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Master's thesis

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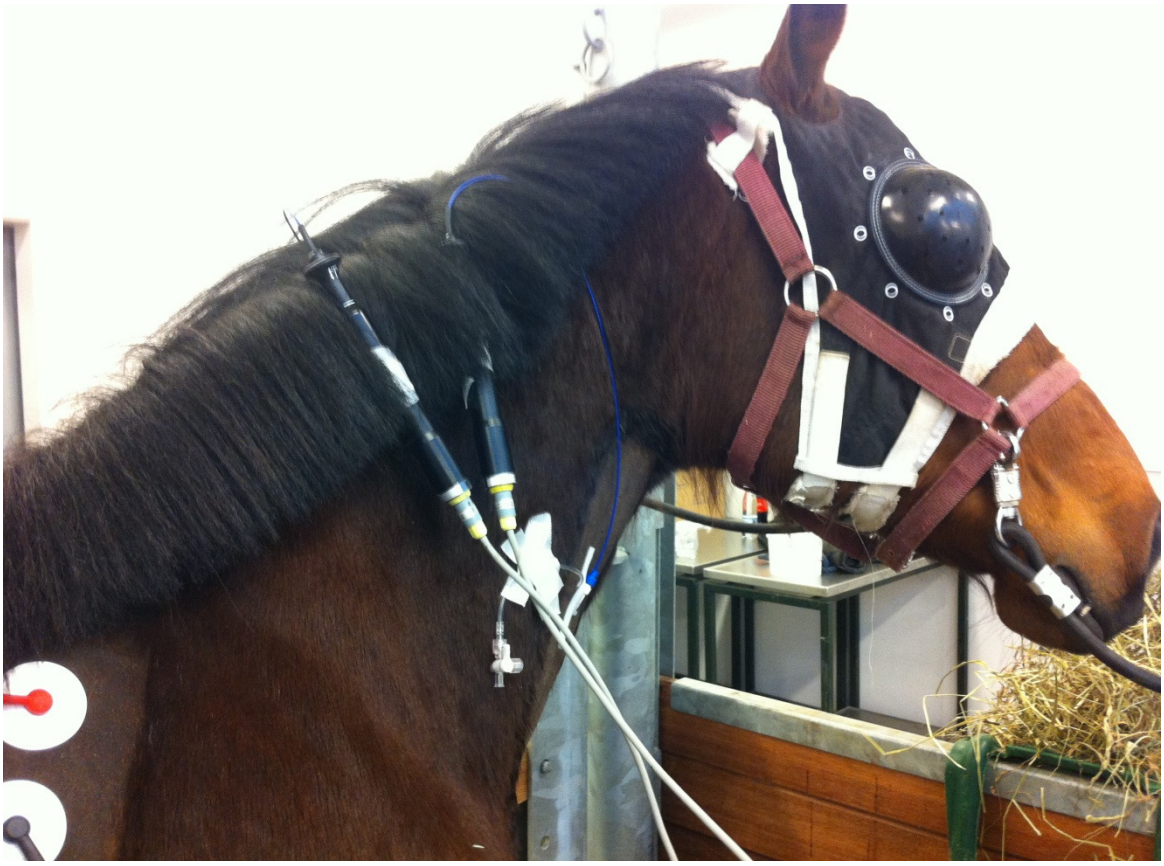
Induced Atrial Fibrillation in the Horse

Evaluation of cTnI and CK-MB after electrical stimulation of the heart



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Abstract

Introduction: Atrial fibrillation is an arrhythmia of the heart causing an irregular heartbeat and decreased performance in the horse. The treatment of choice in horses is oral dosage of quinidine. This treatment is effective but has multiple adverse effects, and thus treatment alternatives are needed. Atrial contraction can be generated by applying electrical stimulus to the atrial myocardium. This stimulus can also induce atrial fibrillation in the healthy heart. The cardiac specific biomarkers cTnI and CK-MB are released into the bloodstream following myocardial ischemia or necrosis, and thus increased values indicate damage to the heart. Dofetilide and ranolazine are anti-arrhythmic compounds used in human medicine to treat atrial fibrillation.

Objective: The objective of this study is to evaluate the levels of cTnI and CK-MB after pacing of the atria and induction of AF as well as after the administration of ranolazine and dofetilide.

Materials and methods: Nine horses were used in this study. A safety procedure and an electrophysiologic procedure were conducted. During the safety procedure the horses were only administered the medical compounds, whereas the electrophysiologic group the first had atrial fibrillation induced and subsequently were administered the compounds. Blood samples were taken at 0, 4 and 24 hours and levels of cTnI and CK-MB were measured.

Results: The results showed three elevated cTnI samples and four elevated CK-MB samples. Horses that were exposed to pacing did not have higher levels of cTnI and CK-MB than those that were unexposed. There was no correlation between the number of times tachypaced or number of procedures the horse underwent and increased levels of cTnI and CK-MB. For cTnI at 24 hours there was a significant relationship between the amount of time the horse was in atrial fibrillation and the levels of cTnI; however the increase was minor. There was no difference in levels of cTnI and CK-MB after administration of the different anti-arrhythmics.

Conclusion: The electrophysiologic procedure is a safe procedure that does not inflict damage to the myocardium even when performed multiple times. The administration of dofetilide and ranolazine does not lead to increased levels of cTnI and CK-MB. Induced atrial fibrillation might lead to an increase in cTnI but the levels were not of clinical importance at the time observed in this study.

Perspective: The verification of the electrophysiologic procedure as a safe procedure is useful in the future research on atrial fibrillation. In future studies a larger and more varied study population is needed. Further studies on CK-MB are needed to determine its importance as biomarkers in horses. Histological and macroscopic examination of the hearts of the horses used in this study could provide interesting and useful results.

Resumé

Introduktion: Atrieflimmer er en hjertearytmi der giver en irregulær hjerterytme og forringet præstation ved heste. Behandling er oral dosering af quinidine. Denne behandling er effektiv, men har mange bivirkninger og der er derfor et behov for nye behandlingsmuligheder. Atriet kan stimuleres til at kontrahere sig ved at tilføre myocardiet en svag elektrisk strøm. Denne stimulus kan også inducere atrieflimmer i det raske hjerte. Biomarkørerne cTnI og CK-MB bliver udskilt til blodbanen efter iskæmi eller nekrose af hjertemuskulaturen. Forhøjede værdier indikerer derfor en skade på hjertet. Dofetilide og ranolazine er antiarytmiske stoffer der bruges i humanmedicin til at behandle atrieflimmer.

Målsætning: Målet med dette forsøg var at evaluere niveauet af cTnI og CK-MB efter pacing af atriet og induction af atriflimmer, samt efter indgivelsen af ranolazine og dofetilide.

Martriale og *metode:* Der blev brugt ni heste til dette forsøg. Der blev udført en sikkerhedsprocedure samt en elektrofysiologisk procedure. Ved sikkerhedsproceduren blev hestene kun tildelt de antiarytmiske stoffer, mens de i den elektrofysiologiske procedure blev induceret med atrieflimmer hvorefter de fik indgivet stofferne. Der blev taget blodprøver før procedure samt 4 og 24 timer efter, og niveauet af cTnI og CK-MB blev målt.

Resultater: Resultaterne viste at kun tre af cTnI-prøverne og fire af CK-MB-prøverne var forhøjede. Heste der var blevet pacet havde ikke højere værdier af cTnI og CK-MB end heste der ikke var blevet pacet. Der var ingen sammenhæng mellem antal gange en hest var blevet pacet eller hvor mange procedurer hesten havde været igennem og forhøjede niveauer af cTnI & CK-MB. Der var signifikant sammenhæng mellem antal minutter i atrieflimmer og niveauet af cTnI ved 4-timers prøven, dette var dog en lille stigning. Der var ingen statistisk forskel mellem niveauer af cTnI og CK-MB efter de forskellige stoftildelinger.

Konklusion: Den elektrofysiologiske procedure er en sikker procedure der ikke påfører skader i myocardiet, selv hvis udført flere gange. Indgiften af dofetilide og ranolazine fører ikke til forøgede niveauer af cTnI og CK-MB. Induceret atrieflimmer kan muligvis føre til en forøgelse af cTnI men niveauet var så lavt at det ikke er klinisk relevant i dette studie.

Perspektivering: Verificeringen af sikkerheden af den elektrofysiologiske procedure kan være nyttig i den videre forskning indenfor atrieflimmer. I fremtidige studier vil en større og mere varieret gruppe forsøgsheste være ønskelig. Studier omkring CK-MB er nødvendige for at fastlægge en referenceværdi for heste. Makroskopisk og histologisk undersøgelse af hjerterne efter forsøgene kunne give interessante og nyttige resultater.

Preface

This thesis is written as a part of the master programme of veterinary medicine at the Faculty of Health and Medical Sciences, University of Copenhagen.

The aim of the project was to evaluate the safety of the electrophysiologic procedure. The results from this thesis can be used by veterinarians and physicians in future research on atrial fibrillation.

Several people have contributed to the making of this project. All the help has been greatly appreciated. Two people deserve extra credit.

Firstly thank you to my supervisor Rikke Buhl for her help and guidance during this project.

A special thank you goes to Helena Carstensen for her incredible work and commitment during the making of this thesis. She has been an enormous help and support and I want to thank her for all the time and energy she has spent on this project.

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1. Introduction

1.1 Introduction to the project

Atrial fibrillation is the most important arrhythmia in horses with exercise intolerance as the symptom seen most often (Holmes *et al.* 1969; Else & Holmes 1971; Deem & Fregin 1982). Quinidine therapy is the drug of choice for cardioversion and is effective > 80% of the time (Deem & Fregin 1982; Reef *et al.* 1995). However quinidine has many toxic and adverse effects (Reef *et al.* 1995). This has led to the need for new treatment options for atrial fibrillation. The horse is a good animal model for atrial fibrillation not only because of its large heart and atria, but also its high vagal tone, all of which favour the presence of atrial fibrillation (Blissitt 1999). Induced atrial fibrillation and electrophysiologic measurements are methods of analysing and testing a new medical compound. Atrial fibrillation is induced by sending an electrical current through the atrial myocardium causing the atria to contract (Scherf *et al.* 1950). Ischemia can occur as a result of atrial fibrillation or pacing of the atria (Turer *et al.* 2011; Parwani *et al.* 2013; van Bragt *et al.* 2014).

Cardiac troponin I (cTnI) and creatine kinase fraction MB (CK-MB) are biomarkers of the heart which are released into the bloodstream following myocardial ischemia or necrosis. If such damage occurs to the myocardial cells an increase in cardiac troponin I and creatine kinase fraction MB can be detected in blood samples (Sobel & Shell 1972; Adams *et al.* 1993b).

A horse model for atrial fibrillation could be very useful in the future research of atrial fibrillation and treatment hereof. It is therefore relevant to study the effect of atrial pacing and induced atrial fibrillation on the equine heart in order to evaluate the safety of the procedure.

This project was a part of a PhD project studying the two anti-arrhythmic compounds ranolazine and dofetilide and their possible synergistic effect on atrial fibrillation.

The aim of this project was to evaluate blood levels of cTnI and CK-MB before and after induction of atrial fibrillation and infusion of the medical compounds to study if any myocardial damage had occurred.

Based on a literature study and the experiments conducted the following hypotheses were made:

- Levels of cTnI and CK-MB will increase in horses which have undergone atrial pacing.
- Levels of cTnI and CK-MB will correlate with the number of times the horse was tachypaced.

- Levels of cTnI and CK-MB will correlate with the number of procedures the horse has undergone.
- Levels of cTnI and CK-MB will be lower in horses that received ranolazine compared to the horses that received dofetilide and a placebo.
- Levels of cTnI and CK-MB will be higher in horses that received dofetilide compared to horses that received a placebo.
- Levels of cTnI and CK-MB will be lower in horses that received the combination of ranolazine and dofetilide compared to horses that received a placebo.
- Levels of cTnI and CK-MB will correlate with the amount of time the horse has been in atrial fibrillation.

1.2 Limitations

This study was a part of a PhD project and thus several aspects of the project are not included in this thesis. The main focus of this study was to evaluate the concentration of cTnI and CK-MB before and after the procedures. The horses' response to the drug administration as well as the electrocardiographic changes due to the drug infusion will not be mentioned unless it is of importance to the interpretation of the cTnI and CK-MB findings. Furthermore the atrial effective refractory period (aERP) measurements made during the procedures and the evaluations of the efficiency of the drugs are not included.

2. Atrial fibrillation

Atrial fibrillation (AF) is an arrhythmia of the heart characterised by rapid, irregular atrial contractions and irregular ventricular beating (Holmes *et al.* 1969).

The rhythm of the heart is normally controlled by the sinus node which generates impulses that depolarise cardiac cells and lead to atrial contraction (Godtfredsen 1999; van Loon 2001; Nattel 2002). AF is initiated by irregular electrical impulses caused by spontaneously firing atrial ectopic foci occurring in atrial tissue within the pulmonary veins, by a single re-entry circuit or by multiple functional re-entrant circuits (Allessie *et al.* 2001; Nattel 2002). Multiple-circuit re-entry is considered the dominant conceptual model for AF (Nattel 2002). The hypothesis postulates that the persistence of AF is dependent on the number of wavelets present in the atria. If the number of wavelets is high the probability of all of them dying out at the same time is small and therefore AF

is maintained. However if there is only a small amount of wavelets present, they will quickly die out and AF will self terminate (Wijffels *et al.* 1995). The number of wavelets depends on the surface area of the atria and the length of the atrial impulse (Wijffels *et al.* 1995). The wavelength of atrial refractoriness is the product of conduction velocity and refractory period (Wijffels *et al.* 1995). Impulses spreading into the atrial myocardium can initiate re-entering wavelets if the wavelength is sufficiently short (Allessie *et al.* 2001). The refractory period of the atrial myocardial cells is considerably shortened in AF at the same time as the atria dilate as a result of loss of contractility. This allows more wavelets to coexist in the atria leading to a maintaining of AF (Wijffels *et al.* 1995; Allessie *et al.* 2001; Allessie *et al.* 2002). This has lead to the term "AF begets AF" (Wijffels *et al.* 1995). The longer AF persists the harder it is to convert to sinus rhythm and prevent recurrence (Allessie *et al.* 2001).

2.1 Atrial fibrillation in horses

AF was first reported in horses in 1911 by Lewis (Holmes *et al.* 1969). Horses have been reported to be more prone to AF because of their high vagal tone causing the refractory period to shorten (Else & Holmes 1971; Blissitt 1999).

The prevalence of AF in horses ranges from 0.34 % to 2.5 % (Holmes *et al.* 1969; Else & Holmes 1971; Deem & Fregin 1982). Out of 18,963 horses admitted to the University of Pennsylvania the prevalence of Standardbreds with AF was significantly higher than other races (Deem & Fregin 1982). However other studies have shown that larger breeds such as draught horses are more disposed to AF because of their large hearts and atria (Holmes *et al.* 1969; Else & Holmes 1971; Godtfredsen 1999; van Loon *et al.* 2000).

AF is diagnosed by auscultation and most importantly electrocardiography (ECG) (Holmes *et al.* 1969; Dukes-McEwan 2002). The ECG findings will show an absence of p-waves which have been replaced by f-waves of varying amplitude, contour and spacing (see figure 2.1). Additionally the R-R intervals will be irregular, while the QRS complex will have a normal conformation (Holmes *et al.* 1969; Kubo *et al.* 1975; Dukes-McEwan 2002). On auscultation the heart beat is irregular and heart sounds vary in intensity (Holmes *et al.* 1969; Deem & Fregin 1982; Manohar & Smetzer 1992).

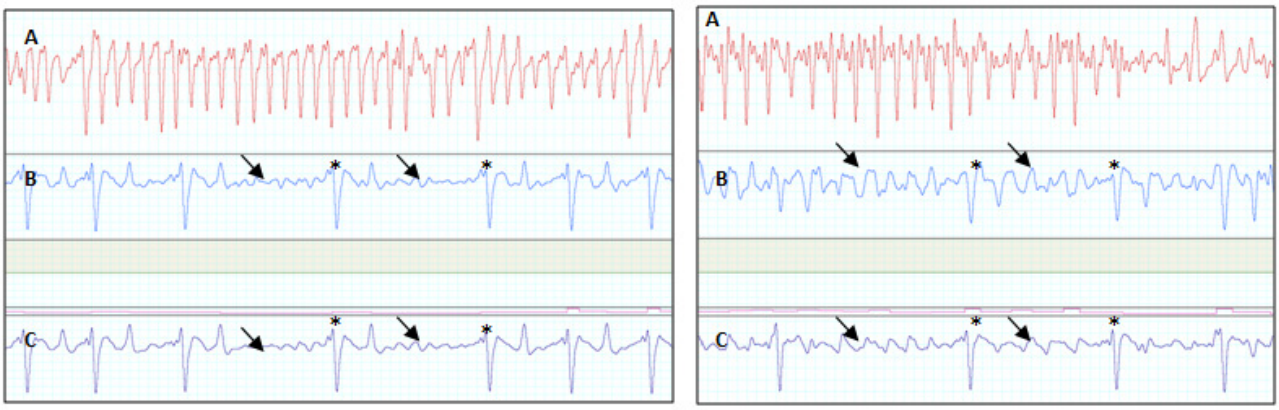

Figure 2.1.

ECG findings in a horse with naturally occurring AF to the left and in a horse with induced AF to the right. The black arrows indicate f-waves. **A** shows the intra atrial electrogram, **B** shows the p-wave lead and **C** shows the standard base-apex lead. Note the irregular interval between QRS complexes (*) in both horses.

Once AF is established, it can lead to electrical and physical remodelling of the heart (Dukes-McEwan 2002; De Clercq *et al.* 2008a). Echocardiography of seven horses with AF sustaining more than four months showed that both atria as well as the left ventricle were dilated (Kovac *et al.* 2003). Similar results were found in a study where 42 horses seven weeks after undergoing cardioversion still showed signs of atrial contractile dysfunction (Decloedt *et al.* 2013).

AF can occur as a result of an underlying cardiac disease, such as mitral or tricuspid regurgitation and cardiomyopathy which can lead to atrial enlargement. Premature beats are often observed in enlarged atria and these can be a predisposing factor in the onset of AF with a properly timed atrial premature beat (Else & Holmes 1971; Manohar & Smetzer 1992). AF without underlying structural cardiac disease has been observed in both humans and horses and is also known as lone AF (Dukes-McEwan 2002). One study claims that cardiac disease in many horses with AF is minimal (Deem & Fregin 1982).

The most frequent symptom of AF in horses is exercise intolerance or reduced performance (Holmes *et al.* 1969; Deem & Fregin 1982; Reef *et al.* 1988; Stewart *et al.* 1990; Reef *et al.* 1995; Blissitt 1999; McGurrin *et al.* 2005). Other signs of AF are exercise induced epistaxis, dyspnea and hyperpnea (Deem & Fregin 1982). However AF has also been diagnosed in horses as an incidental finding without any history of reduced performance or signs of cardiac disease (Holmes *et al.* 1969; Deem & Fregin 1982; Reef *et al.* 1988). In a study of 106 horses with AF, the horses that did not show any clinical signs were used for suboptimal exercise, which indicates that the extent of

exercise intolerance depends on the type of exercise the horse is asked to perform (Deem & Fregin 1982).

The treatment of choice for AF in horses is quinidine sulphate, administered by nasogastric tube (Deem & Fregin 1982; Blissitt 1999). The success rate of quinidine is > 80%, but there are many adverse effects and the drug cannot be administered in horses with congestive heart failure (Deem & Fregin 1982; Reef *et al.* 1988; Reef *et al.* 1995).

Because of this other methods of cardioversion have been sought. Transvenous electrical cardioversion (TVEC) have shown to be an effective and safe procedure for restoring sinus rhythm in horses without underlying cardiac disease (McGurrin *et al.* 2005; De Clercq *et al.* 2008b). However, the procedure can only be performed in full anaesthesia which makes it more costly and complicated compared to oral treatment of quinidine. In addition, TVEC is not without risk as one report of complete atrioventricular block has been reported causing the authors to recommend that a ventricular temporary pacing is provided (van Loon *et al.* 2005).

TVEC is not yet used for cardioversion in horses in Denmark (Haugaard *et al.* 2011).

3. Biomarkers

The ideal biomarker for myocardial damage is present in high concentrations in the heart, but not present in other tissues. It has a rapid release after cardiac injury and persists in plasma. It allows the development of accurate, rapid, and sensitive assays (Adams 1999).

3.1 Cardiac troponin I

Cardiac troponin is a part of the contractile apparatus, the troponin-tropomyosin complex, which is located within all kinds of striated muscles but not in smooth muscle (Collinson *et al.* 2001). The troponin-tropomyosin complex regulates muscle contraction by binding of Ca^{2+} to troponin (Zot & Potter 1987; Collinson *et al.* 2001; Sato *et al.* 2004). Troponin is a complex that consists of three subunits; troponin C (cTnC), troponin I (cTnI) and troponin T (cTnT) (Staprans *et al.* 1972; Collinson *et al.* 2001). cTnC is the Ca^{2+}-binding component, cTnI inhibits the actomyosin ATPase and cTnT binds to tropomyosin and attaches the troponin complex to the thin filament (Zot & Potter 1987). cTnC has two isoforms (fast and slow skeletal), cTnI has three isoforms (one for fast skeletal muscle, one for slow skeletal muscle and one for cardiac muscle) and cTnT has multiple isoforms (Collinson *et al.* 2001). cTnT and cTnI are suitable as serodiagnostic markers for myocardial injury

since the heart and the extracardial muscles express distinct isoforms, whereas cTnC is of little value because of its extracardiac expression in slow skeletal muscle fibres (Undhad *et al.* 2012).
cTnT is expressed in small amounts in skeletal muscle during fetal development, and a re-expression of the fetal form can occur in skeletal muscle during injury (Mair 1997; Babuin & Jaffe 2005). Studies have shown that cTnT might be elevated in patients receiving dialysis for renal failure or myopathies whereas cTnI remains low. This has lead to cTnI being preferred to detect myocardial ischemia in these cases (Mair 1997; Babuin & Jaffe 2005; Undhad *et al.* 2012).
The majority of cTnI is found in the contractile apparatus and is released as a result of proteolytic degradation (Collinson *et al.* 2001). Five to seven percent of cTnI is present in the cytoplasm while the rest is complexed to the contractile apparatus. The amount in the cytoplasm is therefore released rapidly which secures an early diagnostic sensitivity while a continuous release of the remaining cTnI from the contractile apparatus results in a prolonged diagnostic window (Adams 1999). In patients with myocardial infarction increased levels of cTnI were seen until about seven days after infarction, which suggest an ongoing degradation of myofibrils (Cummins *et al.* 1987a). Cardiac biomarkers such as cTnI can occur from a continuum from ischemia to infarction (Turer *et al.* 2011). An increased demand for atrial oxygen during AF leads to a supply-demand mismatch which is comparable to mild to moderate ischemia; which explains why an increase in cTnI is frequently observed in patients with acute AF (Parwani *et al.* 2013; van Bragt *et al.* 2014).
In healthy humans the cTnI-level is zero or so low that it cannot be detected (Collinson *et al.* 2001). Levels of cTnI are elevated 4-6 hours after myocardial damage and peak at around 15-24 hours (Cummins *et al.* 1987a).

Adams *et al.* (1993b) found cTnI to be a highly sensitive and specific biomarker for myocardial injury. They stated that despite severe acute and/or chronic muscle injury an elevation in cTnI did not occur unless there was simultaneous cardiac injury. It has further been found that varying levels of cTnI reflect the extent of myocardial necrosis (Cummins *et al.* 1987a).

3.2 Cardiac troponin I in horses

Because of nucleotide and amino acid sequence similarity between equines and other mammals, commercial cTnI analyzers can be used to detect equine cTnI (Rishniw & Simpson 2005).
The cTnI-level in healthy horses is generally low, < 0.12 ng/mL (Phillips *et al.* 2003; Begg *et al.* 2006; Nostell & Haggstrom 2008; Slack *et al.* 2012). No difference was seen between the cTnI

levels of pastured and race-training thoroughbreds. Nor was there any indication of an increase as a result of age, use and intense training (Phillips *et al.* 2003). The same was found by Slack *et al.* (2012) who also found that there was no increase in cTnI-levels after racing in winning horses. In a study by Nostell & Haggstrom (2008) some horses had mildly elevated cTnI 1-14 hours after racing. Similar results were found in a study where the horses underwent a short-duration, intense exercise on a treadmill. Plasma cTnI concentrations were not significantly different at any time but all individual horses showed a trend towards an increase at both three and six hours (Durando *et al.* 2006).

Even in horses with mitral regurgitation cTnI is not obviously elevated after exercise, which suggests that mitral regurgitation is not associated with myocardial stress or damage (Trachsel *et al.* 2013).

While cTnI is low in healthy horses both pastured, in training and after exercise; an increase in cTnI is correlated with a wide range of cardiac diseases such as dilated cardiomyopathy, endocardiosis, endocarditis and congestive heart failure (Serra *et al.* 2010).

This is confirmed by a study in which cTnI was measured in several horses admitted at the University of Melbourne with signs of cardiac disease. The study found a significant difference in the cTnI-levels in horses with myocardial disease when compared to other horses, both the control group but also horses with cardiac structural lesions or lone arrhythmias. Horses with structural lesions also had a significant higher level of cTnI than healthy horses (Nath *et al.* 2012a).

In a study by Kraus *et al.* (2010) six healthy horses were treated with monensin. Before treatment the horses were healthy and all had a cTnI level of < 0.05 ng/mL. After treatment the cTnI concentration increased and remained elevated at least until the heart rate returned to pre-treatment value. Three of the six horses underwent necropsy and in all of them myocyte necrosis was detected.

Elevated cTnI has also been seen associated with snakebites, abdominal disease, endotoxaemia, atypical myopathy, myocardial contusion and aortic jet lesion (Cornelisse *et al.* 2000; Nostell *et al.* 2012; Nath *et al.* 2012b; Verheyen *et al.* 2012; Gilliam *et al.* 2012; Peters *et al.* 2013).

There has not been found any significant correlation between elevated cTnI and survival in horses (Nath *et al.* 2012a; Verheyen *et al.* 2012). A single measurement of cTnI is not sufficient as a prognostic tool, however serial measurements of cTnI can indicate whether the horse is improving or worsening (Nath *et al.* 2012a).

A study has found that cTnI after transvenous electrical cardioversion was elevated in 7 of 16 horses and the maximum post cardioversion value differed significantly from the pre-cardioversion value. There was no difference in the number of shocks received by horses with increased cTnI and the number of shocks received by horses with cTnI within the reference value. However, the increase observed in the seven horses was so small that the authors questioned whether it was clinically important or not. Had there been myocardial necrosis cTnI would be expected to be significantly higher (Jesty *et al.* 2009). In humans however, cTnI and cTnT were unaffected by electrical cardioversion indicating that no myocardial damage had occurred (Vikenes *et al.* 2000).

3.3 Creatine kinase fraction MB

Creatine kinase (CK) is a tissue enzyme consisting of three isoenzymes, CK-BB, CK-MM and CK-MB (Van der Veen & Willebrands 1966; Adams *et al.* 1993a). CK-BB is mostly found in brain tissue, CK-MM in striated muscle and CK-MB in the cardiac muscle (Van der Veen & Willebrands 1966; Adams *et al.* 1993a). CK-MB is located exclusively in the cytoplasm and is therefore released rapidly after myocardial cell death, but also persists for a short time (Adams 1999). CK-MB levels are elevated following myocardial ischemia and are related quantitatively to release from injured cells (Sobel & Shell 1972). CK-MB has been the mainstay for diagnosis of acute myocardial infarction in humans for nearly 20 years and was considered the test of choice in the 1980's, because it is released in high amounts from the myocytes after cell death (Dolci & Panteghini 2006). A patient with a rising and falling pattern of CK-MB and a peak level above the upper reference range is considered to have myocardial cell death of any cause (Adams *et al.* 1993a).

However CK-MB is produced in skeletal muscle during fetal development causing CK-MB to be reproduced in adult skeletal muscle after injury (Adams 1999; Rosalki *et al.* 2004). This was proven by Cummins *et al.* (1987b) who measured both CK-MB and cTnI in 11 marathon runners. CK-MB was elevated in all runners' post-race samples while cTnI was within normal reference range in both the pre- and post-race samples. This led to the conclusion that the source of elevated CK-MB was of skeletal origin.

18 % of patients who received electrical cardioversion for AF were found to have slightly elevated CK-MB 18-24 hours after the procedure; however cTnI was unaffected (Vikenes *et al.* 2000). Along with the results from Cummins *et al.* (1987b) this further supports the theory that CK-MB is not purely of cardiac origin.

CK-MB can be measured in serum or plasma and can be detected 6-10 hours after myocardial injury and peaks at around 24 hours while returning to normal within 36-72 hours (Adams *et al.* 1993a; Adams 1999). The reference limit of CK-MB is < 5 ng/mL (Vikenes *et al.* 2000). In patients with myocardial infarction the sensitivity of CK-MB measurements are highest around 9-12 hours after the onset of pain (Panteghini *et al.* 1999). Samples should be obtained every 12 hours to secure adequate sensitivity (Adams *et al.* 1993a).

3.4 Creatine kinase fraction MB in horses

In the horse CK occurs in highest activity in the skeletal and cardiac muscle (Thornton & Lohni 1979; Boyd 1983). The proportion of CK-MB in the heart of horses is an area of discussion, as a study has shown that it is accountable for only 3.9% of the activity in the heart, while another study states that CK-MB makes up 30-40% of the total CK in cardiac muscle (Anderson 1976; Argiroudis *et al.* 1982). Contrary to humans no elevation in CK-MB was seen in horses after exercise (Anderson 1976; Cummins *et al.* 1987b).

The reference level of CK-MB in horses is 160-300 U/L (Diana *et al.* 2007). In healthy neonatal foals CK-MB levels have been reported to be 0.4 – 9.3 ng/mL (Slack *et al.* 2005).

In a horse with an aortic jet lesion CK-MB was undetectable in samples taken at admission while levels of cTnI were elevated (Cornelisse *et al.* 2000). However increased levels of both CK-MB and cTnI were reported in a horse with multiform cardiac arrhythmias and multifocal myocardial cell damage caused by piroplasmosis (Diana *et al.* 2007). A significant rise in CK-MB has also been reported in septic neonatal foals (Slack *et al.* 2005). It would appear that measurement of CK-MB is an insensitive marker for detection of myocardial damage and therefore of little diagnostic value in the evaluation of heart diseases in horses (Argiroudis *et al.* 1982; Cornelisse *et al.* 2000).

4. Induced atrial fibrillation

Electrical impulses from the sinoatrial node cause the myocardial cells to depolarise. This can also be obtained by artificial stimulation of the cardiac cells with electrical impulses, called pacing (van Loon 2001).

It is possible to induce atrial fibrillation in the healthy heart by either stimulating the atria with electrical pacing, or by applying acetylcholine to the area of the sinus node (Scherf *et al.* 1950).

The most widely used technique for atrial pacing is transvenous endocardial pacing where a lead or catheter electrode is placed through the vein into the atria (van Loon 2001).

The heart can be paced either by incremental pacing or extrastimulus pacing. With incremental pacing the heart is stimulated in a constant rhythm with a determined rate, whereas extrastimulus pacing introduces one or more premature impulses (van Loon 2001). These extra impulses will result in an extrasystolic contraction and can be used to measure the atrial refractory period or if timed correctly induce atrial fibrillation (Senta & Kubo 1978; Wijffels *et al.* 1995; van Loon 2001).
Every electrical stimulus of the atria triggers an extrasystolic p-wave. However, if the stimulus is applied shortly after one another the atria will not respond to the stimulus (Senta & Kubo 1978). The failure of the stimulus to provoke atrial contraction if applied shortly after one another is explained by the myocardial cells being stimulated in the refractory period and thus being unable to spike a new action potential (Senta & Kubo 1978).

Atrial fibrillation was induced in seven horses by inserting a catheter electrode to the right atrium via the jugular vein. The atrium was then stimulated repeatedly for 15 seconds and AF was triggered (Kubo *et al.* 1975). The same method was successfully used by Senta & Kubo (1978) to induce AF in eight horses.
Several studies have been successful in inducing chronic AF by implanting a pacemaker or suturing electrodes to the atrial wall (Wijffels *et al.* 1995; van Loon *et al.* 2000; Loon *et al.* 2003). In one study a pacemaker was implanted in the right atrium of four ponies. The pacemaker detected sinus rhythm and automatically delivered two second burst stimuli which induced AF. After five days of repetitive AF induction the duration of AF increased (Van Loon *et al.* 2002). Similar results were found in another study by van Loon *et al.* (2000) where a progression in AF duration was seen during the study leading to a sustained AF for more than 24 hours.

In a study on human patients it was impossible to induce AF in patients without any prior history of AF or atrial flutter by using burstpacing for 10 seconds. Conversely AF was successfully induced in 95 % of patients who previously had experienced AF or atrial flutter, and also in 57 % of patients at risk, i.e. patients with structural atrial disease (Brignole *et al.* 1986).

In humans no complications have been observed in procedures where AF was induced by pacing of the atria and the pacing and the arrhythmia have been tolerated well (Brignole *et al.* 1986). The same observation has been made in horses where the procedure can be performed without any sedation and without any adverse reactions by the horse (Kubo *et al.* 1975; van Loon 2001). In a

horse with a pacemaker implanted no behavioural reactions were seen during the pacing of the atria (Van Loon *et al.* 2002). However a release of cTnT has been reported in human patients after pacing induced stress to the myocardium. The increase was below the detection of a conventional assay but could be measured by a high sensitive cTnT assay (Turer *et al.* 2011).

5. Ranolazine

Ranolazine is a medicine originally approved by the US Food and Drug Administration (FDA) for treatment of stable angina pectoris (Pelliccia *et al.* 2012). Ranolazines mechanism of action is inhibition of the late sodium currents (I_{Na}) during AF (Sossalla *et al.* 2010). By inhibition of the sodium currents ranolazine reduces intracellular calcium overload which is arrhythmogenic (Belardinelli *et al.* 2006). The inhibition of the late I_{Na} delays repolarization and leads to an increase in atrial refractory period (Frommeyer *et al.* 2012; Hawwa & Menon 2013; Milberg *et al.* 2013).

Ranolazine has shown potential as treatment for atrial fibrillation (Frommeyer *et al.* 2012; Hawwa & Menon 2013; Milberg *et al.* 2013). In a study by Frommeyer *et al.* (2012) ranolazine reduced the inducibility of atrial fibrillation as provoked by atrial burst stimulation. Ranolazine also significantly lowered the rate of atrial fibrillation in the normal hearts as well as hearts with dilated atria (Milberg *et al.* 2013). In a small group of patients with resistant AF ranolazine was effective in restoring sinus rhythm in the majority of the patients (Murdock *et al.* 2008).

Ranolazine has also been shown to be cardioprotective by decreasing the incidence of myocardial injury in patients during percutaneous coronary intervention. The pilot study showed that pre-treatment with ranolazine reduced procedural injury significantly. Furthermore there was a tendency of lower levels of CK-MB and cTnI in the ranolazine treated group than in the placebo group with a p-value of 0.192 and 0.223 respectively (Pelliccia *et al.* 2012). Ranolazine has shown few adverse effects and no signs of organ toxicity or pro-arrhythmic effect (Jerling 2006; Frommeyer *et al.* 2012).

6. Dofetilide

Dofetilide is a class III anti-arrhythmic drug which prolongs the repolarisation of the myocardium by selectively blocking the time dependent K^+ current (Gwilt *et al.* 1991; Benson & Powless 2003; Elming *et al.* 2003) . Thereby dofetilide lengthens the refractory period of the myocardium without depressing the conduction velocity (Gwilt *et al.* 1991). Clinical trials have shown that dofetilide is

effective in restoring and maintaining sinus rhythm in patients with chronic atrial fibrillation (Elming *et al.* 2003). However dofetilide can induce pro-arrhythmia known as torsades de pointes (TdP) (Sedgwick *et al.* 1995; Benson & Powless 2003; Elming *et al.* 2003). The risk of TdP is dose-dependent alas so is the efficacy (Elming *et al.* 2003). Because of the risk of developing TdP the FDA requires that all practitioners in the U.S. complete an education programme before dofetilide is prescribed. Dofetilide is not approved for use in Europe (Elming *et al.* 2003).

Concurrent use of ranolazine and dofetilide to control AF has shown to be efficacious and safe (Shah *et al.* 2014).

7. Materials and methods

7.1 Horses:

A total of nine horses were used in this study. Six were mares and three geldings. All of the horses were Standardbreds between the age of 5 and 14 years. The study was divided into three groups each with three horses. The first group was a safety trial where the safety of administration of the drugs to horses was evaluated, seeing as neither ranolazine nor dofetilide have ever been administered to horses before. The two other groups underwent a standing electrophysiologic procedure. In all three groups each horse was used for four procedures. One procedure for each administered drug, ranolazine, dofetilide, ranolazine-dofetilide combination and placebo, see table 7.1. The procedures were performed with one week intervals.

Table 7.1

This table shows the distribution of drug administration over the four procedures. For the electrophysiologic group the combination procedure was placed last for convenience. The rest of the administration is randomly distributed.

	Procedure 1	Procedure 2	Procedure 3	Procedure 4
Horse 1	Dofetilide	Ranolazine	Combination	Placebo
Horse 2	Combination	Dofetilide	Ranolazine	Placebo
Horse 3	Placebo	Dofetilide	Combination	Ranolazine
Horse 4	Dofetilide	Ranolazine	Placebo	Combination
Horse 5	Dofetilide	Ranolazine	Placebo	Combination
Horse 6	Placebo	Dofetilide	Ranolazine	Combination
Horse 7	Placebo	Dofetilide	Ranolazine	Combination
Horse 8	Placebo	Ranolazine	Dofetilide	Combination
Horse 9	Dofetilide	Placebo	Ranolazine	Combination

The horses underwent a full physical examination including haematological and biochemical profile, a 24 hour ECG and echocardiogram. This was done to ensure that none of the horses had any structural cardiac diseases or arrhythmias which could interfere with the study. All horses were considered healthy based on the examinations. The horses furthermore underwent a short physical examination on the day of the procedure to ensure they were healthy and that the clinical parameters were within normal references.

The horses were gradually accustomed to standing in a stock over one to two weeks before starting the procedure.

7.2 Electrophysiologic procedure:

On the day of the procedure the horse was placed in a stock. Horses that were anxious wore an eye mask where the right eye was covered (see figure 7.1).

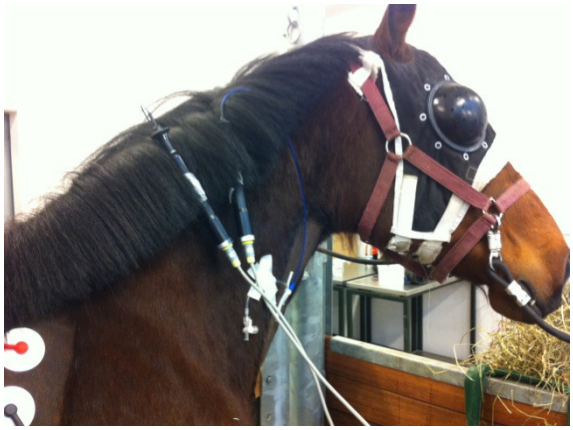

Figure 7.1

An anxious horse wearing an eye mask to prevent it from being spooked by the wires and the electrical equipment

The horse was clipped in a 15 x 15 cm area on the left and right scapula, behind the left elbow and behind the xiphoid to the left of the midline of the abdomen. The clipped areas were washed with mediScrub[1] and wiped with alcohol. The areas were allowed to dry before the ECG holter unit[2] was applied. The negative electrode (red) was placed on the right scapula, the reference electrode (black) was placed on the left scapula, the two positive electrodes (yellow and green) were placed behind the left elbow and left to the midline of the abdomen respectively (see figure 7.2). The ECG holter unit provided two leads. One lead between the red and yellow electrode, and one lead

[1] Medi-scrub, Rovers Medical Devices B.V. Leekstraat 10 – 5347 KV Oss - Netherlands
[2] Televet 100 ECG, Kruuse A/S, Havretoften 4, 5550 Langeskov

between the red and green electrode. The ECG holter unit remained on for 24 hours after the induction of atrial fibrillation.

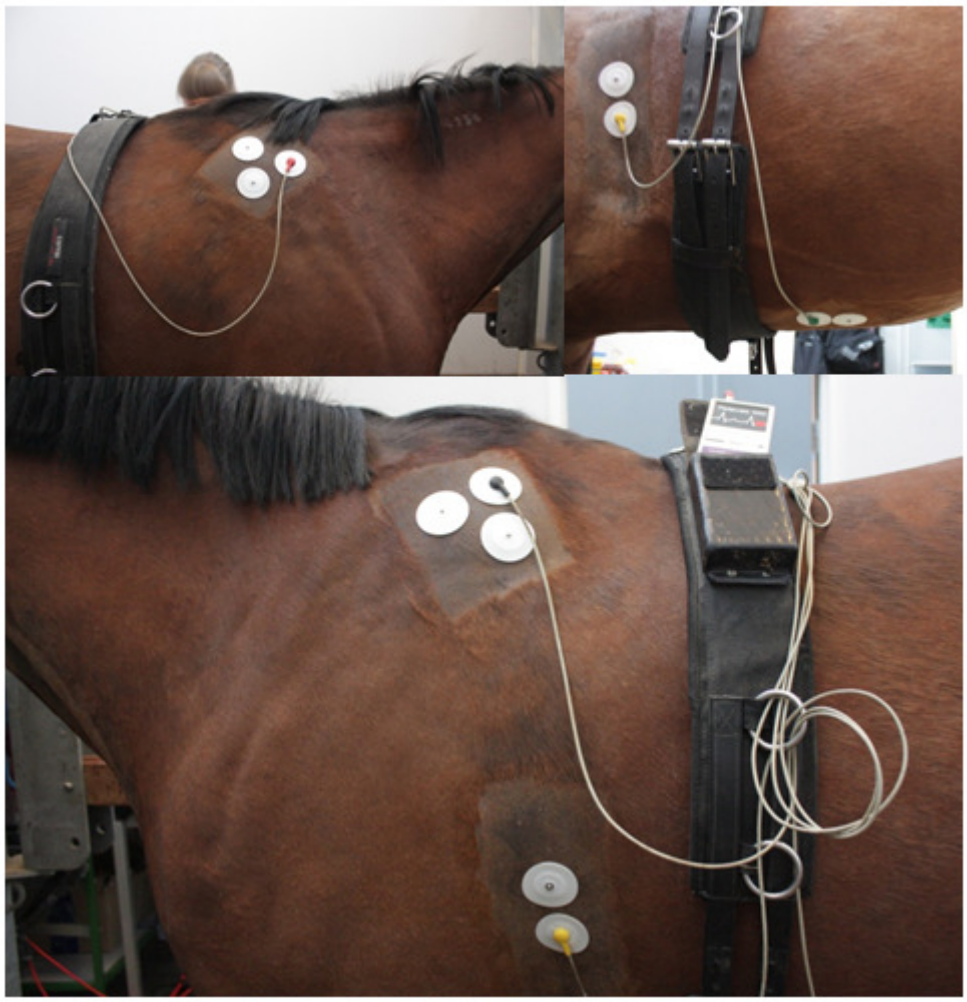

Figure 7.2

The ECG holter unit applied to the horse. The reference electrode (black) on the left shoulder, the negative electrode (red) on the right shoulder and the two positive electrode (yellow and green) behind the elbow and ventral on the abdomen.

An additional two ECGs were placed (see figure 7.3), those being a standard base-apex lead and a p-wave lead.

The positive electrode of the standard base-apex lead was placed left to the midline just caudal to the xiphoid, while the negative electrode was placed dorsally on the right scapula. The reference electrode was placed dorsally on the left scapula.

The positive electrode of the p-wave lead was placed behind the left elbow at the girth region, the negative electrode was placed dorsally on the right scapula and the reference electrode was placed dorsally on the left scapula.

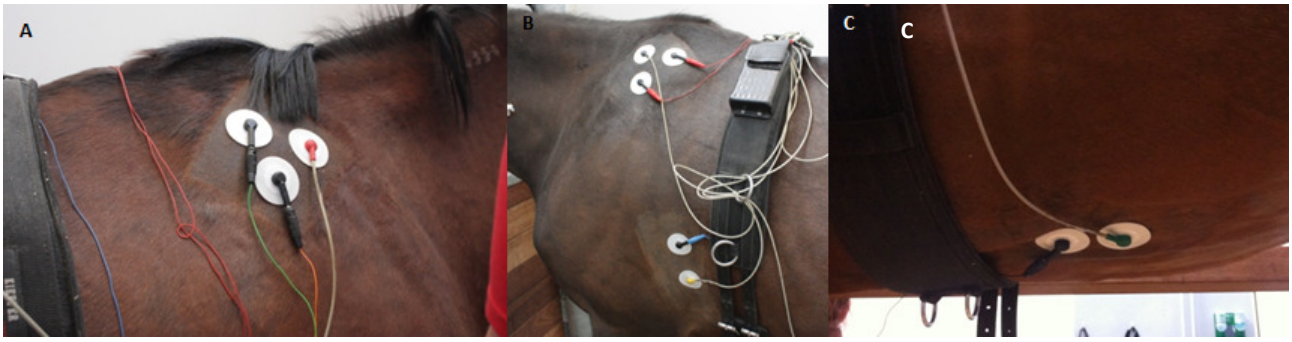

Figure 7.3

The two additional ECG leads applied to the horse. The first picture (A) shows the negative electrode of the base-apex lead (green wire) and the negative electrode of the p-wave lead (orange wire). The second picture (B) shows the two reference electrodes for standard base apex lead and the p-wave lead (red wires) as well as the positive lead of the p-wave lead (blue wire). Picture three (C) shows the positive electrode of the standard base apex lead.

An area over the right jugular vein of approximately 15 x 40 cm was clipped and aseptically prepared. Two local analgesics were placed 10 - 15 cm from each other with approximately 3 mL of mepivacain (Carbocain®[3]) injected subcutaneously at each site. Afterwards two 12 G catheters were placed. A guide wire was inserted through the catheter. When in place the catheter was removed, leaving only the guide wire in the vein. The introducer sheath was then lead over the guide wire and into the vein. If the incision in the skin was too small it would be extended with a scalpel. The guide wire and the stylet were removed and the catheter was rinsed with heparinized saline. The extension to the introducer sheath was attached to the horses with tape or snögg[4]. If it was not possible to place both catheters on the right side of the horse, one would be placed on the left side of the horse. The distance from the introducer sheath to the heart was measured and marked on an 8-polar electrode. The 8-polar electrode was then inserted through the introducer sheath and placed in the right atrium. Once this was done the 8-polar electrode was coupled to the receiver. The electrode was then moved back and forth until the signal of the intracardiac electrogram was simultaneous with the p-wave indicating that the electrode was in the atrium (see figure 7.4).

The electrode was secured by the top of the introducer sheath being tightened. The handle of the 8-polar electrode was taped to the mane of the horse.

[3] Carbocain, AstraZeneca A/S, København S, Danmark
[4] Snøgg, Snøgg Industri AS, P.B. 70, 4671 Mosby, Norge

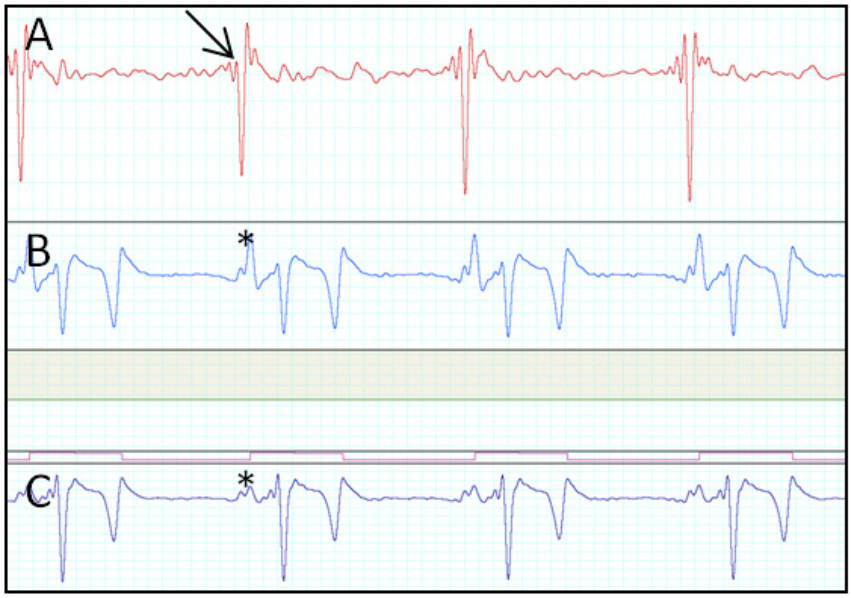

Figure 7.4

Contraction of the atria is reflected as the p-wave (*) on the two ECG leads (B and C) and on the intracardiac electrogram (arrow).

Once both electrodes were successfully placed aERP measurements were performed. These are not described further in this thesis.

After this, atrial fibrillation was induced. Pulse width was set to 2 ms, pacing cycle length was 20 ms and the amplitude was set to 10 mA. Burstpacing was performed for 5-6 seconds. If the horse entered atrial fibrillation no further pacing was performed unless the horse converted back to sinus rhythm. AF was considered persistent if sustained for more than 15 minutes. If this was not the case the tachypacing protocol was repeated. If the horse had not entered sustained AF after 45 tachypacings no further attempts were made and the procedure was terminated.

During each procedure the drug was administered 4 times at 20 minutes interval. Each administration was a higher dose leading up to the horse receiving a total of 0.2 mL/kg for NaCl, 8 µg/kg for dofetilide and 2.4 mg/kg for ranolazine.

If the horse converted to sinus rhythm after the administration of the anti-arrhythmics aERP measurements and tachypacings were repeated. Thus meaning a horse could receive up to 90 tachypacings during one procedure.

The procedure was terminated if the horse still was in AF 30 minutes after last drug administration or tachypacing or if the aERP measurements and tachypacing protocol were completed.

Six horses developed a fever shortly after the end of one or more of the procedures. Some of these horses were treated with 20.000 ie penicillin procain (Penovet®[5]) once or twice a day for three days depending on the horses condition.

7.3 Safety trial:

A safety trial was conducted first, seeing as the drugs dofetilide and ranolazine never had been used in horses previous to this study.

The horse was placed in a stock and an ECG holter unit was placed as described earlier. Two catheters were placed, also described earlier, one on the right side and one on the left side. The right catheter was used for drug infusion while the left catheter was used for blood sampling.

Blood pressure was measured invasive and non-invasive. For the invasive blood pressure the horses were clipped and aseptically prepared in the area over the transverse facial artery. A local anaesthetic gel (Tapin®[6]) was applied in the area and covered with tape about an hour before placement of the catheter. A 20 G catheter was placed in the artery and glued to the skin. The catheter was flushed with heparinized saline and further secured with snögg. The catheter was connected to an electronic pressure transducer[7].

For the non-invasive blood pressure measurement a cuff was placed around the base of the tail. The cuff was also connected to the electronic pressure transducer.

Drugs were infused twice at 20 minutes interval. The second dose was higher than the first dose. The total amount of drug infused is mentioned in the section on the electrophysiologic procedure. Blood samples were drawn from a catheter prior to drug infusion and then four and 24 hours after the second drug infusion. A complete description of blood sampling is made in the following section.

7.4 Blood sampling:

The area over the left jugular vein was clipped and aseptically prepared. Approximately 3 mL of mepivacain (Carbocain ®) was injected subcutaneously over the jugular vein. A 12 G catheter was placed and sutured to the skin with a 2-0 nylon suture. This catheter was only used for blood sampling.

Blood samples were taken prior to electrical pacing, four and 24 hours post induced sustained AF in the electrophysiologic group and before drug infusion, 4 and 24 hours after the last drug infusion in

[5] Penovet Vet, Boehringer Ingelheim Danmark A/S, 2100 København Ø
[6] Tapin 2.5 %, Copyfarm A/S, Energivej 15, 5260 Odense
[7] GE Healthcare A/S, Park Allé 295, 2605 Brøndby

the safety group. Before each blood sample 10 mL of blood was drawn and discharged to ensure that no remnants of old blood or saline was present. Another 10 mL of blood was then taken via the catheter with a syringe and transferred to a lithium heparin tube. The blood samples were put in a bucket of ice for a maximum of seven hours. The blood samples were then centrifuged at 43 RPM for ten minutes. 3 mL of plasma was transferred to cryovials (see figure 7.5) and frozen at -80° C until further analysis.

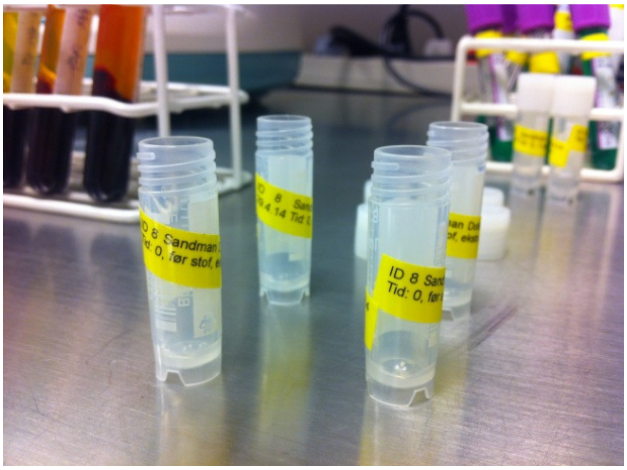

Figure 7.5

Cryovials were labelled with horse ID, horse name, date, procedure and blood sample ID.

7.5 Blood analysis

Cardiac troponin I was tested by a sandwich immuno-analysis using direct chemiluminometric technology. It is a quantitive assay and there is a direct correlation between the amount of cTnI in the sample and the amount of relative light units the system detects. The assay has a detection limit of 0.006 ng/mL.

The CK-MB analysis is a two centre sandwich immune-analysis, which uses direct chemiluminometric technology with constant amounts of two antibodies. The two antibodies are monoclonal mouse anti-CK-MB-antibody and monoclonal mouse anti-CK-BB-antibody. The assay has a detection limit of 0.18 ng/mL.

7.6 Statistical analysis

All data was transferred to an excel document. The statistical analyses were made in R®[8].

A Chi-Square Test was used to estimate the relationship between pacing and levels over reference point of cTnI and CK-MB.

[8] R studio, 250 Northern Avenue, Suite 420, Boston, Massachusetts 02210, USA

Values of cTnI and CK-MB from the same group (e.g. safety group) were compared using a paired t-test, while values between the safety and electrophysiologic group were compared using students t-test. If the values were not normally distributed Wilcoxon signed rank test was applied.

The relationship between the biomarker values from each drug administration would normally be compared using analysis of variance (ANOVA). As the data did not follow normal distribution Kruskal-Wallis test was used instead. Both tests were calculated for all time points for both cTnI and CK-MB.

The association between biomarkers and number of pacings, number of procedures and amount of time in AF respectively was calculated using linear regression. For all statistical analysis a p-value < 0.05 was considered significant.

8. Results

The horses in the study had a mean age of 8.9 ± 3.2 years and a weight of 493.2 ± 35 kg. The horses in the electrophysiology procedure underwent tachypacing 25.8 ± 26.3 times and were in sustained AF for 94.5 ± 67.6 minutes.

In this section only means and standard deviations as well as statistical results will be mentioned, but all data on the horses as well as a table of all values of cTnI and CK-MB can be found in the appendix.

Mean values of cTnI and CK-MB for the two groups at the different time points are listed in Table 8.1. The minimum value observed for cTnI was 0.000 ng/mL while the maximum value was 0.227 ng/mL.

For CK-MB the minimum value was 0.59 ng/mL and maximum value was 6.44 ng/mL.

Table 8.1

The mean concentration and standard deviation of cTnI and CK-MB respectively are here shown for all horses and for each of the two groups at 0, 4 and 24 hours.

Time of blood sampling	cTnI ng/mL Mean ± SD	CK-MB ng/mL Mean ± SD
0 h. all horses	0.006 ± 0.027	2.16 ± 1.16
4 h. all horses	0.008 ± 0.038	2.11 ± 0.94
24 h. all horses	0.006 ± 0.030	2.50 ± 1.41
0 h. electro procedure	0.001 ± 0.002	2.42 ± 1.11

4 h. electro procedure	0.002 ± 0.003	2.22 ± 0.78
24 h. electro procedure	0.001 ± 0.002	2.45 ± 1.18
0 h. safety procedure	0.014 ± 0.047	1.66 ± 1.12
4 h. safety procedure	0.020 ± 0.065	1.90 ± 1.22
24 h. safety procedures	0.016 ± 0.051	2.60 ± 1.84

Three blood samples showed cTnI values over the reference interval whereas CK-MB exceeded the upper reference in five samples. To decide if pacing was a contributing factor to elevated cTnI and CK-MB a chi-square test was used. A horse was put in "disease +" if it had a cTnI level > 0.12 ng/mL or a CK-MB level > 5 ng/mL. Horses were considered exposed if they had been tachypaced. The results are shown in figure 8.1 and 8.2.

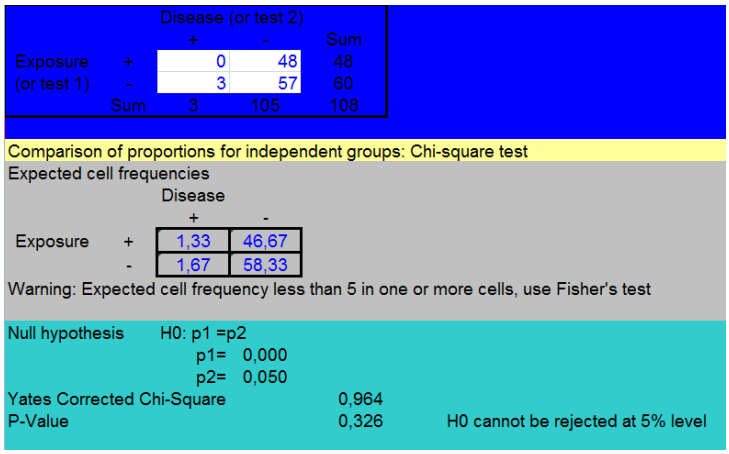

Figure 8.1

The chi-square test results for values of cTnI. Samples taken at 0 hour and samples from the safety procedure were denoted as non-exposed. P-value is 0.33.

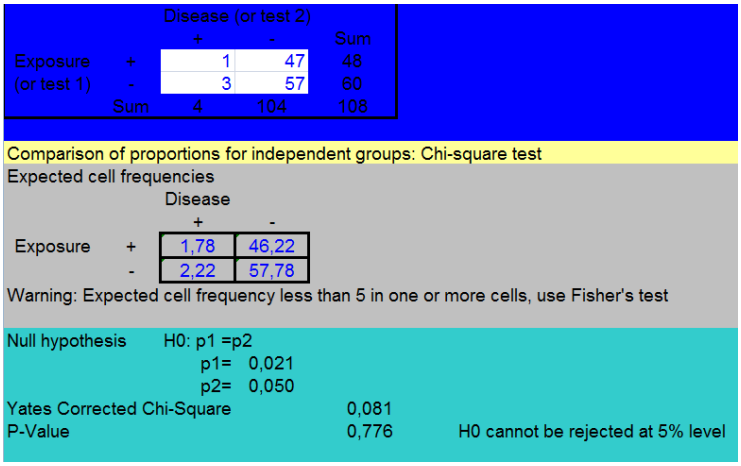

Figure 8.2

The chi-square test results for values of CK-MB. P-value is 0.78.

Levels of cTnI and CK-MB were compared to see if there was any difference from 0 h to 4 and 24 hours, as well as if there was any difference between the electrophysiologic group and the safety group. A t-test was applied for the normal distributed groups, while a Wilcoxon test was used for non-normally distributed data. P-values can be seen in table 8.2.

Table 8.2

This table shows p-values at the different blood sampling points.

Blood sampling time	P-value T-test	P-value Wilcox test
cTnI: 0 h. vs. 4. h. safety	-	0.50
cTnI: 0 h. vs. 24. h. safety	-	0.40
cTnI: 0 h. vs. 4. h. electro	-	0.30
cTnI: 0 h. vs. 24. h. electro	-	0.73
cTnI: 4 h. safety vs. 4 h. electro	-	0.90
cTnI: 24 h. safety vs. 24 h. electro	-	1
CK-MB: 0 h. vs. 4. h. safety	-	0.06
CK-MB: 0 h. vs. 24. h. safety	-	0.04
CK-MB: 0 h. vs. 4. h. electro	0.09	-
CK-MB: 0 h. vs. 24. h. electro	0.88	-
CK-MB: 4 h. safety vs. 4 h. electro	-	0.14
CK-MB: 24 h. safety vs. 24 h. electro	-	0.73

There was generally a decrease in both cTnI and CK-MB at 4 and 24 hours according to the times the horse was tachypaced. However none of these results were significant. The correlation between tachypacing and blood levels of cTnI and CK-MB can be seen in figure 8.3.

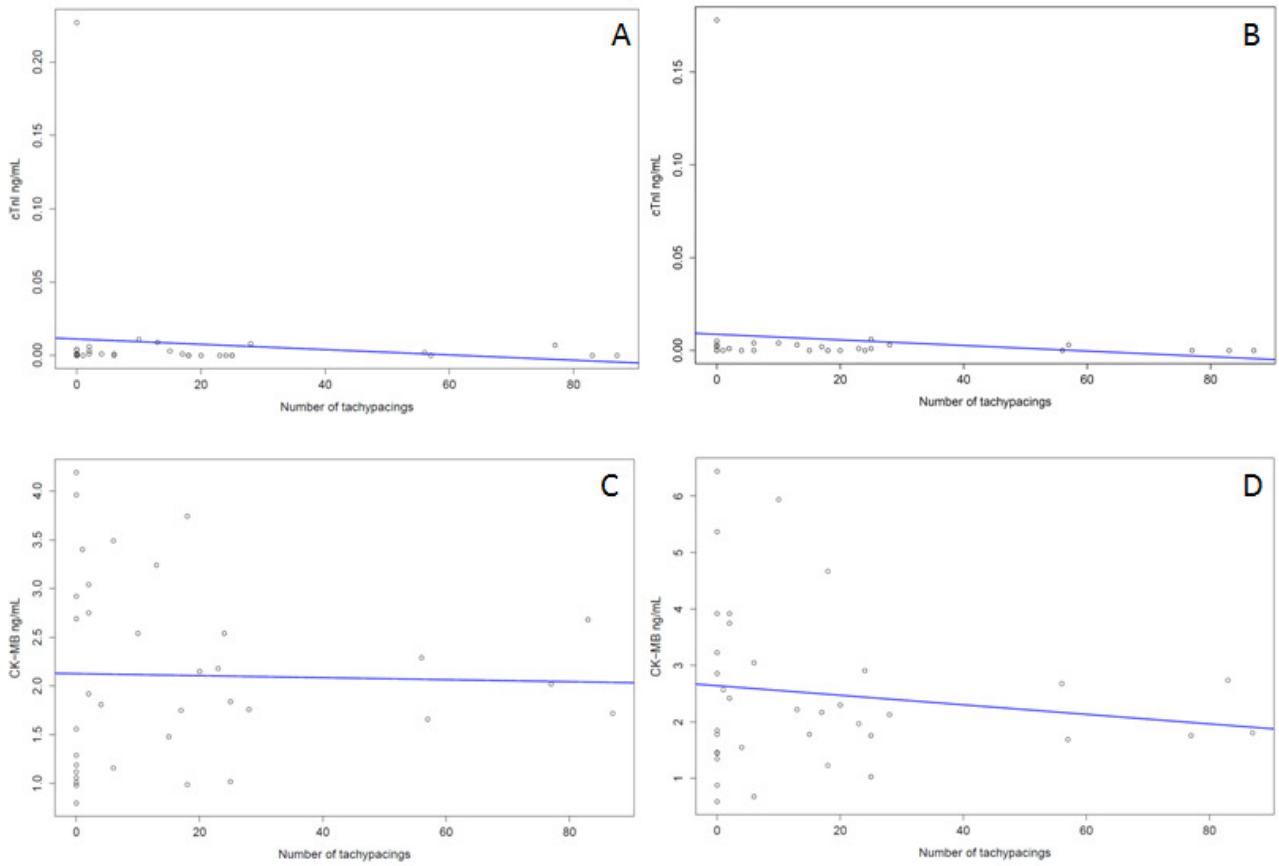

Figure 8.3

The plots reflect the relationship between number of tachypacings and cTnI at 4 hours (A) and 24 hours (B), as well as between tachypacings and CK-MB at 4 hours (C) and 24 hours (D). The linear regression line is reflected in all plots.

The decreases in the biomarkers according to pacing were only minor and at all times insignificant. For cTnI the p-value was 0.49 and 0.47 for 4 hours and 24 hours respectively. For CK-MB it was 0.88 and 0.40 4 hours and 24 hours respectively.

All horses underwent 4 procedures. The correlation between the number of procedures and blood levels of cTnI and CK-MB is shown in figure 8.4.

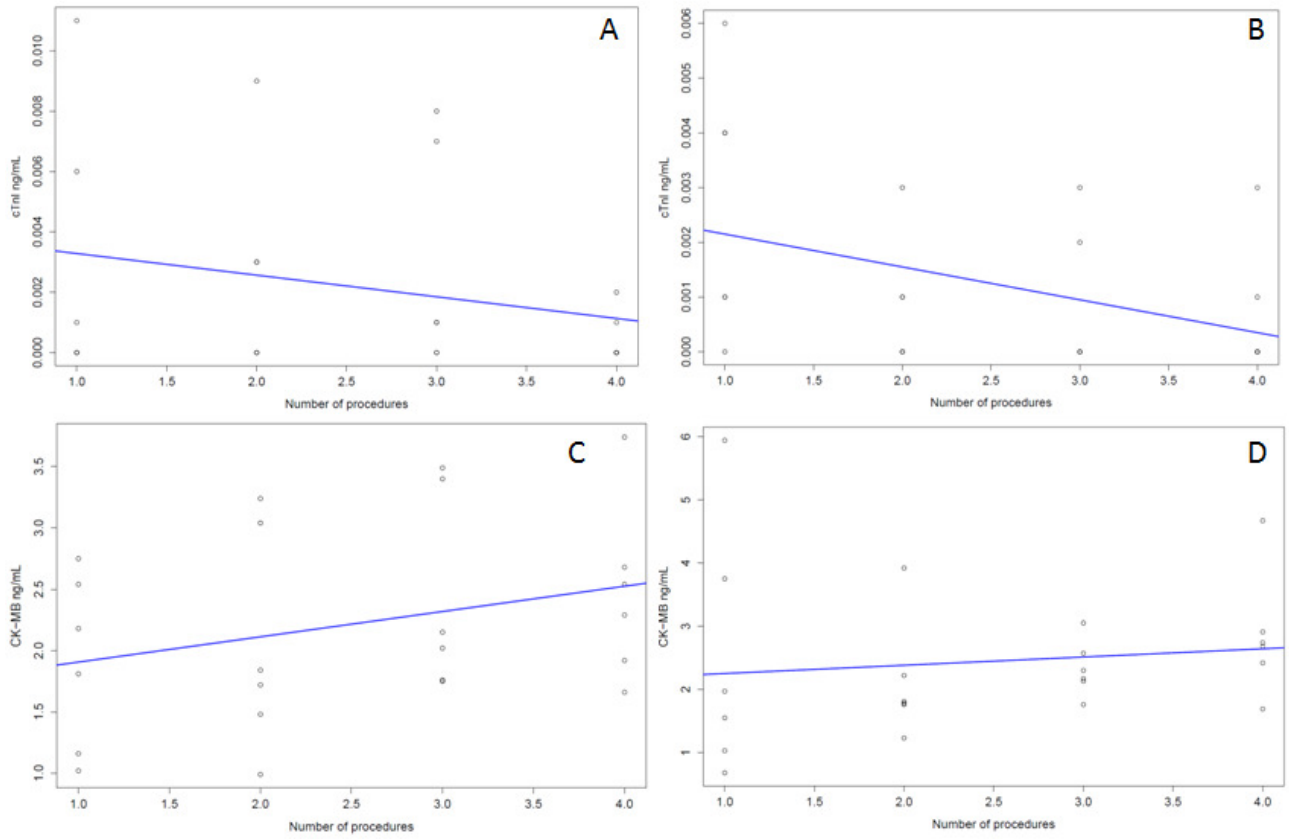

Figure 8.4

The four plots reflects the relationship between number of procedures and levels of cTnI and CK-MB at 4 hours (A,C) and 24 hours (B,D). The blue line is the linear regression line.

For cTnI there is a decrease at both 4 and 24 hours. At 4 hours it is insignificant ($p = 0.25$) where as a significant decrease is seen for cTnI at 24 hours ($p = 0.05$).

This is in contrast to CK-MB where an increase is seen at both 4 and 24 hours, but these are insignificant ($p = 0.15$ and $p = 0.56$).

Even though the decrease for cTnI at 24 hours is significant the decrease is still very minor, i.e. only 0.0006 ng/mL pr. procedure.

The horses were administered two different drugs, a combination of these two as well as a placebo. The mean and standard deviation of each administration is listed in table 8.3.

Table 8.3

Mean values of cTnI and CK-MB for each of the drug administrations. Data includes both the safety and electrophysiologic group.

Drug administration	cTnI ng/mL Mean ± SD	CK-MB ng/mL Mean ± SD
Ranolazine	0.002 ± 0.002	2.12 ± 1.20
Dofetilide	0.016 ± 0.054	2.24 ± 1.03
Combination	0.001 ± 0.001	2.41 ± 1.09
Placebo	0.007 ± 0.031	2.26 ± 1.43

The different drug administrations were calculated using a Kruskal-Wallis test. Normally an analysis of variance (ANOVA) would have been used, but that requires normally distributed data which these results were not. The mean of each drug administration did not differ significantly from each other at any time point for either cTnI or CK-MB. P-values can be seen in table 8.4

Table 8.4

This table shows the p-value for the Kruskal-Wallis test of the four drug administration groups.

	cTnI 4 h	cTnI 24 h	CK-MB 4 h	CK-MB 24 h
P-value	0.32	0.33	0.80	0.79

The association between blood levels of the biomarkers and the number of minutes the horse was in sustained AF is shown in figure 8.5.

During two procedures the ECG holter unit did not record the ECG after the procedure was terminated and the precise amount of time the horse was in sustained AF could not be measured. This happened with two different horses and the samples following those procedures are not included in the calculations.

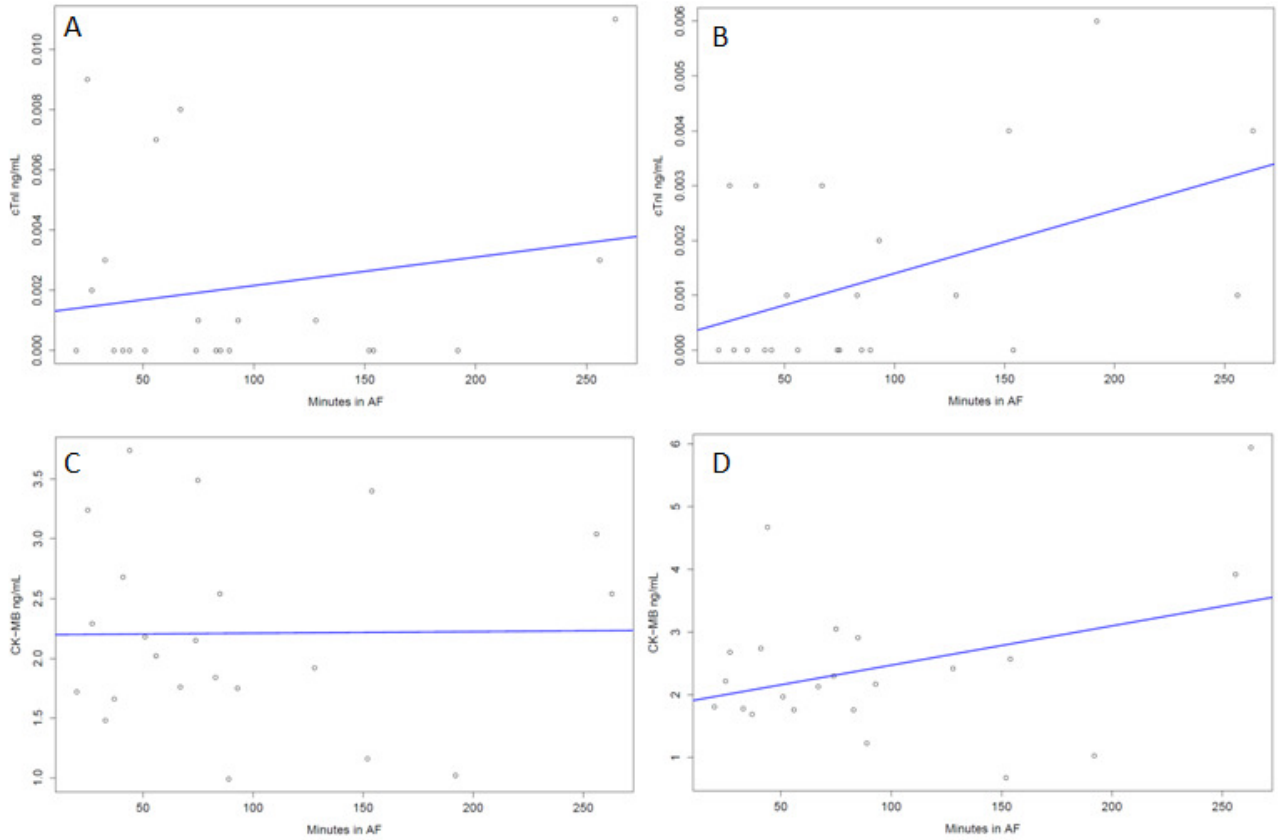

Figure 8.5

The correlation between minutes in AF and levels of cTnI (A,B) and CK-MB (C,D) is shown here. A and C is at 4 hours while B and D is at 24 hours.

For cTnI and CK-MB at both 4 and 24 hours an increase is seen relative to the amount of time the horse was in sustained AF. At 4 hours the increase was insignificant ($p = 0.38$ and $p = 0.96$ for cTnI and CK-MB respectively). But at 24 hours the increase is significant for cTnI ($p = 0.03$) and nearly significant for CK-MB ($p = 0.09$).

9. Discussion

This study showed that it was possible to place electrode catheters in the right atrium, receive capture and induce AF in all horses without any sedation of the horse, and with only few signs of discomfort which disappeared once the electrode was replaced. However there is still the question of whether or not the electric stimuli provided to the atrium results in damage to the myocardium. It has also been reported that acute AF results in mild to moderate ischemia (van Bragt *et al.* 2014), thus leading to two factors that could potentially damage the heart.

The aim of this study was therefore to evaluate the damage inflicted on the heart by the electrical stimulation and the induction of AF as well as the anti-arrhythmics given.

Only three of the samples were above reference value for cTnI (>0.12 ng/mL). All of the elevated samples were obtained from the same horse. The peculiar part is that this particular horse (Horse ID. 2) did not undergo the electrophysiologic procedure as it was a part of the safety study group. Two of the elevated samples were 4 and 24 hours after the administration of dofetilide while the last was the pre sample before administration of placebo. The placebo procedure was two weeks after the dofetilide procedure.

For CK-MB it was hard to evaluate the results since it was not possible to find any reference values for horses. A reference value of 160-300 U/L was reported by (Diana *et al.* 2007) but unfortunately it is not possible to convert this unit to ng/mL which is the unit the samples in this study were measured in. For healthy neonatal foals the values of CK-MB have been reported to be up to 9.3 ng/mL (Slack *et al.* 2005). The reference value of CK-MB in humans is < 5 ng/mL (Vikenes *et al.* 2000) and accordingly I have decided to use 5 ng/mL as the upper reference limit.

Four samples of CK-MB were above 5 ng/mL. The samples came from four different horses. Two of them were from the safety group while the other two were from each of the two groups that underwent the electrophysiologic procedure. Three of the samples were obtained 24 hours after drug administration/induction of AF, however only one of them is from the electrophysiologic procedure. The last sample was a pre procedure sample.

The only horse that had elevated CK-MB and cTnI at the same time was horse ID. 2, 24 hours after the dofetilide procedure.

Horses that were exposed to tachypacing were not more likely to have levels of CK-MB or cTnI over the reference value, than the horses that only received drug administration in the safety trial and the hypothesis was rejected. It was not possible to find any literature mentioning the values of CK-MB and cTnI after atrial pacing which makes it difficult to put these results in perspective. There are results from one human study on levels of cTnT after atrial pacing (Turer *et al.* 2011). Here a significant increase was seen following atrial pacing. In spite of a large relative increase in cTnT it was only patients with chronic coronary artery disease who had levels above the upper normal reference. The study does not mention the amount of energy delivered during the atrial pacing. All the patients in the study by Turer *et al.* (2011) had stable angina. Thus it is difficult to compare these results with results from healthy subjects such as the horses in this study.

Values of cTnI after TVEC are available from studies in both humans and horses (Vikenes *et al.* 2000; Jesty *et al.* 2009). In humans no increase has been observed whereas in horses an increase has been noted in some horses. The maximum of joules delivered in TVEC in a single shock in a horse was 360 J (Jesty *et al.* 2009) which is markedly higher than in this study where the horses were tachypaced with 12 J. In the study on TVEC they delivered up to 9 shocks, while in this study one horse was tachypaced 87 times. The maximal cumulative energy delivered in one horse during the TVEC procedure was 2,200 J. For the horses in this study the maximal cumulative energy based only on tachypacings were 1,044 J. This is still a much lower number and it is therefore not surprising that this study resulted in lower levels of cTnI. Like the results in this thesis, Jesty *et al.* (2009) found no correlation between the amount of shocks delivered or the cumulative energy delivered and the levels of cTnI.

Furthermore the results showed that in the electrophysiologic group the values of CK-MB and cTnI did not differ significantly from the pre-procedure samples at neither 4 nor 24 hours post AF induction. Also, the 4 hour values from the electrophysiologic procedure did not differ from the 4 hour values from the safety procedure. The same applies to the 24 hour samples. This again supports the conclusion that the heart is not significantly affected by pacing; at least not to a degree that causes myocardial necrosis or ischemia.

The only significant difference between the different time marks was for CK-MB when the 0 hour values were compared to the 24 hour values in the safety group. A tendency towards significance ($p = 0.06$) was also seen for CK-MB in the safety group between 0 and 4 hours. Why these values are increased is difficult to explain as these horses did not undergo atrial pacing or induced AF. It could be because of the drug administration; however as will be described later there is no difference in CK-MB levels between the different drug administrations. But seeing as cTnI is not significantly increased at either 4 or 24 hours in the safety group, it is unlikely that the CK-MB increase is related to true myocardial damage. Increase in CK-MB because of muscle damage is seen in humans (Cummins *et al.* 1987b). The increase seen in the safety group could be caused by muscle damage due to lack of blood supply because the horses were unable to move around for several hours. But the horses in the safety procedure spend a considerable shorter amount of time in the stock than the horses in the electrophysiologic procedure. The horses in the electrophysiologic procedure did not have a significantly higher value of CK-MB at 4 and 24 hours and therefore it would seem unlikely that the increase in CK-MB in the safety group is because of muscle damage.

Contrary to the assumptions in the hypothesis, the number of times a horse was tachypaced during a procedure did not have an elevating effect on cTnI or CK-MB at neither 4 nor 24 hours post AF induction. One horse was tachypaced as much as 87 times during a procedure before entering sustained AF. One would think that such a high number of pacings would result in an increase of cardiac biomarkers. Still this horse did not have an increased level of cTnI or CK-MB at either 4 or 24 hours. It should also be mentioned that the horses did not only receive tachypacing but were also subjected to incremental pacing and extrastimulus pacing. The thought was that tachypacing was more inclined to cause necrosis or ischemia in the myocardium and thereby result in an increase in cardiac biomarkers than the incremental pacing. Therefore only the number of tachypacing was used for the statistics.

The number of procedures the horse underwent was hypothesised to have a cumulative effect on CK-MB and cTnI. This was not observed and the hypothesis was rejected. A slight, but insignificant decrease was seen in cTnI at the 4 hours sampling point. At the 24 hour sample a significant ($p=0.05$) decrease was seen in cTnI. Even though the decrease is very minor (0.0006 ng/mL per procedure) this is still a very peculiar result. The first thought that comes to mind is that after having undergone several procedures, the horses were more susceptible to the pacing and therefore entered AF more quickly than during the other procedures. However this was not the case. A more likely explanation is that the pacing did not have any effect and that the lower values from the fourth procedure are pure coincidence. It is unlikely that a similar result would be found in a larger study population.

For CK-MB a slight increase was seen at both 4 and 24 hours post induction, however the increase was not significant. If the assumption that cTnI decreases after 24 hours concurrent with the number of procedures is true, this would indicate that the heart is less likely to be damaged when exposed to several procedures, and as such it would be expected that CK-MB would follow the same tendency. Even though the results on the relationship between number of procedures and levels of CK-MB are insignificant they still are contrary to the cTnI results, which is very unlikely if lesser damage to the heart after multiple procedures is correct.

Overall it could be concluded that horses that had undergone atrial pacing did not have significantly higher levels of CK-MB and cTnI. There was no association between number of procedures or

number of tachypacings and an increased level of the cardiac biomarkers. These results indicate that this procedure is safe to perform. However there are limitations to this study. First of all the study population is quite small with only nine horses of which three did not undergo atrial pacing and induced AF. This makes the statistical analysis very uncertain. A larger study group could provide more conclusive results. That being said the results did not show any elevations in cTnI after pacing and only one sample had a level of CK-MB exceeding the reference value after pacing. Thus it would be unlikely that even in a larger population a significant increase in cTnI and CK-MB would be seen after pacing.

Secondly all horses served as their own control, meaning there was no real control group. Although it is an advantage that the horses post procedure values were compared with their own pre procedure values, but a real control group could have provided an insight to the range of cTnI and CK-MB in healthy horses that have not been exposed to the procedure and provided a better understanding of the changes seen in cTnI and CK-MB after the procedures. The safety group could be considered a control group since they did not go through atrial pacing and AF; however they did receive the anti-arrhythmics and even though the data shows no significant changes in the levels of CK-MB and cTnI in response to the anti-arrhythmics, these drugs could still possibly affect the levels of CK-MB and cTnI.

The verification that the electrophysiologic procedure is safe to perform is an important result for future research of AF. If a significant increase in cTnI and CK-MB had been observed, thereby indicating myocardial injury it could be discussed whether or not it would be unethical to perform procedures such as the one carried out in this study. Even though the horse might not have a behavioural response to pacing indicating pain it is still controversial to perform a procedure that inflicts damage to the horse. Another aspect is that myocardial damage could have an influence on the treatment tested which could lead to unreliable results.

Ranolazine has been reported to have a cardioprotective effect and to have a decreasing effect on cTnI after cardiac surgery in human patients (Pelliccia *et al.* 2012). It was therefore interesting to see if such results could be recreated in this study. However a lack of high levels of cTnI and CK-MB in general and especially after pacing makes this difficult to evaluate. The different drug administrations were still compared. There was not observed a difference in either cTnI or CK-MB between the drug administration groups at any time point, indicating that the anti-arrhythmic

treatment did not cause harm to the heart. All hypotheses concerning drug administrations were rejected.

Even though there was no statistical difference between the drug administrations one interesting result did occur. Horse ID. 2 had a large increase in cTnI after the administration of dofetilide. Since the horse did not undergo the electrophysiologic procedure one can only assume that the increase is due to dofetilide. To investigate this further the horse's ECG was examined. After the administration of dofetilide the horse's t-wave went from biphasic to negative. Nevertheless this was not only observed in this horse. All the horses had changes in the t-wave after the administration of dofetilide. But only one horse other than Horse ID. 2 had a negative t-wave after receiving dofetilide. This other horse had a cTnI concentration of 0.001 ng/mL after the dofetilide procedure.

In addition some ventricular premature complexes (VPC) and supraventricular premature complexes (SVPC) were seen in horse ID. 2. Arrhythmias such as VPC and SVPC are not uncommon in the horse; however VPC can be caused by myocardial disease or lead to ventricular tachydysrhythmias (Verheyen *et al.* 2010). Both VPCs and SVPCs are seen on the 24 hour ECG that the horse had before enrolling in the study (see figure 9.1). It is therefore unlikely that the arrhythmias have any relation to the dofetilide administration. However it is a peculiar finding and the combination of the high levels of cTnI and multiple VPCs observed could be an indication that this horse had an early heart disease that was not detected.

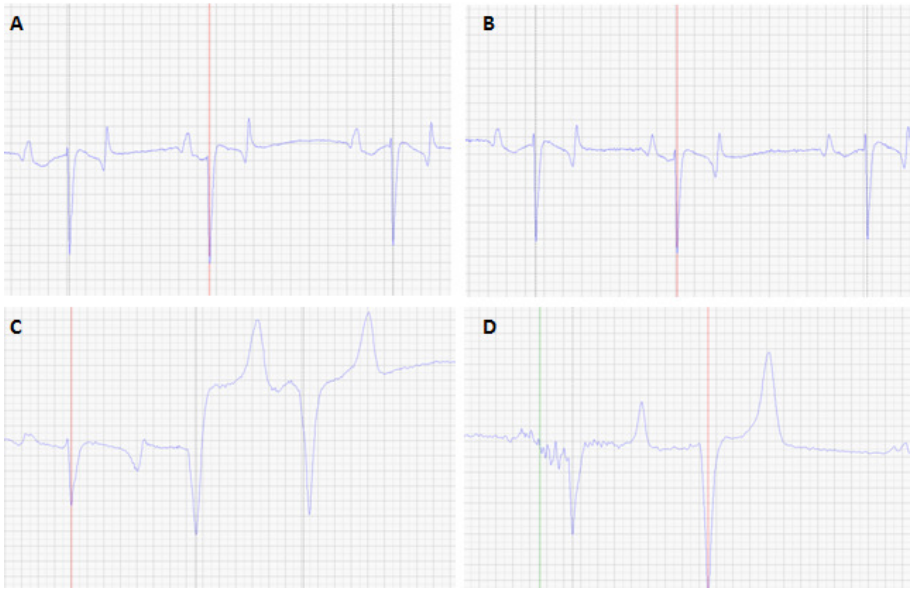

Figure 9.1

Occurrence of SVPC before (A) and after (B) the dofetilide procedure. C shows two VPCs before the dofetilide procedure. D shows a VPC nine hours after the administration of dofetilide.

Another unusual finding from the same horse is that it also had a cTnI over reference point before the placebo procedure. At 4 and 24 hours after the placebo procedure the levels were 0.000 ng/mL. No explanation could be found.

As acute AF has been associated with ischemia and higher levels of cTnI in humans (Parwani *et al.* 2013; van Bragt *et al.* 2014; Lippi *et al.* 2014) it was ideal to investigate whether or not the amount of time the horse has been in AF had an effect on cTnI and CK-MB.

There was a significant positive correlation between the level of cTnI after 24 hours and the number of minutes the horse was in AF ($p = 0.03$). For CK-MB after 24 hours the p-value was 0.09 which indicates that this might not be an incidental finding. At four hours an increase was also seen, however this was insignificant. This is not surprising. The horses were in AF both before and after drug administration. This means that at the four hour sampling point the horse might only have been in AF a short amount of time. Another reason could be that the biomarkers usually are released 4-6 hours after myocardial increase and does not peak until 24 hours. If the source of myocardial injury is the AF then this would influence cTnI and CK-MB levels later than four hours after induction.

The hypothesis that the amount of time in AF was correlated to the level of biomarkers was confirmed for cTnI at 24 hours, but rejected for cTnI at 4 hours as well as CK-MB at 4 and 24 hours.

Even though none of the cTnI samples and only one of the CK-MB samples are over the reference limit the correlation between AF and levels of cTnI and CK-MB is still an interesting result. Increased levels of cTnI have been seen in horses with AF (Nath *et al.* 2012a) and acute onset of AF in humans is often associated with increased values of cTnI (Parwani *et al.* 2013; Lippi *et al.* 2014). The maximum of time a horse was in AF during this study was 256 minutes. If the horses had been in sustained AF for a longer period of time, levels of cTnI and CK-MB above the reference values might have been recorded. Theoretically based on the linear regression line at the 24 hours sampling the horse should be in AF for more than 7 days to reach a cTnI level of 0.12 ng/mL. For CK-MB the horse should theoretically be in AF for 8.4 hours to reach a level of 5 ng/mL. This reflects the very slight increase in cTnI according to minutes in AF. It can therefore be discussed how clinical relevant the results are. Even though cTnI is significantly correlated to minutes in AF at 24 hours the increase seen during the time span of this study leads to a cTnI level that is nowhere near the

upper reference level of 0.12 ng/mL. On this basis it could be said that the increase is not of clinical importance.

There is of course some bias to these results. First of all it was not possible to calculate the exact amount of time two of the horses were in AF because the ECG holter unit did not register the ECG after the procedure was terminated. The results after these two procedures are therefore not included in the statistical analysis. This reduces the already small amount of data.

Another limitation is that only the episodes of sustained AF (> 15 minutes) were included in the statistical analysis. This decision was made based on convenience and on the hypothesis that if AF had an effect on cTnI and CK-MB this effect would not occur after only one or two minutes of AF. Some of the horses had several incidences of AF that only lasted few minutes. If all episodes of AF had been included the results might be different.

This study is generally characterized by a lack of statistical significant results. Very few test results were actually above the level that is considered to be normal for healthy horses. There is of course a large bias in the interpretation of CK-MB value since it was very difficult to find literature describing CK-MB in horses. The reference value used is based on human literature and the true value for horses might be very different. This may have led to a certain degree of misinterpreting of the CK-MB values. However CK-MB has been reported to be of little value in diagnosing heart disease in horses (Argiroudis *et al.* 1982; Cornelisse *et al.* 2000). So even though it is interesting to observe the effect of the procedures on CK-MB, the values of cTnI are attributed more value. Although human cTnI assays have been reported to be useful for measuring cTnI in horses, the human assays still need validation in equine medicine (Rishniw & Simpson 2005; Rossi *et al.* 2014). The lack of validation of the assays gives rise to uncertainty in the reference intervals. The literature used in this thesis reported different reference values. The highest reported reference value was used in this study, but the variations in the samples are so slight that a lower reference value for cTnI would not have resulted in more samples being above reference value. The lack of validation of the commercial assays could influence the results made in this study. The results may be influenced by the anticoagulant use in the blood sampling tubes as well as the sampling procedure and time (Rossi *et al.* 2014). Still the blood samples were all handled in the same manner thereby minimizing the bias between the samples in this study. The problem is rather that it becomes difficult to compare the results from this study to other studies that used different assays, blood sampling tubes and collection time points. Until there is further evidence on the use of human cTnI

assay in equine medicine the best solution is to minimize bias in the study and to be critical when comparing results with other studies made.

Still the lack of results above reference value made the statistical analysis difficult, because even when the results were significant the difference was still so minor that there was no clinical importance. If any of the factors in this study had damaging effect on the heart a clear increase of cTnI and CK-MB would have been expected. All positive correlation found in this study, however significant, are possible incidental. Even if they were truly significant they were still of no clinical importance.

10. Conclusion

The objective of this study was to evaluate blood levels of cTnI and CK-MB before and after atrial pacing and induced AF to see if the electrophysiologic procedure resulted in myocardial damage and thereby evaluate the safety of the procedure. Furthermore the effect of the anti-arrhythmic drug administration on cTnI and CK-MB was evaluated.

It can be concluded on basis of the results obtained during this study that no elevation above reference value in the cardiac specific biomarkers cTnI and CK-MB was seen following a standing electrophysiologic procedure including tachypacing and induced AF. There was no significant association between the number of tachypacings or number of procedures and increasing levels of cTnI and CK-MB.

There was no significant difference in the levels of cTnI and CK-MB after administration of the different anti-arrhythmics. However one horse did have increased levels of cTnI 4 and 24 hours after the administration of dofetilide and even though some VPCs were observed it most likely does not have a connection to receiving dofetilide.

It could be concluded that atrial pacing as well as induction of AF was a safe procedure that did not cause damage to the myocardium even when performed multiple times. Experimentally induced AF might cause an increase in cTnI but it was of no clinical importance at the amount of time observed in this study.

11. Perspective

Electrophysiological procedures are useful tools in the research on AF. They provide an insight to the electric remodelling that occurs after AF and offers a method to induce healthy animals with AF. Experimentally induced AF can be used to test different anti-arrhythmics. It is therefore

essential that the procedure is safe to perform. This study concluded that in this case the procedure indeed was safe to perform. This is not only of interest in veterinary medicine but also in human medicine where AF is a problem especially in older patients. A good understanding of AF and its' pathophysiology is crucial to the future of treatment and prevention of AF.

In potential future studies a larger study group would provide more results and thereby a more reliable statistical analysis. All the horses in this study were Standardbreds, which gives a very one sided result. It is not impossible that other types and races of horses would respond differently to the stimulus applied. A more varied study population would therefore also be needed to fully verify the results from this study. In future studies it would also be interesting to measure the values of cTnI and CK-MB in horses with experimentally induced AF lasting more than a few hours to see if the values increase over time.

Increased levels of cTnI have been associated with AF in horses, but no studies have measured cTnI in a large population of horses with AF. It would be interesting to see what the mean cTnI level was in horses with naturally occurring AF compared to horses with induced AF.

One thing affecting this study is the uncertainty of CK-MB levels in horses as well as the usefulness of this marker for myocardial damage in the horse. Further studies reflecting the normal values of CK-MB in horses both before and after exercise as well as studies on the CK-MB response to true myocardial damage are needed. Of course it can be debated if there is a need for such studies, when cTnI has shown to be both specific and sensitive in detecting myocardial damage in humans and horses. A standardisation and validation of the cTnI assays would be more useful.

Since there were high values of cTnI and CK-MB that could not be explained it would have been interesting to have examined the hearts of the subjects, both macroscopically and histologically, after the study was completed. This could have provided information on the location and extent of the damage, if any was observed.

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13. Appendix

Appendix I

ID	Clab ID	Troponin	CK_MB
Noble Heart Ranolazine før stof	**1**	0.003	0.59
Noble Heart Ranolazine 4 timer	**2**	0.001	1.06
Noble Heart Ranolazine 24 timer	**3**	0.003	0.59
Noble Heart Dofetilide før stof	**4**	< 0.000	1.00
Noble Heart Dofetilide 4 timer	**5**	< 0.000	0.98
Noble Heart Dofetilide 24 timer	**6**	< 0.000	0.88
Noble Heart kombi før stof	**7**	< 0.000	1.21
Noble Heart kombi 4 timer	**8**	0.001	1.29
Noble Heart kombi 24 timer	**9**	< 0.000	6.44
Noble Heart placebo før stof	**10**	< 0.000	1.05
Noble Heart placebo 4 timer	**11**	0.001	1.56
Noble Heart placebo 24 timer	**12**	< 0.000	1.46
Smilla Ranolazine før stof	**13**	0.002	4.24
Smilla Ranolazine 4 timer	**14**	0.004	4.19
Smilla Ranolazine 24 timer	**15**	0.005	3.23
Smilla Dofetilide før stof	**16**	0.004	3.29
Smilla Dofetilide 4 timer	**17**	0.227	3.96
Smilla Dofetilide 24 timer	**18**	0.178	5.37
Smilla kombi før stof	**19**	0.001	2.13
Smilla kombi 4 timer	**20**	0.001	2.69
Smilla kombi 24 timer	**21**	< 0.000	3.92
Smilla placebo før stof	**22**	0.162	2.28
Smila placebo 4 timer	**23**	< 0.000	2.92
Smilla placebo 24 timer	**24**	< 0.000	2.86
Sweet Image Ranolazine før stof	**25**	< 0.000	0.86
Sweet Image Ranolazine 4 timer	**26**	< 0.000	0.80
Sweet Image Ranolazine 24 timer	**27**	0.002	1.35
Sweet Image Dofetilide før stof	**28**	< 0.000	1.01
Sweet Image Dofetilide 4 timer	**29**	< 0.000	1.01
Sweet Image Dofetilide 24 timer	**30**	< 0.000	1.85
Sweet Image kombi før stof	**31**	< 0.000	0.95
Sweet Image kombi 4 timer	**32**	< 0.000	1.19
Sweet Image kombi 24 timer	**33**	< 0.000	1.45
Sweet Image placebo før stof	**34**	< 0.000	1.25
Sweet Image placebo 4 timer	**35**	0.004	1.12
Sweet Image placebo 24 timer	**36**	0.002	1.78
Hirse Ranolazine før stof	**37**	< 0.000	1.32
Hirse Ranolazine 4 timer	**38**	0.003	1.48
Hirse Ranolazine 24 timer	**39**	< 0.000	1.78

Hirse Dofetilide før stof	**40**	< 0.000	1.67
Hirse Dofetilide 4 timer	**41**	0.001	1.81
Hirse Dofetilide 24 timer	**42**	< 0.000	1.55
Hirse kombi før stof	**43**	0.006	1.64
Hirse kombi 4 timer	**44**	< 0.000	1.66
Hirse kombi 24 timer	**45**	0.003	1.69
Hirse placebo før stof	**46**	< 0.000	1.63
Hirse placebo 4 timer	**47**	0.001	1.75
Hirse placebo 24 timer	**48**	0.002	2.17
Line Ranolazine før stof	**49**	< 0.000	3.98
Line Ranolazine 4 timer	**50**	0.003	3.04
Line Ranolazine 24 timer	**51**	0.001	3.92
Line Dofetilide før stof	**52**	< 0.000	2.66
Line Dofetilide 4 timer	**53**	0.006	2.75
Line Dofetilide 24 timer	**54**	0.001	3.75
Line kombi før stof	**55**	< 0.000	1.98
Line kombi 4 timer	**56**	0.001	1.92
Line kombi 24 timer	**57**	0.001	2.42
Line placebo før stof	**58**	< 0.000	4.69
Line placebo 4 timer	**59**	< 0.000	3.40
Line placebo 24 timer	**60**	< 0.000	2.57
Ingrid Ranolazine før stof	**61**	0.003	1.96
Ingrid Ranolazine 4 timer	**62**	0.008	1.76
Ingrid Ranolazine 24 timer	**63**	0.003	2.13
Ingrid Dofetilide før stof	**64**	< 0.000	2.51
Ingrid Dofetilide 4 timer	**65**	0.009	3.24
Ingrid Dofetilide 24 timer	**66**	0.003	2.22
Ingrid kombi før stof	**67**	< 0.000	2.02
Ingrid kombi 4 timer	**68**	0.002	2.29
Ingrid kombi 24 timer	**69**	< 0.000	2.68
Ingrid placebo før stof	**70**	< 0.000	2.69
Ingrid placebo 4 timer	**71**	0.011	2.54
Ingrid placebo 24 timer	**72**	0.004	5.94
Lindy Ranolazine før stof	**73**	0.006	3.66
Lindy Ranolazine 4 timer	**74**	0.001	3.49
Lindy Ranolazine 24 timer	**75**	< 0.000	3.05
Lindy Dofetilide før stof	**76**	0.007	1.92
Lindy Dofetilide 4 timer	**77**	< 0.000	1.84
Lindy Dofetilide 24 timer	**78**	0.001	1.76
Lindy kombi før stof	**79**	< 0.000	2.78
Lindy kombi 4 timer	**80**	< 0.000	2.54
Lindy kombi 24 timer	**81**	< 0.000	2.91
Lindy placebo før stof	**82**	0.003	2.05

Lindy placebo 4 timer	**83**	< 0.000	1.16
Lindy placebo 24 timer	**84**	0.004	0.68
Sandman Ranolazine før stof	**85**	< 0.000	0.94
Sandman Ranolazine 4 timer	**86**	< 0.000	0.99
Sandman Ranolazine 24 timer	**87**	< 0.000	1.23
Sandman Dofetilide før stof	**88**	< 0.000	2.69
Sandman Dofetilide 4 timer	**89**	< 0.000	2.15
Sandman Dofetilide 24 timer	**90**	< 0.000	2.30
Sandman kombi før stof	**91**	0.003	5.60
Sandman kombi 4 timer	**92**	< 0.000	3.74
Sandman kombi 24 timer	**93**	< 0.000	4.67
Sandman placebo før stof	**94**	< 0.000	1.25
Sandman placebo 4 timer	**95**	< 0.000	1.02
Sandman placebo 24 timer	**96**	0.006	1.03
Devil Ranolazine før stof	**97**	< 0.000	1.81
Devil Ranolazine 4 timer	**98**	0.007	2.02
Devil Ranolazine 24 timer	**99**	< 0.000	1.76
Devil Dofetilide før stof	**100**	< 0.000	2.16
Devil Dofetilide 4 timer	**101**	< 0.000	2.18
Devil Dofetilide 24 timer	**102**	0.001	1.97
Devil kombi før stof	**103**	< 0.000	2.77
Devil kombi 4 timer	**104**	< 0.000	2.68
Devil kombi 24 timer	**105**	< 0.000	2.74
Devil placebo før stof	**106**	< 0.000	1.64
Devil placebo 4 timer	**107**	< 0.000	1.72
Devil placebo 24 timer	**108**	< 0.000	1.81

Appendix II

	Noble Heart	Smilla	Sweet Image	Hirse	Line	Ingrid	Lindy	Sandman	Devil
Age in years	9	5	12	14	6	11	10	5	8
Sex	F	F	F	F	M	F	M	M	F
Ranolazine									
Weight in kg	469	540	536	491	506	447	480	513	456
Tachypacings	0	0	0	15	2	28	6	18	77
Minutes in AF	0	0	0	33	256	67	75	89	56
Dofetilide									
Weight in kg	466	545	547	485	500	455	471	508	435
Tachypacings	0	0	0	4	2	13	25	20	23
Minutes in AF	0	0	0	111*	113*	25	83	74	51
Combination									
Weight in kg	462	551	544	490	515	460	487	532	443
Tachypacings	0	0	0	57	2	56	24	18	83
Minutes in AF	0	0	0	37	128	27	85	44	41
Placebo									
Weight in kg	470	537	539	493	510	453	469	504	445
Tachypacings	0	0	0	17	1	10	6	25	87
Minutes in AF	0	0	0	93	154	263	152	192	20