trotting and "hacking" (a slow canter), a fast work and a walking off period. In the fast work the horses were allowed to go at a good speed for around 600-1000 meters, but they did not get pushed or ridden out as in a race.

The stored ECG recordings from Standardbreds were obtained during harness racing and are a mix of continuous ECG recordings during rest, exercise and recovery and separate recordings from rest and exercise plus recovery (Pedersen *et al* 2013). The recordings from Arabian Horses and the Shagya- Arabian Horse are separate recordings of 24 hours resting ECG and an exercise ECG obtained during interval training with a rider on. Recordings of the Icelandic horses are continuous recordings from a standardized program starting with a resting period followed by exercise by hand without a rider. The North-Swedish Trotting Horses recordings are continuous from rest followed by interval harness racing exercise. Recordings from the Warmblood- breeds are either from Dressage or Show- Jumping horses (Barbesgaard *et al* 2010). They are as well a mix of continuous recordings from rest followed by an exercise protocol and a recovery period and separated recordings during rest and during exercise. The electrodes were placed in a similar way for all horses.

4.4 ECG analysis

All ECG tracings were analysed using the Televet[a] software. The time intervals were measured manually with on-screen calibers[d] as described by Pedersen *et al* (2013). Eight periods with a stable HR were identified to minimize the possible effect of QT lag and -hysteresis (Pedersen *et al* 2013). The eight periods represent HRs from resting HR to maximal HR of the individual recording. The HR of each period was determined as an average of 10 consecutive beats (see Figure 12). Recording time, in the software program[a], and screenshots, as seen in Figure 12, were saved from each of the eight periods to facilitate control and recognition of the same period.

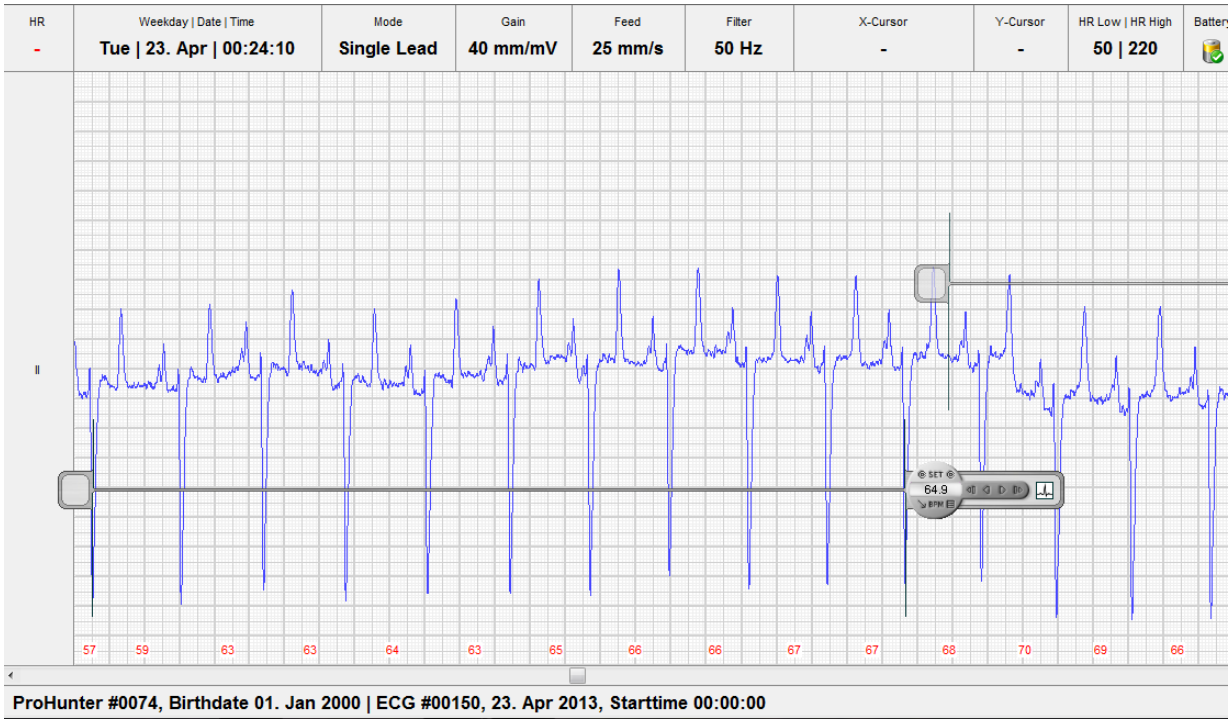

Figure 12: Screenshot showing the determination of HR with on- screen calibers[d] in ten consecutive heartbeats. The recording time is shown in the upper left corner. Recording is from a Thoroughbred gelding with a paper speed of 25 mm/s and a gain of 40 mm/mV.

In the five following complexes the QT intervals were measured from the earliest onset of the QRS complex to the end of the T-wave (T_{end}), as seen in Figure 13a. This gives a total of 40 measurements for each horse (8 periods * 5 measured QT interval). Recording time (see Figure 12) showed in the software program[a] was also noted for every QT interval measurement to facilitate recognition of the same complexes again.

The T_{end} was determined visually as the point where the downward leg of the T- wave reaches the isoelectric baseline, considering shape of the wave and background noise (for more detailed information see section 4.5. In addition the intervals between the onset of the Q-wave and the peak of the T-wave (QT_{peak2}) were measured, as seen in Figure 13C. In case of a bifid T-wave, both peaks were measured as QT_{peak1} versus QT_{peak2} (see Figure 13B + C). QT_{peak1} were left blank if not present and then QT_{peak2} corresponds to QT_{peak}. T_{end} was always measured after the second peak in case of a bifid T-wave (see Figure 13A).

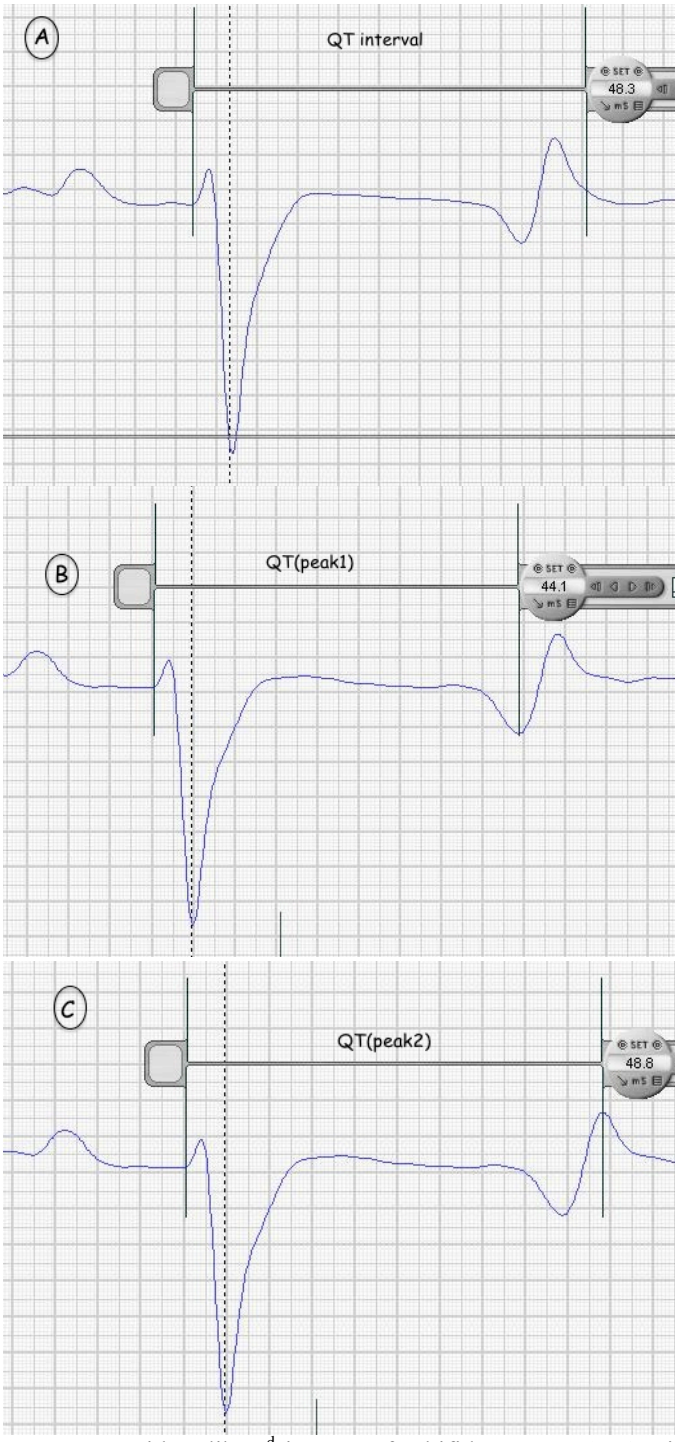

Figure 13: On- screen measurement with calibers[d] in case of a bifid T-wave; **A)** QT interval; **B)** QT_{peak1}; **C)** QT_{peak2}. Recordings from a North Swedish Trotting horse gelding with a paper speed of 200 mm/s and a gain of 40 mm/mV.

All measurements were performed by the author in lead II with a feed of 40 mm/mV and a paper speed of 200 mm/s. Before starting to measure the QT interval in this study, the author underwent a short training session with PhD student Philip Juul Pedersen who has four years experience of measuring QT interval.

4.5 QT measurements in this study

The T-wave morphology in horses varies and the offset of the wave can therefore be difficult to determine (Ayala *et al* 1999; Menzies-Gow 2001; Pasławska *et al* 2012a). Because the ECG tracings are obtained partly during exercise, motion disturbance from the horses will give turbulence on the isoelectric line. This further will complicate the interpretation of T_{end}. Another difficulty is present at high HR where the T- and P-wave merge together (Verheyen *et al* 2010b).

The following Figure 14-19 will give a description of where the QT intervals have been measured based on examples of different morphologies seen in this study. All figures are from lead II with a paper speed of 200 mm/s and a gain of 40 mm/mV.

One or two notches at the downward leg of the T-wave, see Figure 14 *A + B:*

When there are two notches the T_{end} is measured after the second notch. If only one notch, T_{end} is measured at this as long as it does not give a T_{end} situated so far up the downward leg that the QT interval will get a much lower value than the preceded measurement did. See Figure 18 for cases where the notch is situated to far up.

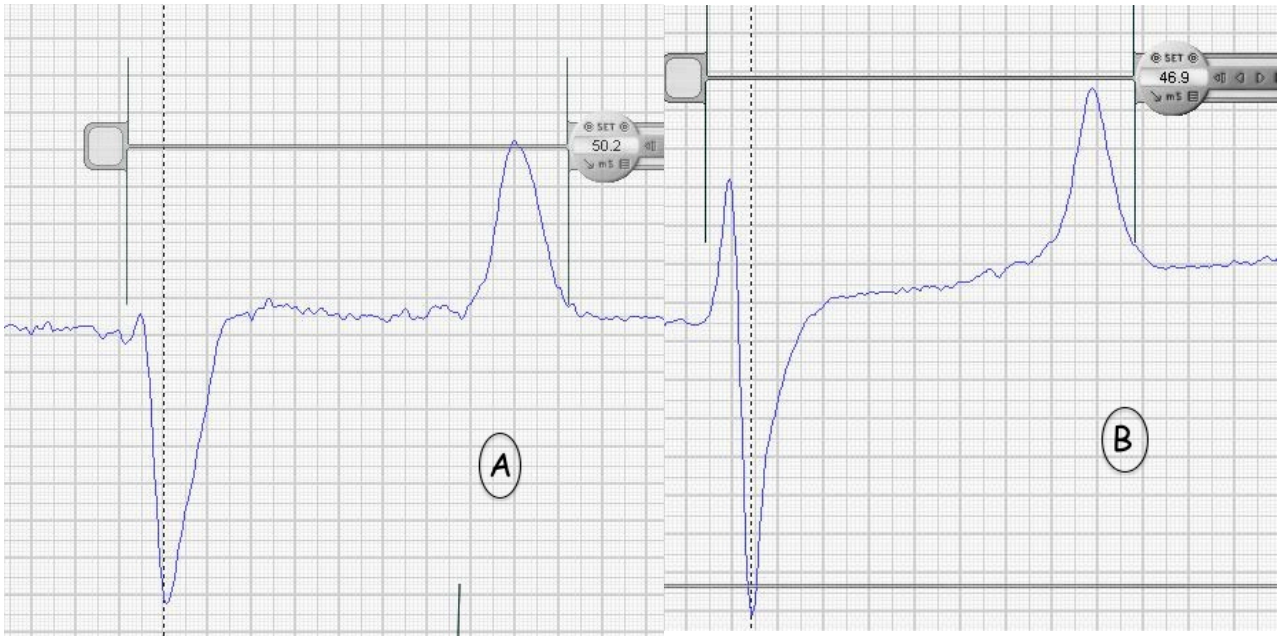

Figure 14: Where to interpret T_{end} when there are one or two notches at the downward leg. **A:** When two notches T_{end} is measured at the second notch (right leg of the caliber[d]). **B:** If only one notch, T_{end} is measured at this (right leg of the caliber[d]).

Flat Q- wave, see Figure 15*:* The QT interval is measured from the beginning of the flat period just before the downward deflection or from the first notch or deflection of the baseline prior to the S-wave.

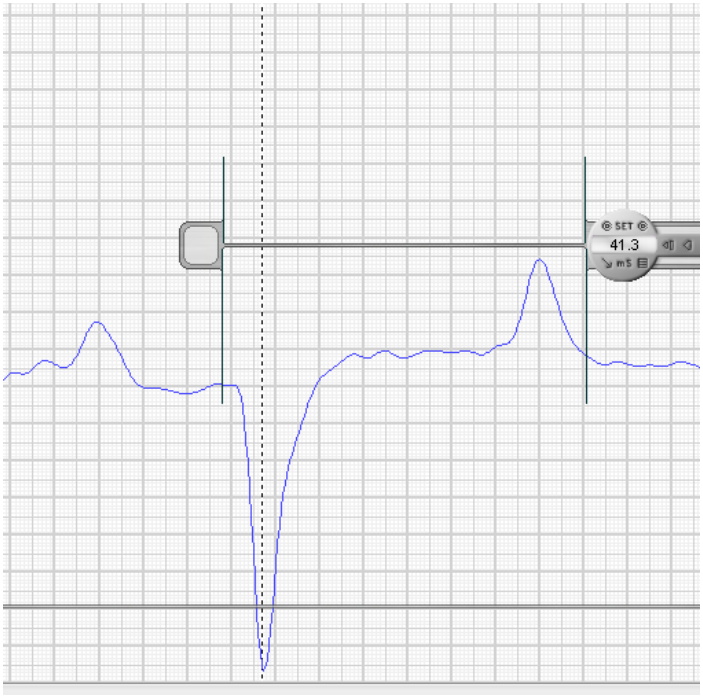

Figure 15: The QT interval is measured from the beginning of the flat period before the downward deflection (left leg of the caliber[d]). This correlates with the first smaller deflection/notch of the baseline prior to the S-wave.

Double peak of the T-wave and no Q-wave, see Figure 16*:* T_{peak} is measured between the two peaks if they are of the same height, otherwise the highest point is used for T_{peak} measurement. In addition the Q- wave is here absent (often described in horses (Verheyen *et al* 2010a)) and the QT interval is therefor measured from the beginning of the S- wave.

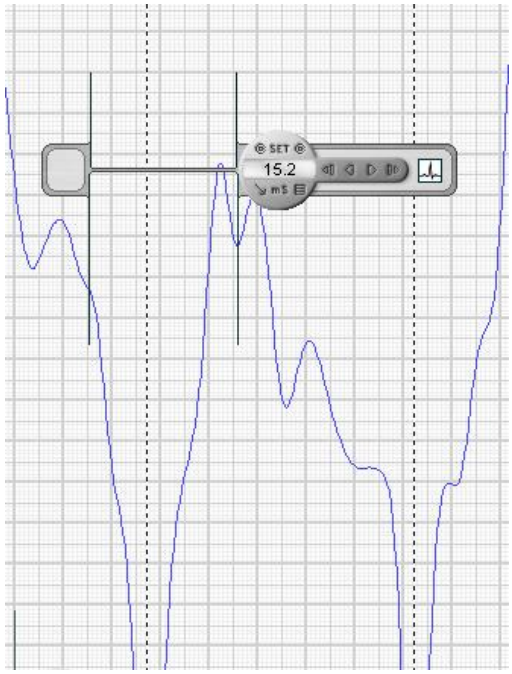

Figure 16: The QT interval is measured from the first deflection after the P-wave (left leg of the caliber[d]). T_{peak} is measured between the two peaks if they are of equal size (right leg of the caliber[d]), otherwise the highest peak is used.

Negative deflection of the T- wave, see Figure 17*:* T_{end} is measured when the upward leg of the T-wave reaches the isoelectric baseline.

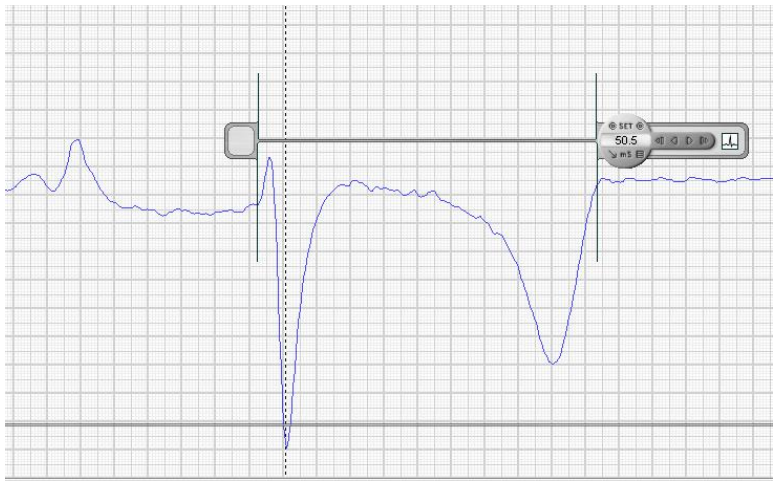

Figure 17: Negative T-wave, T_{end} is measured where the upward leg reaches the isoelectric baseline (right leg of the caliber[d]).

Flattening of the T-wave, see Figure 18*:* If T_{end} is measured at the first notch it gives a too short QT interval (based on the preceded value). Therefore the continuing of the downward leg of the T-wave is theoretical constructed (red dotted line in Figure 18) and T_{end} is measured where this construction would reach the isoelectric baseline.

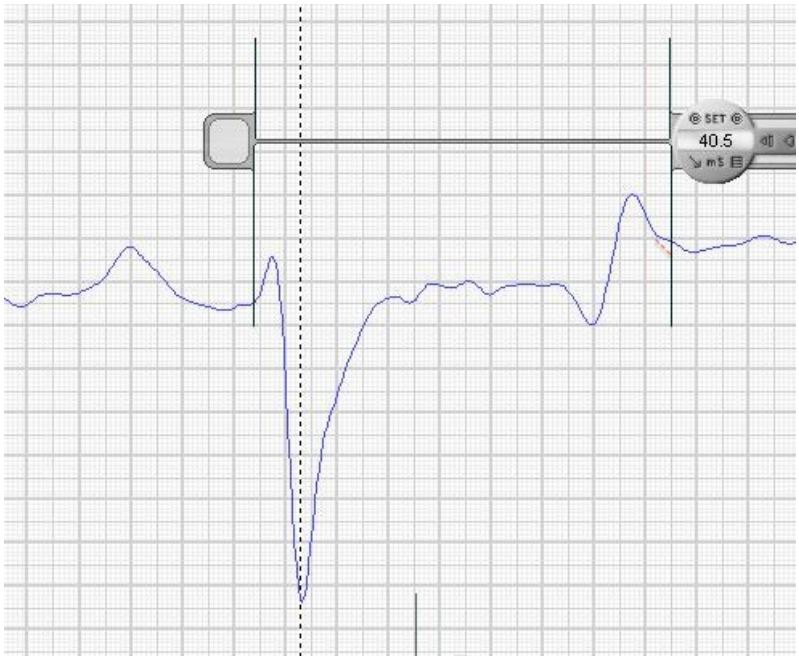

Figure 18: Theoretical construction of the downward leg of the T-wave. T_{end} is measured where this construction reaches the baseline (right leg of the caliber[d]).

T-wave with a "bend", see Figure 19*:* In cases where the first notch will give a to short QT interval, the T_{end} is measured where the downward leg of the "bend" reaches the baseline.

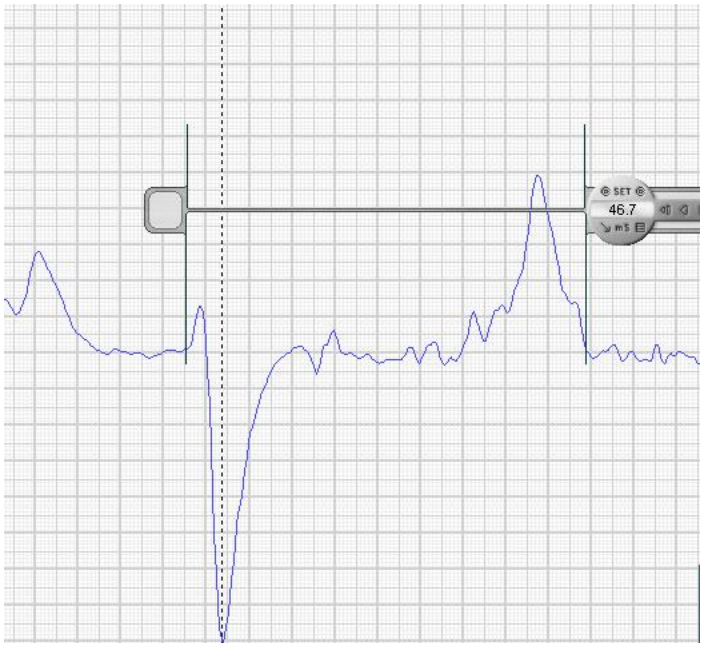

Figure 19: T_{end} is measured where the downward leg of the "bend" reaches the baseline (right leg of the caliber[d]).

4.6 Data analysis

The values of HR, QT_{peak1}, QT_{peak2}/$QT_{peak,}$ QT_{end} and recording time were transferred to a data management program[e]. The HR values were converted to a calculated RR interval (RR = 60/HR), the measured QT_{end} values were converted to seconds (QT_{sek} = measured QT_{end}/1000) and T_{peak} -T_{end} interval in seconds (T_pT_e) where calculated as $T_pT_e = (QT - QT_{peak2})/1000$.
These values where in turn transferred to SAS[f] for statistical analysis and to GraphPad Prism[g] for statistical analysis and graphic presentation. The values transferred to SAS[f] (for coding see Appendix II) underwent a "proc nonlin method" analysis, which provided the values for $Slope_1$, $Slope_2$ and RR_{bend} (see section 4.6.1).
To allow statistical analysis of the slopes and bending points of the breed differentiated lines a variable, called RR_{step}, was introduced and defined by:

$$RR_{Step} = \begin{cases} 0, & RR \le RR_{bend} \\ RR - RR_{bend}, & RR > RR_{bend} \end{cases}$$

To estimate the specific RR_{step} values for each RR measurements a SAS program[f] was constructed and used for automatic calculations (for coding see Appendix II)
Data was further analysed using a "proc mixed" model in SAS[f] (for coding see Appendix II) with random effect of horse and repeated measurements over time within horses. The errors were estimated by an independent component and a component with Gaussian decreasing correlation. The model consisted of a factorial design including type (corresponding to breed), RR, RR_{step} and interactions of RR*type and RR_{step}* type.

4.6.1 QT correction:

The piecewise linear regression model, presented by Pedersen *et al* (2013), was used for HR correction of the QT interval. This model is described by two straight lines that join at a bending point (RR_{bend}) and the model can be given by the two equations:

$QT = Slope_1 * RR + intercept_1$ for $RR \le RR_{bend}$
$QT = Slope_2 * RR + intercept_2$ for $RR \ge RR_{bend}$

Since the bending point is given, the intercepts are redundant and the model can be reduced to three parameters ($Slope_1$, $Slope_2$ and RR_{bend}).

All the QT intervals were plotted against the RR values in the GraphPad Prism[g] software and analysed as a non- linear regression model with fitted breed differentiated regression lines.

4.6.2 T_pT_e and RR relationship:

The T_pT_e values were plotted against RR in GraphPad Prism[g] and a linear regression fit on these data was performed to provide the possible dependence of T_pT_e on RR. GraphPad Prism[g] was also asked to perform an alternative to an ANCOVA analysis to test whether the slopes from the different breeds differed.

All the results were considered significant if they had a p- value <0.05.

5. Results

All of the 58 geldings were included in the results. One Thoroughbred horse suffered from epistaxis after pulling up. The horse went later through an endoscopic evaluation and the veterinarian did not find any obvious signs of illness and the horse was therefore included in the study. The Shagya-Arabian Horse was analysed together with the Arabian Horses. The age of the horses ranged from 3-21 years and mean age were 7.3 years. 8 out of the 58 horses were over 10 years old and only 3 were over 15 years old (for an overview of the age of each horse and mean age of each breed see Appendix I)

5.1 QT interval

In all different breeds the relationship between the QT- and RR interval can be described by a piecewise linear regression model. Analysis of the data gives a goodness of fit of $0.92<r^2<0,98$ for all seven breeds. In Figure 20 the QT intervals of the different breeds found in this study are plotted against RR and the breed differentiated fitted piecewise regression lines are created from the solution estimates shown in Table 1.

In Appendix III there is a larger version of only the breed differentiated regression lines without data points. There are also figures showing the data points and fitted curves of the breeds individually with the corresponding 95 % prediction bands (encloses the area where you can expect that 95 % of future data points will be situated).

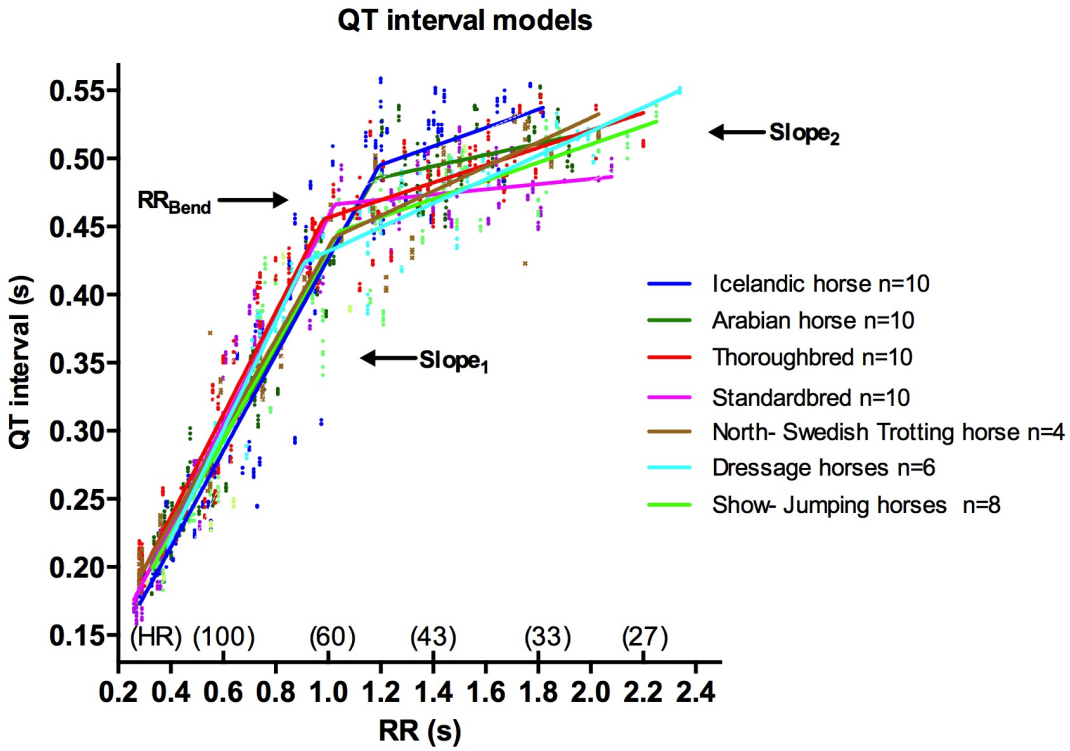

Figure 20: Breed differentiated plots of the QT/RR intervals with fitted ($0.92<r^2<0{,}98$) piecewise linear regression lines. HR= heart rate.

The estimated values (with 95 % confidence interval, CI) for $Slope_1$, $Slope_2$, and RR_{bend} of the model are shown in Table 1. The estimates of RR_{bend} were used to calculate RR_{step}.

Table 1: The solution estimates with 95% CI for $Slope_1$, $Slope_2$ and RR_{bend} from the breed differentiated piecewise linear QT regression lines. H. = Horses

Breed	$Slope_1$ [CI]	$Slope_2$ [CI]	RR_{bend} [CI]
Arabian H.	0.334 [0.322; 0.346]	0.0416 [0.0259; 0.0573]	1.173 [1.139; 1.207]
Thoroughbred	0.376 [0.362; 0.390]	0.0640 [0.0506; 0.0775]	0.9819 [0.9503; 1.014]
Standardbred	0.380 [0.368; 0.393]	0.0189 [0.0061; 0.0318]	1.025 [0.9983-1.051]
Icelandic H.	0.353 [0.337; 0.370]	0.0678 [0.0332; 0.102]	1.194 [1.142; 1.246]
Dressage H.	0.400 [0.374; 0.425]	0.0879 [0.0793; 0.965]	0.9087 [0.8767; 0.9407]
Show-Jumping H.	0.347 [0.330; 0.365]	0.0669 [0.0527; 0.0810]	1.040 [0.9990; 1.081]
North- Swedish H.	0.337 [0.317; 0.357]	0.0897 [0.0710; 0.108]	1.023 [0.9637; 1.083]

As can be seen in Figure 20 the Icelandic Horses seem have the longest QT interval at lower HR but marginally the shortest at higher HR. The curve of the Arabian Horses and the Icelandic Horses bends at a lower HR and the curve of the Dressage Horses bends at a slightly

higher HR compared to the others breeds. The RR_{bend} for all breeds lies in the HR range of 54.5- 71.6 bpm. $Slope_2$ of the Standardbred seems markedly more flat compared to the other breeds and do also have a lower value. The values and fitted lines of $Slope_1$ all lays very close until the different bending points.

The result of the "proc mixed" analysis is shown in Table 2. The resulting p- values show that breed has a significant influence on both slopes (RR*Type) and on RR_{bend} (RR_{step}*Type). This supports the visual difference between the curves.

Table 2: The result of the "proc mixed" analysis, showing a significant effect of type (breed) on slopes and RR_{bend}.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Type	6	188	19.99	<0.0001
RR	1	1271	10969.3	<0.0001
RRstep	1	1318	3066.70	<0.0001
RR*Type	6	1272	161.11	**<0.0001**
RRstep*Type	5	1326	3.20	**0. 0071**

Figure 21 shows the data points for horses > 15 years old. As it can be seen, all the data points from these individuals lay within the 95 % prediction band

Figure 22 shows the data points for the Thoroughbred horse that suffered from epistaxis when pulling up from the fast work. As can be seen in the figure, there are a few data points outside the 95 % prediction bands.

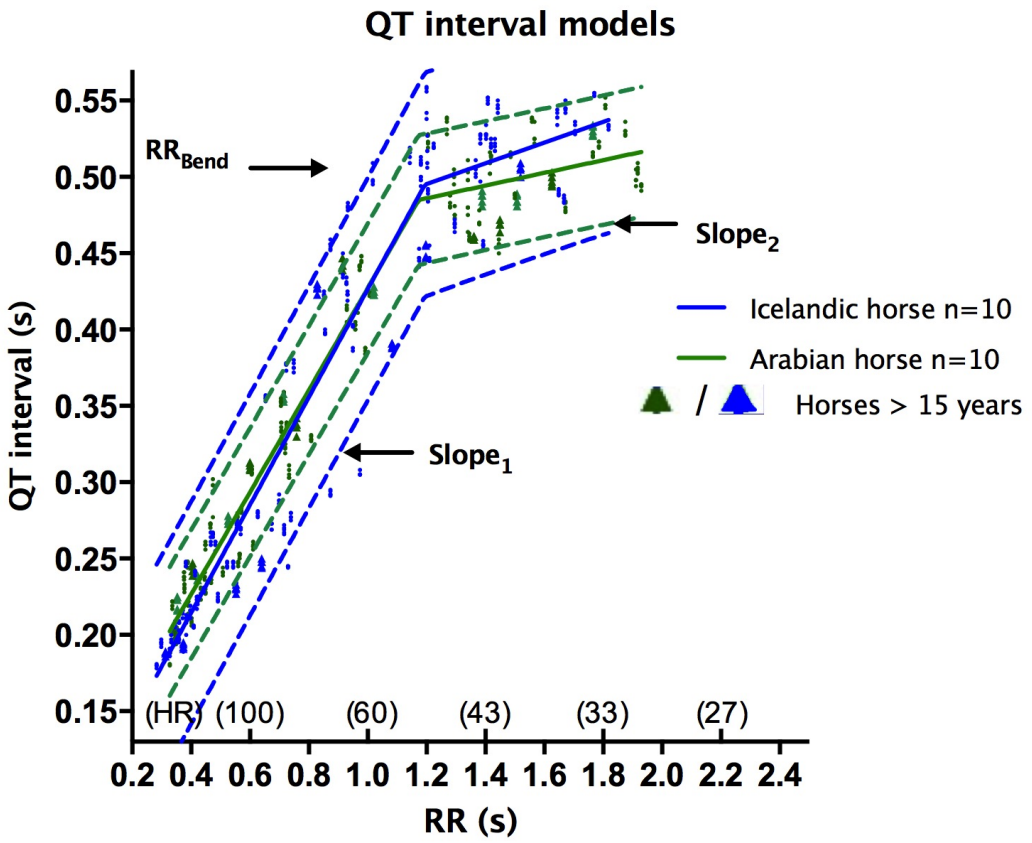

Figure 21: Showing the placement of data points for horses <15 years old (dots) and > 15 years old (triangles). The dotted lines represent the 95 % prediction bands.

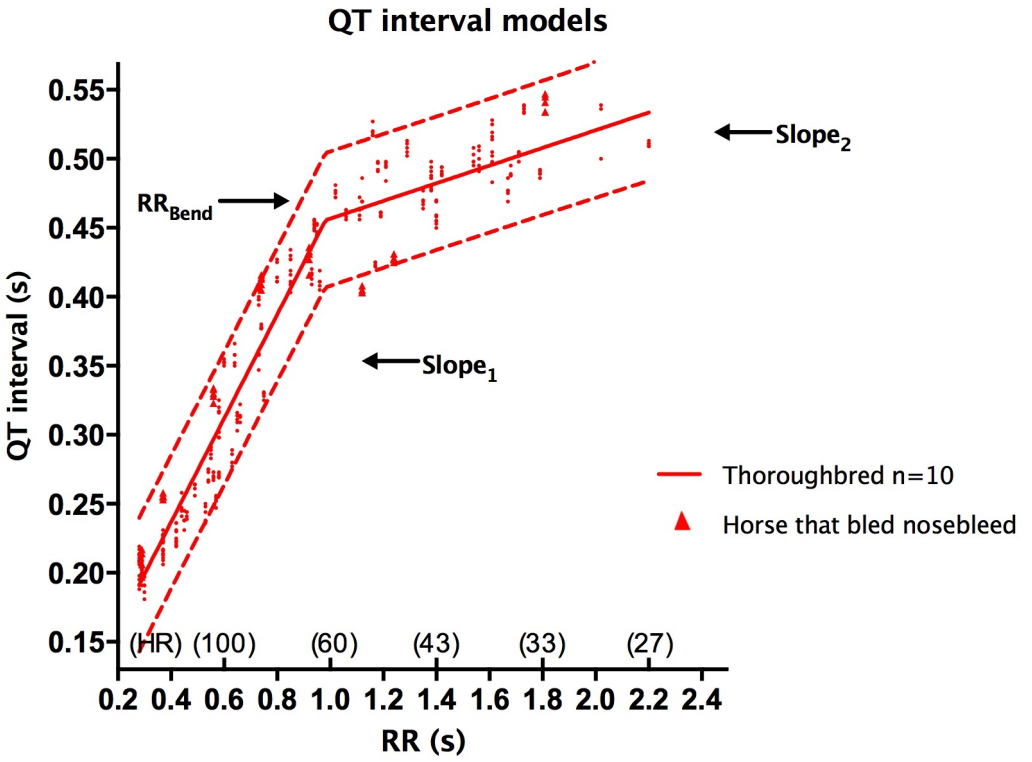

Figure 22: The triangles shows the data points for the horse that bled nosebleed. The dotted lines represent the 95 % prediction bands.

5.2 T_pT_e interval

The linear relationships of T_pT_e interval and RR for the different breeds are shown in Figure 23. All of the slopes, except the one from the North-Swedish Trotting Horses, have a significant deviation from zero.

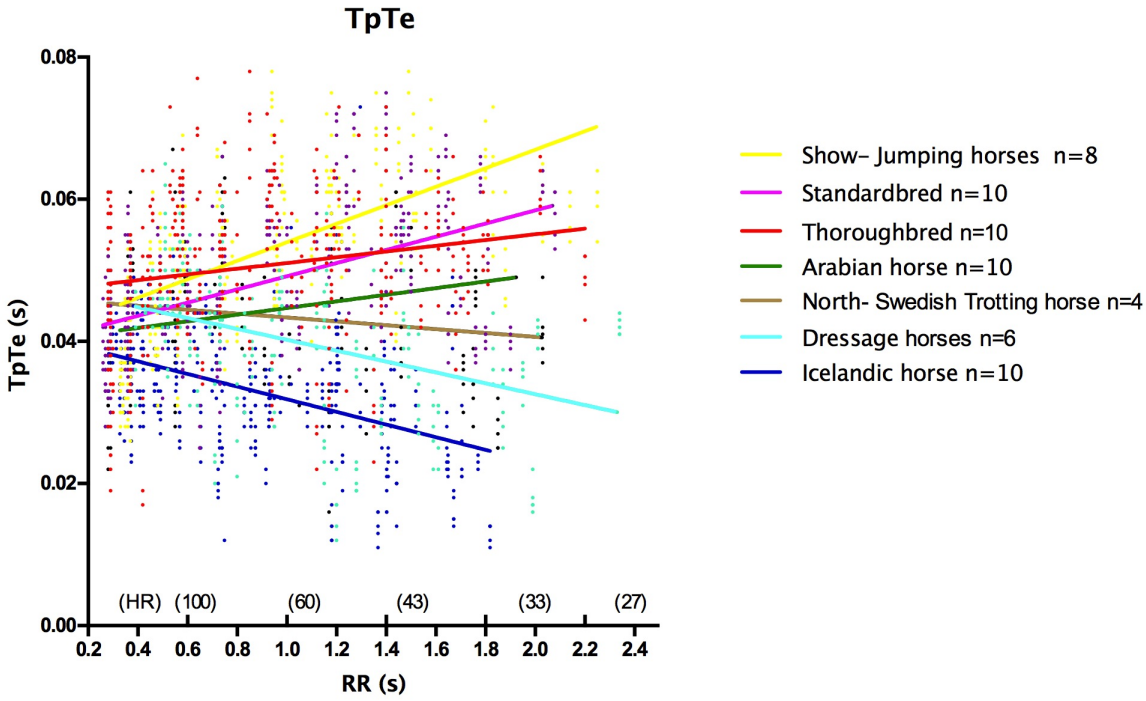

Figure 23: The different T_pT_e intervals plotted against RR. Only the North- Swedish Trotting horse has a slope with a deviation not significant from zero.

Table 3 provides a summary of all P-values, deviation from zero and r^2 of the T_pT_e/RR breed differentiated regression lines.

Table 3: A summary of statistical values from the T_pT_e/RR plotted breed differentiated linear regression lines. H. = Horses

Breed	P-value	Deviation from zero	r^2
Arabian H.	< 0.0001	Significant	0.0733
Thoroughbred	0.0002	Significant	0.0346
Standardbred	< 0.0001	Significant	0.203
Icelandic H.	< 0.0001	Significant	0.198
Dressage H.	< 0.0001	Significant	0.101
Show-Jumping H.	< 0.0001	Significant	0.340
North- Swedish H.	0.1714	Not Significant	0.0118

The r^2 value indicate how much of the dependence between T_pT_e and RR that is explained by the RR interval (for example 7.3 % in Arabian horses). It can be seen that in Show- Jumping horses, Standardbreds and Icelandic Horses the RR interval do explains more of the T_pT_e dependency of RR than in the other breeds. When letting GraphPad Prism[g] perform an analysis comparable to ANCOVA to test whether the slopes of the linear regression lines of each breed differ the resulting p-value is < 0.0001, indicating a highly significant difference between the slopes.

When performing a single linear regression fit for all data points from the T_pT_e measurements against RR (see Figure 24) the resulting fitted line is visually much flatter than the individually lines for each breed, and RR only explains 1.2 % of the T_pT_e dependency on the RR interval. But it has still a significant deviation form zero (p-value: < 0.0001, r^2 0.0121).

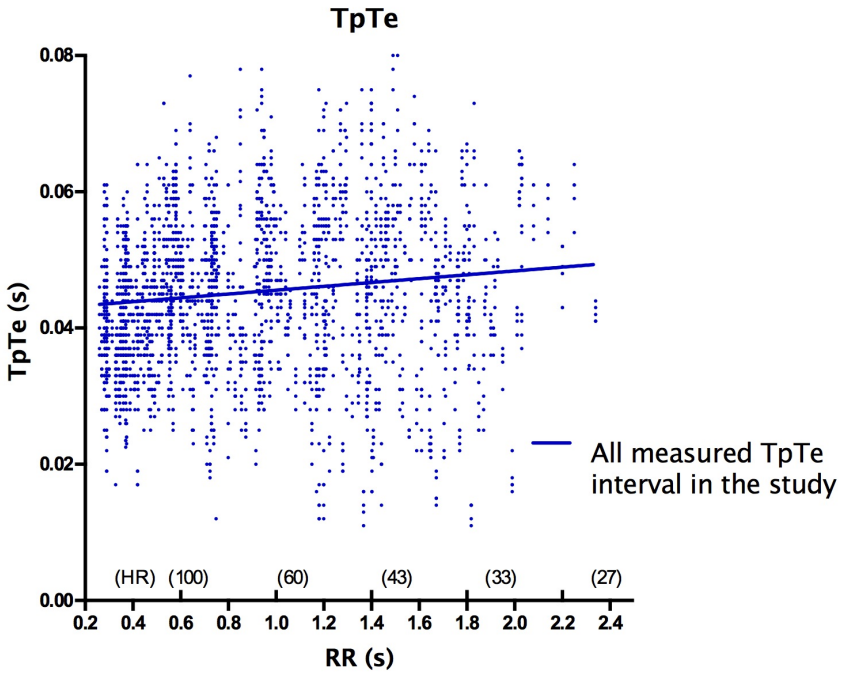

Figure 24: All data points from the measured T_pT_e interval in the study plotted against RR with the resulting fitted linear regression line (p-value <0.0001, r^2 0.0121).

6. Discussion

This is the first larger study comparing QT and T_pT_e intervals in different horse breeds at HR ranging from resting to maximal levels. Earlier studies of QT interval in horses are made within a specific (often low) HR range and/or include a specific breed (Lannek *et al* 1951; Fregin 1982; Ayala *et al* 1995; Pasławska *et al* 2012a; Pasławska *et al* 2012b; Schwarzwald *et al* 2012; Pedersen *et al* 2013). In comparison with the earlier studies of the QT interval the advantage of this study is that it includes seven different breeds consisting of individuals in different athletic condition, different temperament and of different sizes. This gives a better representation of the natural horse population. In addition QT interval is measured over the whole HR span of horses and the higher HR periods are due to a natural raise in pulse following exercise in settings that the horses are accustomed to.

6.1 The relationship between QT- and RR intervals in horses

The piecewise linear regression model showed in Figure 20, is consistent with the result in the study by Pedersen *et al* (2013) where the relationship between QT interval and RR in race- fit Standardbreds is best described by a similar piecewise linear regression model. The model is a contradiction to the linear regression relationship between QT interval and HR described in humans (Rajappan *et al* 2003). In humans did the use of a non- linear model only led to minimal improvement of the fit (Jensen *et al* 2004). But it was shown by Pedersen *et* al (2013) that the piecewise linear regression model fitted the data from horses better than different commonly used human correction formulas. Even if there, as seen in the results, is a significant influence of breed on the slopes and RR_{bend}, the pattern with a $Slope_1$, $Slope_2$ and a clear bending point (RR_{bend}) are same for all breeds. This further supports that the piecewise linear regression model is descriptive of the relationship between QT- and RR interval in horses.

$Slope_1$ represent the QT intervals of the period from RR_{bend} to maximal HR and $Slope_2$ is representing the QT intervals of the period from resting HR and until RR_{bend}. $Slope_1$ is steeper than $Slope_2$, indicating that the QT interval shortens faster at HR higher than the bending point. $Slope_1$ of all breeds seem to be in a very close connection, which indicates a tendency of smaller variation in the length of the QT interval at HR beyond the bending point. As seen in Figure 20, $Slope_2$ has a tendency of higher inter- breed variation than $Slope_1$, which is indicative of a higher variation in the length of the QT interval at HR up to the bending point.

The data points do also seem more spread around the different curves at this part and the 95 % prediction bands (see Appendix III) and CI (data not shown) are wider than at $Slope_1$. This graphically shown tendency of higher inter- breed variation of $Slope_2$ than of $Slope_1$ is similar to the graphically shown tendency of inter- gender variation seen in the study by Pedersen *et al* (2013).

As seen in the results, the RR_{bend} for all breeds lies in the HR range of 54.5-71.6 bpm with the Icelandic- and Arabian Horses in the higher end and Dressage horses in the lower. As seen for Slope2, RR_{bend} seems to an inter- bred variation that is greater than $Slope_1$. The value of RR_{bend} for Standardbreds in this study (1.025) does not correlate very well with the value (0.818) in the study by Pedersen *et al* (2013). The values of RR_{bend} for all breeds actually lays higher in this study than in the study by Pedersen *et al* (2013). The reason for this will be discussed in section 6.1.1. The reason for the greater inter- breed variation in length of QT interval at lower HR, the variation of the RR_{bend} and the significant influence of breed on slopes and RR_{bend} is unknown and further studies are needed.

6.1.1 Explanation and variations of the piecewise linear regression model

The biophysical explanation behind the piecewise linear regression model remains so far unexplained and it is therefore difficult to give an explanation for the difference in $Slope_2$ and RR_{bend} between breeds. It has been speculated that the bending point represents the HR were the slow delayed rectifier potassium channel (KCNQ1) become significant (Pedersen *et al* 2013). The KCNQ1 channels is found in both human and equine cardiac tissue (Finley *et al* 2002). They are activated upon β adrenergic receptor activation which also increase the HR, the entry of extracellular Ca^{2+} and the contractility, but the APD and thereby the QT interval shortens (Cunningham 2002; Marx *et al* 2002). Another theory is that I_{Kur} contributes to the rate-related adaption of the APD required for the wide range of HR in horses at rest and exercise (Finley *et al* 2003). I_{Kur} is expressed in ventricular mycoytes in horses in contraction to other species (hereby humans) where it is primarily seen in atrial myocytes (Nerbonne 2000). This difference in expression might be an explanation of the non- linear relationship between QT- and RR intervals in horses compared to the linear relationship described in humans (Rajappan *et al* 2003). The inter- bred variation of $Slope_2$ and RR_{bend} might be due to small differences in the expression level of these channels in different breeds.

6.2 Facts influencing the results of the measurements

6.2.1 Measuring errors

The ECG data for the Arabian Horses followed by the Icelandic Horses and the Standardbreds were the first data to be analysed. It is also $Slope_2$ of these three breeds that seems to differ mostly from the others (without being statistically tested). It can be discussed whether the difference between the QT interval represented by $Slope_2$ and RR_{bend} in these breeds are due to mistakes of interpreting the onset of the Q- wave and/or the offset of the T- wave. At lower HR the ECG curve is almost without motion disturbance with an easily recognized onset of the Q- wave and off T_{end}. It is therefore not probable that the differences are due to misinterpretations in this HR range. Because the author was inexperienced at the start of the study and then gained more and more experienced for each horse that was measured, the order of which the breeds were measured might have influenced the result. Since the first one to be measured were the Arabian Horses followed by the Icelandic Horses it is conceivable that inexperience combined with less good quality of the ECG tracings from these breeds, rather than purely misinterpretations of the QT intervals, have had an unwanted influence on their respective $RR_{bend}/Slope_2$. This possible influence of inexperience might have been less if it from the start of the study was decided to use either the threshold or the tangent method described in human medicine, since these methods are thoroughly described (Lepeschkin *et al* 1952; Couderc *et al* 2005; Panicker *et al* 2009; Salvi *et al* 2011). But no matter of how thoroughly the measuring technique is described, more or less variability between observers will exist because there will always be a need for a personal choice of where to draw a tangent or choice where the end of the T- wave is. This personal choice will always be present as long as the preferred measuring method is manually and not computerised. On the other hand did Pedersen *et al* (2013) show that inter- observer variability among inexperienced observers of ECG tracing from Standardbreds was within the range described in human medicine for experienced observers. It was further more or less the same inter- observer variability as intra-observer variability (Pedersen *et al* 2013). This indicates that the observer's experience should not have a major influence on the results of the measurements. It is though important to remember that the ECG recordings from the Standardbreds were the ones with best quality and the ones that were easiest to interpret so the resulting inter- and intra- observer variability could be different in the breeds with ECG tracings of inferior quality.

The different $Slope_2$ of the Standardbreds compared to the other breeds should though not be due to difficulties or inexperience in measuring the QT interval because a combination of

ECGs of good quality and a relative gained experience of the author at this time. It should also be mention that $Slope_2$ of Standardbreds (0.0189) do not only differ from the other breeds in this study but also from the $Slope_2$ (0.073) in the study by Pedersen *et al* (2013) where the same data was used but the measurements were made by another observer. Because of the much flatter appearance of the curve from Standardbreds, compared to the other breeds in this study, the three lowest HR range were measured once again a few weeks after the first measuring. There was immediately no major difference of the steepness of the curve and the curve currently described (data not shown). This further supports the theory that the different $Slope_2$ of the Standardbreds is not due to inexperience of the observer. Normal men is shown to have a spontaneous variability of the QT interval of average 76 ms over a 24 hour period (Morganroth *et al* 1991). The same variability was shown when the 24 hour period was divided into 8 hours periods. It can be speculated that the same natural variation can be seen in animals and it could be a part of the explanation to the difference between the $Slopes_2$ for Standardbred geldings in this study and the study by Pedersen *et al* (2013). The difference might also be a natural difference due to that the measurements are made in different HR periods or simply a result of the existing inter- observer variability (Panicker *et al* 2009; Pedersen *et al* 2013).

In the end it could though still be interesting to see if the results of this study changed if more experienced observers remade the measurements.

6.2.2 Verifying the onset of the Q- wave and the offset of the T- wave

Variations in the morphology of both Q- and T- waves complicates the measurement of the QT interval because the variations troubles the determining of the correct onset and offset. In the literature it is described that horses often have a RS morphology rather than a QRS morphology (Verheyen *et al* 2010b). This statement corresponds to the observations in this study where the Q- wave often was not definable (data not shown). The onset of the QRS complex was especially difficult to interpret in the Icelandic Horses because they often lacked both Q- and/or R- wave or had a very small and flat R- wave. Without being statistical tested the spreading of the data points from the Icelandic Horses seems to be greater than in the other breeds. It is possible that this spreading is due to difficulties in determining the starting point of the measurements when the Q- and/or R-wave were absent or had an abnormal morphology. Because of the variable morphology it is natural that the measurement gets more inconsistent since it gets a personal decision for each new variant of QRS complex where to

start the measurement compared to if there had been a normal Q- or R-wave to determine the starting point.

In both veterinary- and human medicine it is a challenge that there does not exist any guidelines of which method to use for determination of the T_{end} (Al-Khatib *et al* 2003; Panicker *et al* 2009; Salvi *et al* 2011). In addition difficulties of determining the T_{end} is described in human medicine (Lepeschkin *et al* 1952; Couderc *et al* 2005; Goldenberg *et al* 2006). These difficulties in determination of the T_{end} were also observed in this study and were common for all breeds. It was not a major problem at lower HR except in cases with a bifid T- wave with a very small second peak where it could be difficult to determine whether it was a bifid T- wave or just fluctuations of the baseline. The problems of determining the offset were greater at HR around and above 75 bpm. In this HR range, motion disturbance from the horses increase and hereby also the fluctuations of the baseline and the T_{end} got more difficult to determine due to U- waves and altered T- wave morphology. When reaching HR around and over 200 bpm the T- and P- wave tends to merge together. The phenomenon of merging T- and P- wave is described in horses before (Verheyen *et al* 2010a) and it was avoided to measure in areas with this phenomenon unless there was a clear notch that marked the end of the T-wave/beginning of the P- wave.

The variation in T- wave morphology found in this study is well described as a natural phenomenon in horses and was not further analysed (Ayala *et al* 1999; Ayala *et al* 2000; Menzies-Gow 2001; Verheyen *et al* 2010a).

6.2.3 Influence of QT lag and/or QT hysteresis

Two factors that influence the QT interval are QT lag and QT hysteresis and their influence on the results in this study can be discussed. *QT lag* is described as delayed adaption of the QT interval following changes in the RR interval (Pueyo *et al* 2003). *QT* hysteresis is characterised by longer QT interval at a given RR interval when the HR is increasing and shorter at the same RR interval when the HR is decreasing (Pelchovitz *et al* 2012). The effects of QT lag in this study design should be minimal because the measurements are done at periods with constant HR (Pedersen *et al* 2013). The mechanism of QT hysteresis is known to be influenced by the autonomic-, and especially by the parasympathetic nervous system in humans (Pelchovitz *et al* 2012). The autonomic nervous system has a high parasympathetic tone in horses and have a strong influence on equine cardiac function. Therefore the presence of QT hysteresis in this study is not unlikely and may be significant, especially in the cases of continuous recordings from rest and during exercise where the HR continuously changes.

There could possibly be a difference in the level of the parasympathetic tone between the different breeds that explain the variation in the length of the QT interval seen in this study.

6.3 Relationship of T_pT_e- and RR- intervals

The T_pT_e interval is a sensitive measurement of the transmural dispersion of repolarisation in the ventricles and in humans the interval is shown to be independent of HR, age and sex (Porthan *et al* 2007; Haarmark *et al* 2010). Human studies have reported that prolongation of T_pT_e, as prolongation of QTc, is a risk factor for developing ventricular arrhythmias and SCD (Porthan *et al* 2007; Panikkath *et al* 2011). In the race-fit Standardbred, the dependence on HR is reported to differ between genders (Pedersen *et al* 2013). In stallions and geldings T_pT_e were dependent on HR in contrast to mares where T_pT_e was independent of HR, as described in humans (Porthan *et al* 2007; Haarmark *et al* 2010). It was also shown that the gender differentiated slopes of the regression lines were significantly different (Pedersen *et al* 2013). As seen in the results for this study, the North Swedish Trotting Horses were the only breed where the T_pT_e interval was independent of HR, in the other breeds the interval were markedly dependent (see Table 3). The study group of the North Swedish Trotting Horses is though smaller (n=4) than the other study groups and the resulting curve might change if more horses were included in the study. The variations in T_pT_e explained by RR of Standardbred geldings in this study ($r^2 = 0.203$) correlates well with the value for the geldings ($r^2 = 0.20$) in the study of Pedersen *et al* (2013). This is interesting since it indicates that the difference in measurements of the QT interval between this study and the study by Pedersen *et al* (2013) are originating from the onset of the QT interval until T_{peak} and not from the described difficulties of determining the T_{end} (Lepeschkin *et al* 1952; Couderc *et al* 2005; Goldenberg *et al* 2006).

The difference between all slopes, and thereby between breeds, is highly significant. An interesting result was also that the directions of the slopes are different. In Show- Jumping horses, Standardbreds, Thoroughbreds and Arabian Horses T_pT_e is decreasing with higher HR whereas T_pT_e of the Icelandic Horses, Dressage horses and The North- Swedish Trotting Horses are increasing with higher HR. The reason for this is currently unknown and need to be investigated further. The T_{peak} is normally easily identified and the inter- breed variation should rather be due to difficulties in determining T_{end} than misinterpretation of the T_{peak}.

When analysing all data points as one model HR/RR only explains 1.2 % ($r^2 = 0.0121$) of the dependence between T_pT_e and RR (see Figure 24), which is lower than r^2 from the individual analysis of all breeds except from the North Swedish Trotting Horses. The regression line seems much flatter than the individual curves indicating a less dependency on HR, which indicates that the T_pT_e interval of a mixed group of horses is independent of HR. The breed differentiated analyse in this study shows that there is a difference between breeds, indicating it may not be ideal to analyse a mixed group of horses as a single model but there is a need for further studies including more than ten horses of each breed before the results can be validated. Due to the inconsistent results in horses in this study compared to some of the results in the study by Pedersen *et al* (2103) further studies are needed to conclude whether the T_pT_e interval is dependent on HR and on breed or not.

In human medicine T_pT_e has been suggested useful in evaluating ventricular repolarisation in cases where the QTc interval is normal or not valid (Panikkath *et al* 2011). This might also be an alternative in horses where the onset of the QRS complex is difficult to determine due to lacking Q- and/or R- waves. It is shown that the T_pT_e interval is significantly prolonged in horses after sedation with Detomidine, whereas the QTc intervals in the same horses were not significantly prolonged (Buhl *et al* 2013). This may indicate that the T_pT_e interval, as in humans, can be useful for evaluating ventricular repolarisation when the QTc interval is normal.

6.4 Physiological parameters and age of the horses

Athletic condition, physical status, age and size of the horses are not included in the analysis of the results of this study.

6.4.1 Athletic conditions of the horses

The athletic condition of the horses varies from race- fit Standardbreds and North-Swedish Trotting Horses; nearly race- fit Thoroughbreds and well trained endurance Arabian Horses to much less trained Icelandic Horses and Dressage and Show-Jumping horses. The “athlete’s heart” is known in both horses (Buhl *et al* 2005) and humans (Sharma *et al* 1999; Pluim *et al* 2000; Rajappan *et al* 2003). It is a state of physiological LVH deriving as a natural response to training but without pathologic decrease in cardiac function (Pluim *et al* 2000; Rajappan *et al* 2003; Buhl *et al* 2005). In non-athlete humans LVH is associated with a prolonged QT

interval and thereby an increased risk of developing arrhythmias and SCD (Haider *et al* 1998; Porthan *et al* 2007). The "athlete's heart" in humans is however not associated with QT prolongation, since the QT interval is shown to shorten similar in both athletes and untrained controls (Rajappan *et al* 2003). It is though not investigated whether horses with "athlete's hearts" have normal or prolonged QT interval. Observation of the regression lines in Figure 20 shows that the Icelandic Horses, which are at the lower end of the athletic condition scale, actually have the longest QT intervals at low HR, and marginally the shortest at higher HR. Even if this difference is not statistically tested the observation still supports the choice not to include athletic conditions into the analysis. This observation together with the human observation of normal QT interval in athletes indicates that the variation in the length of the QT interval between breeds is not due to difference in athletic condition. There is though a need for further studies of the QT interval of horses in different athletic condition before it can be conclusive.

6.4.2 Size of the horses

The choice to exclude the size of the horses from the analysis of the results is based on the findings in the study by Schwarzwald *et al* (2012) who investigated the relationship of HR and length of ECG intervals to body mass in horses and ponies. They found that QT interval was related to body weight (BWT) and to the preceding RR interval. After correction for HR the QTc was found to be nearly independent of BWT, suggesting that the relationship to BWT were mainly due to differences in HR and not to BWT itself (Schwarzwald *et al* 2012). It was also reported that on a population level, small equine breeds tends to have slightly faster HR than larger breeds and this would, rather than the BWT, explain the differences in QT interval between them (Schwarzwald *et al* 2012). The breeds included in this study do primarily represent larger horses and ideally some smaller pony breeds should be included to get a better representation of the natural horse population.

6.4.3 Age of the horses

The study population is a relatively young homogenous age group including only three horses > 15 years. In humans QTc is shown to increase with age (Reardon *et al* 1996) and it could be speculated that the data points from these three horses over 15 years lay further away from the regression line and thereby affect the analysis. If the data points from these three horses are observed separately, they all lie fairly close to their respective regression line and within the

95 % prediction bands (see Figure 21). There is therefore no reason to exclude these horses from the study.

The homogenous age study population is though not so representative for the whole population of horses. The average life span of horses in Denmark was in 2011 said to be 14 years (Hestemagasinet. dk; Boas 2011). Compared to this study's average of 7.3 years it would have been appropriate to include older horses to raise the average age of the study population and thereby get a study- group more representative for the whole horse population. Because most of the ECG recordings were obtained before this study and due to a limited time frame it was not possible to get a more representative group.

6.4.4. Physiological status of the horses

It had been appropriate to perform a basic clinical evaluation of the horses included in the study. Since some of the ECG recordings originally have been obtained and used in earlier studies, the health status and possibly cardiac abnormalities in some of the horses is unknown. It can therefore not be excluded that some horses included in this study suffered from a subclinical, mild cardiovascular disease. The materials from the Thoroughbreds included in the study were obtained by the author but these horses did not either underwent a clinical evaluation. But neither the trainer nor the exercise rider did complain of any health issues or reduced performance before or after the fast work, with exception of a single horse with epistaxis after pulling up from the fast work. This horse underwent later an endoscopic evaluation and the veterinarian did not find any obvious signs of illness. It is therefore chosen to include the horse in the study. The data points from the QT interval measurements of the Thoroughbred with epistaxis do not lay in very close connection with the regression line and few of them lay outside the 95 % prediction band (see Figure 22). It could therefore be speculated that the physical condition did influence the QT-interval. Some of the data points from other horses do though also lay in close connection to the prediction bands, so including this single horse's data points does not change the overall conclusion.

7. Conclusion

The main finding in the study is that horse breed has a statistically significant dependency on $Slope_1$, $Slope_2$ and RR_{bend} of the piecewise linear regression model. It was shown a tendency of smaller differences in the length of the QT interval between breeds at higher HR than at lower HR. At lower HR the Icelandic- and Arabian Horses seem to have longer QT intervals than others, whereas Standardbreds seem to have shorter. The first hypothesis of no differences in the length of the QT interval between breeds is therefore rejected. There is though a strong tendency of agreement between some of the breeds. The explanation for the differences between breeds is not conclusive, it might be due to misinterpretation of the measurement or natural physiological differences in the QT interval or cardiac physiology between the breeds.

The piecewise linear regression model as a description of the relationship between QT- and RR intervals in horses presented by Pedersen *et al* (2013) is supported in this study. The model has the same characteristic ($Slope_1$, $Slope_2$ and RR_{bend}) for all breeds included in this study and thereby is the second hypothesis accepted.

The relationship between T_pT_e intervals and RR intervals were shown to follow a linear regression model. All regression lines of the different breeds except from The North- Swedish Trotting Horse had though a significant deviation from zero, indicating that the T_pT_e interval is dependent on HR in these breeds. When analysing all the measured T_pT_e intervals as a single model the dependency of HR were less than in the breed differentiated analysis. This result in combination with a significant difference between the individual regression lines of each breed indicates that the dependency on HR of the T_pT_e interval may differ between breeds and analysing a mixed group of horses as a single model may thereby not be ideal. The third hypothesis cannot be accepted since the results show that the T_pT_e interval have a tendency of being dependent on both HR and breed.

8. Perspective

The results of this study can be used in order to further build a platform of QT interval characteristic in horses. It could be interesting to expand the study and include pony breeds and older horses/ponies and compare their measurement values to the result of this study to gain information of all types of horses in the natural horse population. The results of this study supports a piecewise linear regression fit of the QT/RR relationship, indicating this model should be used in future studies. It could also be interesting to include stallions and mares in further studies to furthermore investigate if the differences between sexes in Standardbreds, as seen in the study by Pedersen *et al* (2013), are valid for other breeds as well.

In order to increase the consistency of the measurements the different Q- and T- wave morphology should be investigated by experienced cardiologists/ observers of ECGs to see if experience changes the results and to determine a more defined template of where to start and end the measurements of the QT interval. This can give an idea of any practitioner can perform the measurements or if cases need to be referred to a specialist.

The QT interval in horses can be useful for monitoring horses treated with drugs known to prolong the QT interval in humans (such as quinidine). If the interval is measured before the start of the treatment and then monitored under the treatment, the horse can act as its own control.

Whether horses with episodes of syncope or sudden death also have a higher risk of a prolonged QT interval, as seen in humans, is unknown, but would be interesting to investigate. It is difficult to investigate in cases of sudden death due to the lack of pathologic findings at necropsy, but it might be possible in horses with episodes of syncope as soon as there have been stated valid reference values. It could also be interesting to perform genetic testing of horses with episodes of syncope or, if possible, horses that suffered sudden death to investigate if any mutations known in humans also exist in horses. Genetically testing of relatives to these horses might give information of any possibly inherited mutations.

There is further a need for research to characterise the biophysical explanation behind the piecewise regression line with its characteristic bending point. Is the bending point a marker of significant contribution of KCNQ1 channels or the I_{Kur} current? And is it a difference in the expression of potassium channels between breeds that gives slightly different values of the bending point?

Finally there is also need for more studies on the relationship of the T_pT_e interval and the RR interval in order to confirm or reject the theory of T_pT_e's independency of HR, sex or breed.

9. Manufactures Addresses

[a] 1 Televet 100, Televet 100 version 5.1.0, Roesch & Associates Information Engineering GmbH, Frankfurt am Main, Germany

[b] 2 KRUUSE ECG electrodes, Jørgen KRUUSE, Langeskov, Denmark

[c] Snögg, Jørgen KRUUSE, Langeskov, Denmark

[d] Cardio Calipers 3.3, ICONICO, New York, NY, USA

[e] Microsoft Excel for Mac 2011 version 14.3.5, Microsoft Corporation, Redmont, WA, USA

[f] SAS system version 9.2 for Windows, SAS Institute Inc, Cary, NC, USA

[g] GraphPad Prism v 6.0 for Mac OS X and v5.04 for Windows, GraphPad Software, Inc, San Diego, CA, USA

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Appendix I- Number and age of horses of the different breeds

Horse	Breed	Age	Horse	Breed	Age
Bastian	Arabian horse	11.4	Simbad	Thoroughbred	8
Shetan Shadwan	Arabian horse	14.3	Sikorsky	Thoroughbred	6
Portal	Arabian horse	9.6	MajesticEleven	Thoroughbred	6
Gashin El Din	Arabian horse	8.1	TheKicker	Thoroughbred	4
Shaman	Arabian horse	19.4	SirWinston	Thoroughbred	3
Facas	Arabian horse	7.4	Local Chief	Thoroughbred	3
Shajan	Arabian horse	15.3	CarlRasDanaLim	Thoroughbred	3
Vermut	Arabian horse	13.6	Flyer	Thoroughbred	8
Abuzed	Arabian horse	9.4	Hovsa	Thoroughbred	4
Sulivan	Shagya-Arabian	11.3	ProHunter	Thoroughbred	4
	Mean age:	12.0		**Mean age:**	4.9
Fagri-Ørn	Icelandic Horse	10	Thuram	Standardbred	6
Matti	Icelandic Horse	6	Lindy Later	Standardbred	5
Birk	Icelandic Horse	6	Staro Yes (MR)	Standardbred	5
Autur fra Nyberg	Icelandic Horse	9	Tampico Stud	Standardbred	6
Fønix fra Nyberg	Icelandic Horse	5	Little Rainbow	Standardbred	3
Gimmstein	Icelandic Horse	6	Rubis Ingo	Standardbred	6
Emil fra Nyberg	Icelandic Horse	6	Best Friend	Standardbred	6
Glæsir	Icelandic Horse	21	11 (5. rec)	Standardbred	5
Feykir fra Nyberg	Icelandic Horse	5	37 (1. rec)	Standardbred	5
Raudur	Icelandic Horse	7	17 (1. rec)	Standardbred	6
	Mean age:	8.1		**Mean age:**	5.3
2dv	Danish Warmblood	8	21	German Oldenburger	10
2dv	Danish Warmblood	8	22	Belgian Warmblood	9
14dv	Danish Warmblood	9	23	Brandeburger	8
14dv	Danish Warmblood	9	25	Holsteiner	8
3dv	Danish Warmblood	9	26	Danish Warmblood	10
3dv	Danish Warmblood	9	27	German Oldenburger	8
6dv	Danish Warmblood	9	30	Danish Warmblood	13
7dv	Danish Warmblood	?	34	Oldenburger	10
10dv	Danish Warmblood	7		**Mean age:**	9.5
	Mean age:	8.5			
			Total:		
TyriStegg	North Swedish Trotting Horse	6	Mean age:	7.3	
Aaby Knecten	North Swedish Trotting Horse	8	Number of horses	58	
Valle Otto	North Swedish Trotting Horse	7			
Åsgards Ty	North Swedish Trotting Horse	5			
	Mean age:	6.5			

Appendix II- SAS coding

SAS[f] coding for obtaining $Slope_1$, $Slope_2$ and RR_{bend}:

```
ods html;
ods graphics on;
proc nlin method = gauss data = sasuser.Malin ; by type;
     parms m1 = 0.230
           m2 = 0.100
           bend = 0.5
           a  =  0.400  ;
      der.m1 = (rr - bend) ;
      if rr gt bend then der.m1 = 0 ;

      der.m2 = (rr - bend) ;
      if rr le bend then der.m2 = 0 ;

      der.bend = -m1 ;
      if rr gt bend then
      der.bend = -m2 ;

      der.a = 1 ;

     f = a + m1 * (rr - bend) ;

     if rr gt bend then
     f = a + m2 * (rr - bend) ;

     model Qtsek = f ;
output out=Qtsekpred predicted=yp residual=Qtsek_r;
run ;
ods html close;
```

SAS[f] coding for obtaining RR_{step}:

```
data sasuser.Malin;
  set sasuser.Malin;
  RRstep=0;
  if RR > 1.173 and type=1 then RRstep=RR-1.173;
  if RR > 1.194 and type=2 then RRstep=RR-1.194;
  if RR > 1.025 and type=3 then RRstep=RR-1.025;
  if RR > 0.9087 and type=4 then RRstep=RR-0.9087;
  if RR > 0.9819 and type=5 then RRstep=RR-0.9819;
  if RR > 1.040 and type=6 then RRstep=RR-1.040;
  if RR > 1.023 and type=6 then RRstep=RR-1.023;
  run;
```

SAS[f] coding for evaluate the dependence of breed (Type) on slopes and RR_{bend}:

```
title 'Malin model 1';
ods html;
ods graphics on;
proc mixed data=sasuser.Malin;
class HorseID Type;
model  Qt=  type  RR  RRstep  RR*type  RRstep*type  /  ddfm=satterth  residual
solution cl outpm=estimates;
random HorseID;
repeated / type=sp(gau)(Time) subject=HorseID local;
run;
ods html close;
```

Appendix III- Larger figure of the QT-/RR interval model and figures of individual breed models with 95 % prediction bands

Figure 1 is a larger version of the breed differentiated fitted piecewise regression lines for the relationship between QT- and RR intervals.

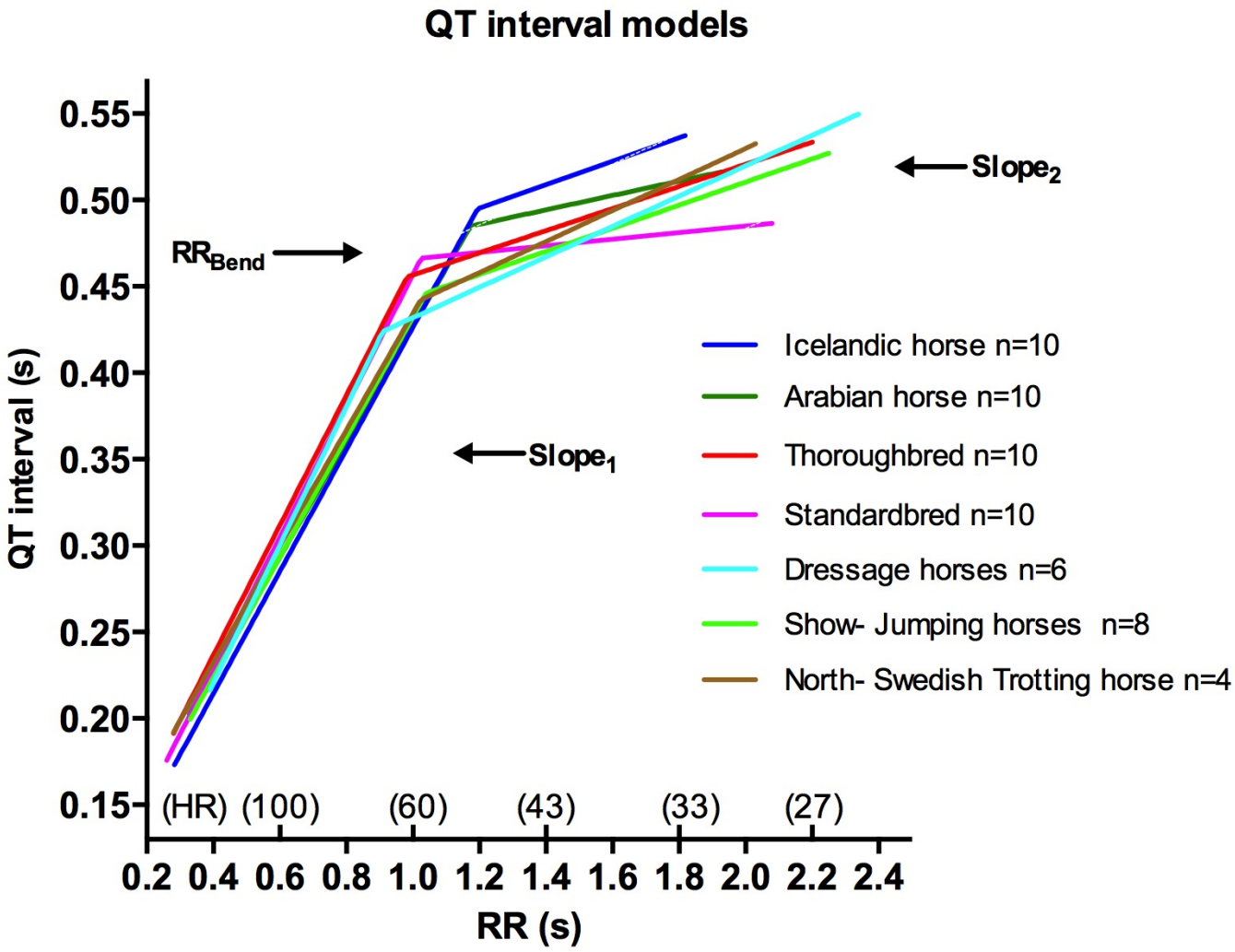

Figure 1: Larger figure showing the breed differentiated fitted piecewise regression lines for the relationship between QT-/RR interval without the data points.

Figure 2-7 shows the fitted piecewise regression line for each breed with the 95 % prediction bands as the dotted line.

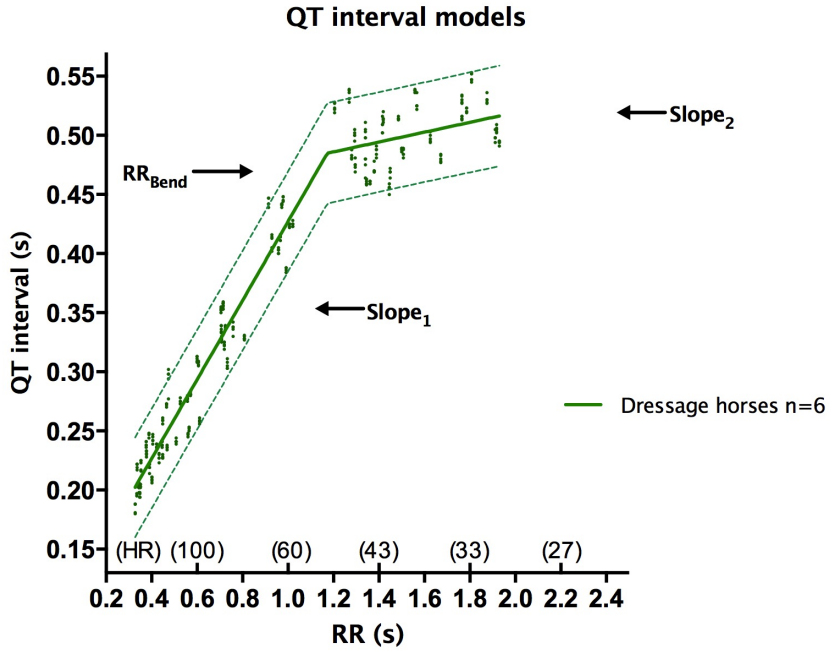

Figure 2: The fitted regression line for the Arabian Horses, with the 95 % prediction bands.

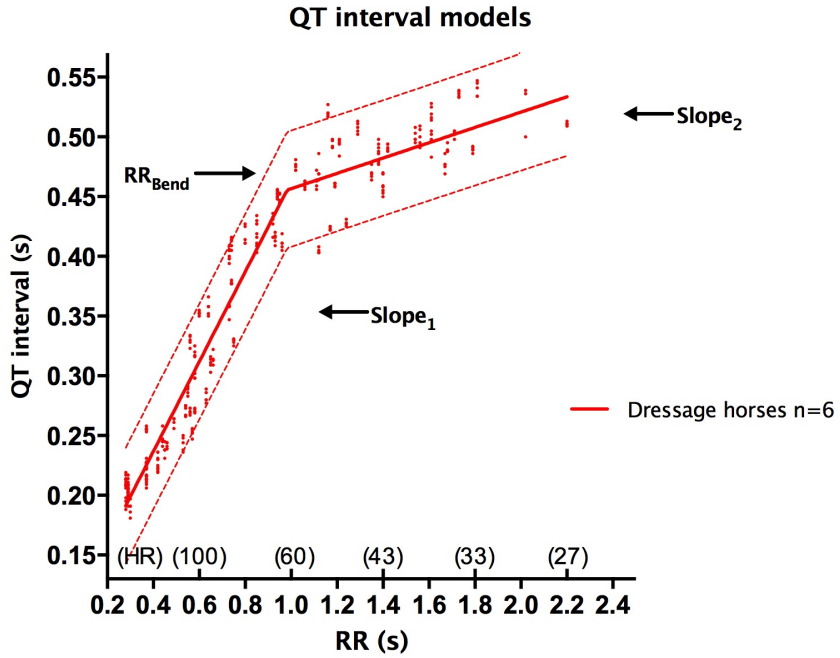

Figure 3: The fitted piecewise regression line for Thoroughbreds, with the 95 % prediction bands

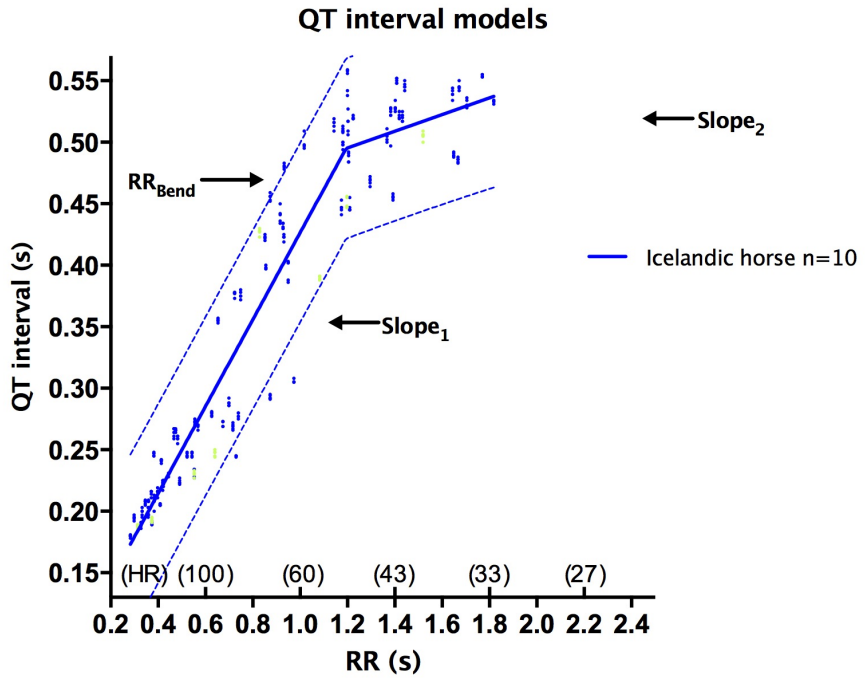

Figure 4: The fitted piecewise regression line for the Icelandic Horses, with the 95 % prediction bands.

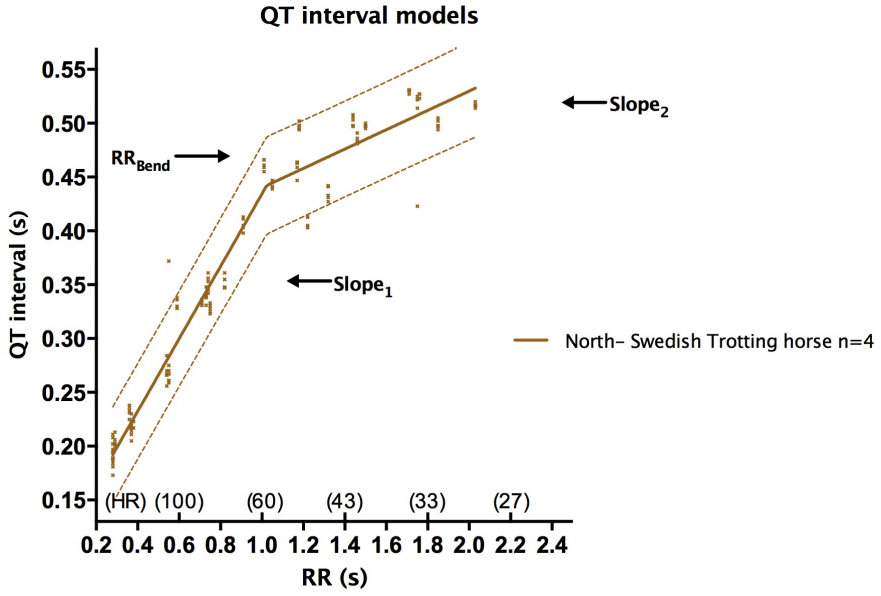

Figure 5: The fitted piecewise regression line for the North Swedish Trotting Horses, with the 95 % prediction bands.

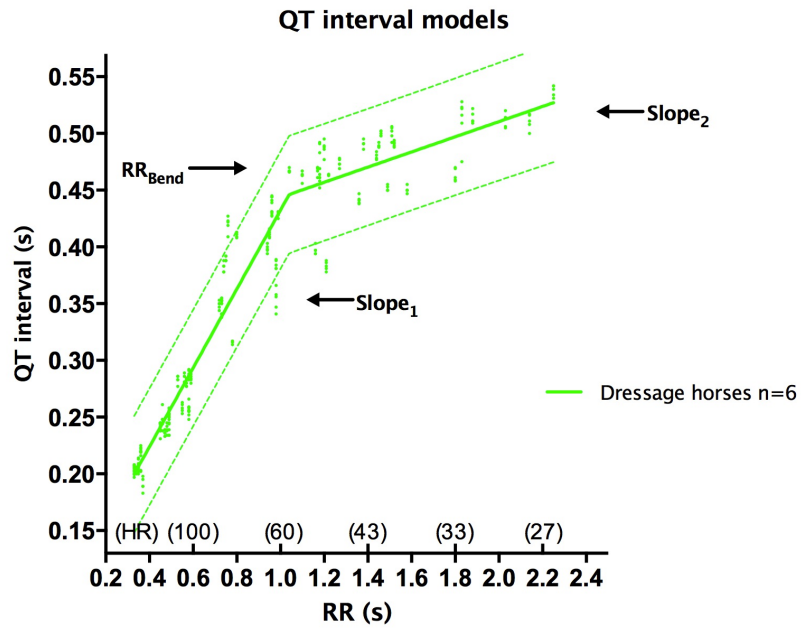

Figure 6: The fitted piecewise linear regression line for the Show- Jumping Horses, with the 95 % prediction bands.

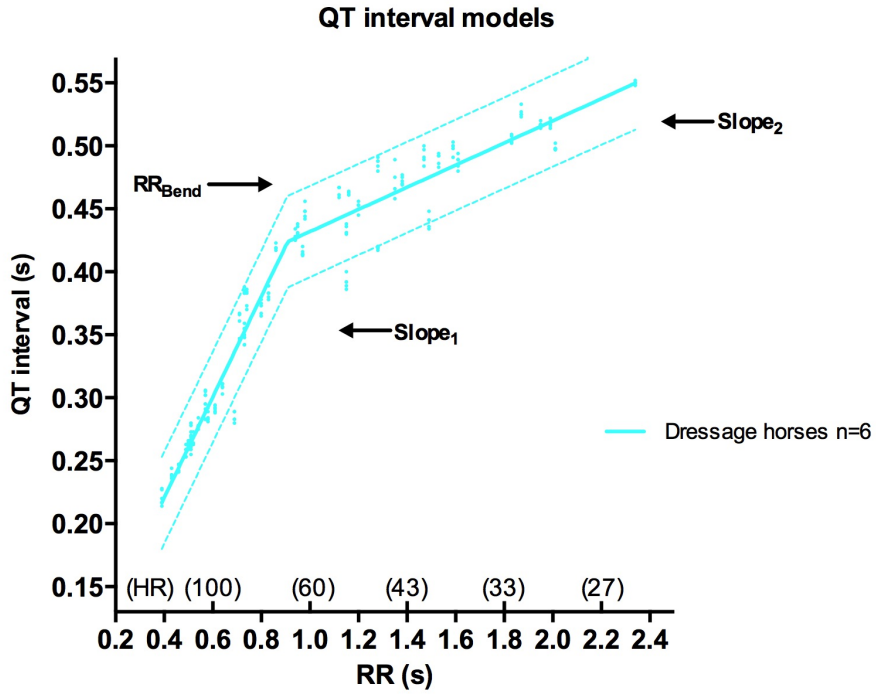

Figure 7: The fitted piecewise linear regression line for the Dressage Horses, with the 95 % prediction bands.