Heart Rate Variability in Endurance Horses
How is the HRV affected by rest and different training settings?

Research Project
Faculty of Veterinary Medicine, Utrecht University
Department Equine Sciences

C.C.C. Vendrig, BSc

Supervisor Netherlands:
Inge D. Wijnberg DVM, PhD, Dipl ECEIM

Supervisor USA:
Meg M. Sleeper VMD, Dipl DACVIM

Preface

This study was performed in the United States of America, at a farm in a small village in New Jersey. The farm housed fourteen horses, but we only used nine of them for the research. The horses we used have all been trained for the purpose of endurance-riding on a high level.
In the Netherlands, at the University of Utrecht, all students have to complete a research project to become more familiar with doing research, collecting data, using literature and integrating the information in a report.

The study was performed in the United States because Veterinarian Meg Sleeper, working at the University of Pennsylvania, Philadelphia, USA, gave us the opportunity to use her horses and find out more about heart rate variability in endurance horses. A contribution has been made by my co-worker Monique van Leeuwen. Together we worked on the data collection. We both worked on the validation of the Polar system and each of us worked on a different hypothesis applying to HRV in horses.

General Introduction

Consecutive heartbeats in a healthy heart sound regular, but these are not regular on a millisecond basis. Normally the inter-beat-interval varies all the time, which is physiological.[1] This variation in inter-beat-interval is regulated by the autonomic nervous system; the constant shifting of the parasympathetic and sympathetic nervous system activity affects firing of the sinoatrial node in the heart, making the intervals vary constantly.[10,21]

Heart rate variability
Heart rate variability (HRV) refers to the heart variability on a beat-to-beat basis; the changes of the intervals between consecutive beats.[9] As mentioned before, these beat-to-beat changes vary constantly and depend on the influence of the autonomic nervous system on the heart. HRV is evaluated by time- or frequency domain analysis; the variance of beat-to-beat intervals is analysed.[1,9] It seems that the more variability there is, the more capable the heart is of adapting to different situations. Less variability is an indicator of less adaptability and even of disease.[1] The recognition that HRV is an indicator of disease, has led to its increasing use to validate the health of the body and the progression of a current disease.[1,3]

Autonomic nervous system
The parasympathetic nervous system is known as the 'resting system', which slows the heart rate. This is due to the effect of acetylcholine, the neurotransmitter released from the synaptic membrane in parasympathetic nerves.[1,10] The sympathetic nervous system, on the other hand, elevates the heart rate and increases the contractility of the heart.[1,10] This is very useful in fright, flight and fight-circumstances. This part of the nervous system involves the neurotransmitter noradrenalin, which is metabolized and reabsorbed slower than the neurotransmitter acetylcholine.[1,10]
In a resting horse, for example, the parasympathetic nervous system is the dominant acting system.[2] During exercises, with for example a heart rate of 120-130 beats per minute, control is mainly by humoral mechanisms.[24]

There are two common methods to collect the HRV data from a heart rate recording device: time domain analysis and frequency domain analysis. Both types of signal processing have their advantages and drawbacks.

Frequency domain analysis

Frequency domain analysis is a more complicated method compared to the time domain analysis, but frequency domain analysis results in a more specific overview of the sympaticovagal balance.[1] In frequency domain analysis, the activity of the parasympathetic and sympathetic nervous system is expressed in Hertz. Before getting the power spectrum output (in which the activity of the nervous system is expressed), the recorded ECG-data has to be examined and corrected and normal-to-normal beats have to be collected (what is also required for time domain analysis). The intervals between normal consecutive beats (in milliseconds) are analysed by the Fast Fourier transformation technique and the amount of cyclical variation present at different frequencies can be detected.[1] The variations are plotted against the frequency in which the variation occurs, where the area under the curve will give the amount of cyclical variability at different frequencies.[1] The power (variance distributed as a function of frequency) is expressed in Hertz in which 0.10 Hz is six cycles per minute.[1,9]
For example, the high frequency (parasympathetic nervous system and breathing rate) is 0.07-0.6 Hz and the low frequency (sympathetic nervous system) is 0.01-0.07 Hz.[2, 4,7]
Keselbrener et al[8], investigated the effects of blockades in the nervous systems and discovered that the high frequency (HF) power can be abolished by vagal blockade (parasympathetically mediated; abolished by atropine or scopolamine) and the low frequency (LF) power can be abolished by sympathetic blockade (β-blocker propranolol, atenolol) as well as vagal blockade.[8] With this knowledge, it is assumed that the low frequency power is not only mediated by the sympathetic nervous system, but by the parasympathetic nervous system as well.[8] Not every researcher agrees with this statement, because the outcome of the power spectrum changes under different circumstances, which is outlined in the guidelines of the Task Force.[9]

Time domain analysis

Time domain analysis gives a more easily interpretable dataset about HRV, compared to the frequency domain analysis.[9] The disadvantage of this method is that a less specific dataset, with regards to the activity of the parasympathetic- and sympathetic nervous system, is gathered. With the time domain analysis, data such as the standard deviation of normal-to-normal RR-intervals (SDNN) and the root mean square of successive difference (RMSSD) as calculations from intervals between normal beats, are obtained.[1,4] SDNN should not be obtained from recordings with different durations, because the value obtained is dependent on the length of the recording: total variance of HRV increases with the length of the recording, meaning that different recording-durations will give different results.[9,10]
When HRV is measured in a 24-hour period, the frequency and time domain analyses are strongly correlated. For example; RMSSD reflects high frequency and the SDNN reflects total power (ultra low frequency, very low frequency, LF and HF).[9] Time domain analysis is recommended over frequency domain analysis for long-term recordings, because of physiological mechanisms; the components of frequency domain analysis are not stable enough for long-term recordings and an average of the data will not give reliable information.[9] To conclude, frequency domain analysis is recommended for short-term recordings and the Task Force states that a five-minute recording is suitable. In time domain analysis, the use of RMSSD and SDNN in short-term recordings is also possible, but frequency domain analysis is preferred because it will give more information about the sympaticovagal balance.[9,10]

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Abstract

Aim of the study: To validate the Polar system for the use of HRV-data collection by comparing it with an ECG-telemetric system, considered as the gold standard method.
Materials and Methods:

- Horses: nine (n=9) horses, being Arab or Half-Arab, mares as well as geldings. The horses have all been trained for endurance riding. The horses lived in a pasture and were privately owned. The ages ranged from four to twenty years old.
- Equipment: The Polar equipment used was the RS800 CX-model (Polar® Electro Oy, Kempele, Finland). The ECG telemetric system was a 3-lead Spacelab Holter system (Del Mar Reynolds Medical, Hertford, UK).
- Experimental Design: one week of pilot measurements and one week of actual recordings. The horses were measured five days in a row in a state of rest, walk and trot. The Polar device and the ECG were attached to the horses and started simultaneously.
  Data analysis: The Polar data was exported to a computer by an infrared device. Using Polar Protrainer 5 software, the recordings were analysed and three correction methods were applied: uncorrected (polar 1), low filter correction (polar 2) and moderate filter correction (polar 3). These three correction methods were applied to the three gaits. The SD and RMSSD of rest, walk and trot in three different corrections were exported into an Excel file.
  The ECG-recordings were analysed by using Impresario-software. The SD and RMSSD values were exported to the same Excel file as the Polar data.
- Statistical analysis: PASW, version 18 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. For the statistical analysis, a regression analysis was performed with a linear mixed model with random horse effect and with fixed effects: Day, gait, ECG and the gait-ECG interaction. Polar 1, Polar 2 and Polar 3 were set as covariance.

Results: Results obtained by the RMSSD showed a higher R^2 compared to R^2 of the SD scatter diagrams. With the use of linear mixed models, significant correlation was confirmed in RMSSD models of the Polar 1 and the 2 setting during rest and walk.
At rest, two horses had second-degree AV-block, which disappeared during walk and trot. During walk of the Polar 1 and the Polar 2 setting an R^2 of respectively 0.695 and 0.717 in contrast to the resting values of the Polar 1 and the Polar 2 setting with an R^2 of respectively 0.600 and 0.458, was calculated.
SD, on the other hand, only showed significant correlation with ECG-results after extraction of the two horses with second-degree AV-block in the Polar 2 and the Polar 3 setting. Nevertheless, the scatter plots of both the Polar 2 and the Polar 3 setting still displayed a low R^2 after extraction of these two horses.
Conclusions: Polar RS800 CX-model with a low filter correction (Polar 2) in the walking-gait was chosen to be used instead of an ECG-telemetric system for gathering HRV-data in the form of RMSSD for practical reasons.
Practical relevance: The Polar equipment is cheaper and easier in use. Additionally, for sports it is a more practical option compared to the ECG telemetric system.

Chapter I: Validation of the Polar system

Introduction

Polar versus ECG

Radespiel-Tröger et al[19], performed a study with humans and compared an inexpensive Polar-device with a Powerlab gold standard ECG-telemetric system and concluded that there is agreement in time domain results between the two devices. They stated that the Polar system is especially adequate for measurements made by family doctors and athletes, because the equipment is affordable.[19] Comparing a Polar-device with a twelve-lead ECG, Nunan et al[20], also found agreement in a specific dataset, namely the total amount of RR-intervals. The HRV data, however, seemed not to be in agreement compared to the ECG-results.[20]

In another study, performed by Marchant-Forde et al[21], a Polar device and a Telemetric ECG system were used at the same time to measure HRV in pigs. Their aim was to investigate the accuracy and precision of the Polar recorder, to categorize abnormal beats and to ascertain the impact of these abnormal beats in time and frequency domain analyses.[21] They discovered abnormalities in the Polar data, which were not found in the ECG, suggesting an error in the Polar system. However, they concluded that correction (by hand, after calculation of the differences with the ECG-dataset) of the errors found in the Polar data or data free of errors will give results compatible with ECG results.[21]

The articles of Radespiel-Tröger et al[19] and Marchant-Forde et al[21], for example, suggest the Polar system can be used to measure HRV. Parker et al[22], on the other hand, performed a study in horses with results suggesting the Polar system does not give accurate HRV results. In their study, they investigated Polar versus ECG in stabled, standing at rest and loose in the field horses. They concluded that the more movement of horses there is, the more the Polar data differed from the ECG data and that the Polar system cannot be used interchangeably. In the horses at rest, however, there is some similarity between the two systems. After correcting the Polar data (using the same method as described in Marchant-Forde et al[21]) in horses standing at rest, the data showed less comparison with ECG data than with the uncorrected Polar data. On the other hand, in stabled horses, the opposite appeared significant; corrected Polar data were more similar with ECG data than uncorrected Polar data.[22] These results are conflicting and suggest that the correction-method is probably not valid.[22]

In a study of Wallen et al[23], on humans, the same Polar device was used as will be used in the current study (Polar RS800 and a 3-lead ECG). They found an inadequate error identification in the Polar software (Polar Protrainer 5, moderate filter power, minimum protection zone of 6 beats/minute) and an overestimation of HRV. They stated that the Polar is not suitable for identification of abnormal beats and they recommended to use the ECG-telemetric system instead of the Polar system for HRV-data analyses.[23]

Chapter I: Validation of the Polar system

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Aim of the study

There is still not a good agreement on the use of a Polar-device instead of the gold standard ECG-telemetric system. Polar data seems not to correlate significantly to ECG derived data, therefore different correction methods were tested. It is a fact that the Polar system is less expensive than the ECG-telemetric systems, making it a good alternative for athletes and family doctors, but only if the data are valid! Otherwise useless and possibly false conclusions can be drawn, which could have enormous consequences, i.e. people with heart diseases, where the outcome of the HRV can be used for prognosis of life (described in Chapter III). In equine sports these devices are easy to use under the saddle and may in the future be used to base training schedules on HRV-outcomes (this will be outlined in Chapter II).
In this study, the Polar system will be compared with an ECG telemetric system and three correction methods will be used, applying the Polar Protrainer 5 software. Using no correction at all and two different types of correction-levels, it will hopefully give a permanent answer to whether the Polar system is valid or not.

> *Hypothesis*: by using the correct filter for data correction (low or moderate), the HRV data collected by the Polar system correlate significantly to ECG-derived HRV data.

The question complementary to this hypothesis is: what is the influence of exercise on the validity of the results obtained with the Polar RS800 CX? By comparing the articles mentioned in the introduction, there is still not a clear conclusion on whether to use the Polar system instead of an ECG-telemetric system. The Polar system is cheaper, uses less sensitive electrodes and does not allow visual identification of beats. However, we think that with a proper correction method, it is possible to gather Polar-data that reflect reality enough to permit using the Polar derived data instead of the ECG derived data.

Chapter I: Validation of the Polar system

Materials and Methods

Horses
In this study nine (n=9) privately owned horses, trained for endurance riding, were used. Included in the study were: five mares (n=5) and four geldings (n=4) being of Arabian (n=7) and Half-Arabian (n=2) breed. The ages ranged from four to twenty years of age (mean 9 ± 4.67 SD). The groups mentioned in table 1 below, were not of any significance for the current study. It is, however, essential in Chapter II.

Table 1: Overview of the horses used in the study

Group	Number of horses	Mean age ± SD	Gender
1	3	11 ± 0.82	2♀, 1 ⚦
2	3 1 Total: 4	6 ± 0.00 20 9.5 ± 6.06	1♀, 2 ⚦ 1 ⚦ 1♀, 3 ⚦
3	2	5 ± 1	2♀
Total	9	9 ± 4.67	5♀, 4 ⚦

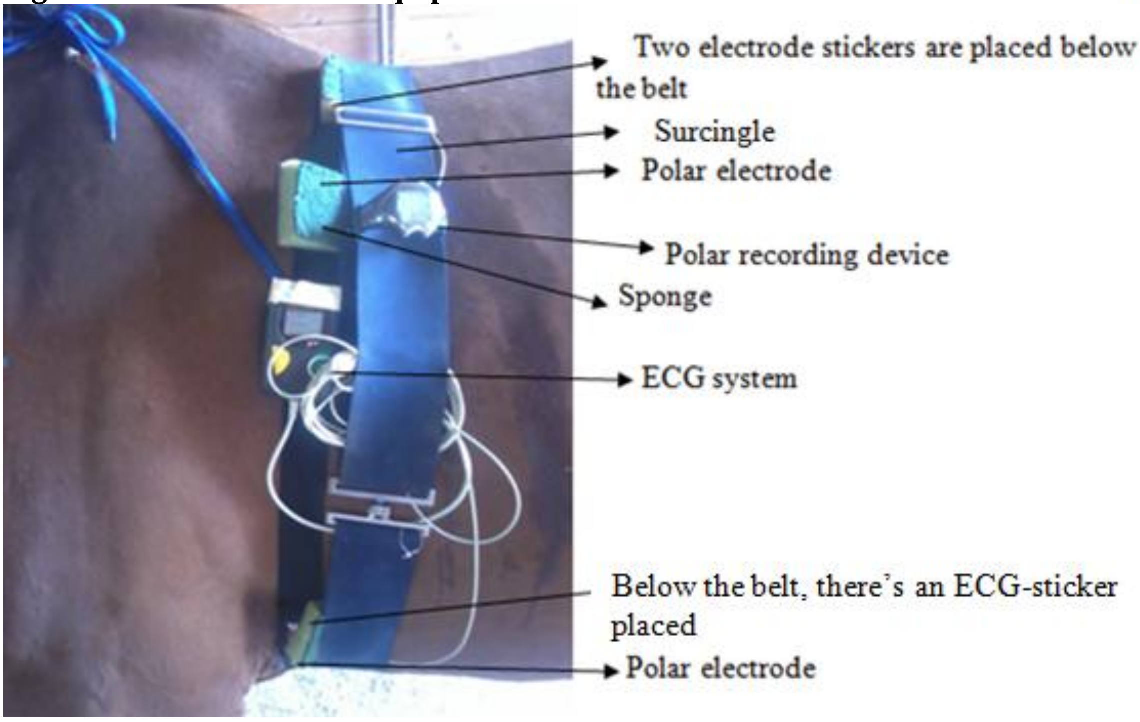
Table: displays the group horses used with their mean age, SD (standard deviation) and gender. ♀ = mare, ⚦ = gelding

All horses were kept in a pasture during the whole year and had free access to water.
The horses in group 1 were housed in a different pasture than the horses in group 2 and 3. The horses in group 2 and 3 shared a pasture together with a few other horses, which did not participate in this study. In the dry season and winter, extra hay will be supplied. The horses in group 1 and group 2 were fed twice a day with concentrate adjusted to their needs. The horses in group 1 were getting higher fat diet (11% fat), compared to the horses in group 2 (6% fat). One horse in group 1 was getting a low carbohydrate, high fat diet (10%). All horses were trained by the same person starting at an age of three to four years.

Electrode placement and equipment
The horses were shaved at the electrode-placement locations. The skin was moistened with warm water and electrode-gel was applied to the skin. For the Spacelab Holter system (Del Mar Reynolds Medical, Hertford, UK), one electrode sticker was placed behind the left elbow and two electrode stickers were placed below the withers also on the left side of the horse. The two Polar belt-electrodes were placed at the left lower and upper side of the thorax.

The Polar equipment used was of the RS800 CX-model (Polar® Electro Oy, Kempele, Finland). The ECG telemetric system was a 3-lead Spacelab Holter system (Del Mar Reynolds Medical, Hertford, UK). Extra tape, sponges and surcingles were placed to cover the electrodes and gave them better stability to reduce movement artifact. The recording device of the Polar equipment was fixed onto the surcingle, preventing interference with other running devices attached to other horses (the signal of the Polar system in the belt could reach a distance of ten meters to the Polar recording device). See figure 1 for the placement of the equipment.

Figure 1: Placement of the equipment



Placing of the equipment on the horse.

Experimental design pilot study

The current study was performed in two weeks, within the first week being a pilot study, where pilot measurements were recorded. This first week was useful to discover the best electrode-placement, getting used to the equipment and software, allowing the horses to become acclimatized to the procedure, ruling out different disturbing factors and to choose the method with the least amount of errors seen in the recordings.

Disturbing factors like feeding, loud noises (cars, tractors, lawn-mowers), other horses walking by, etcetera were ruled out as much as possible in the week of actual recordings. All activities that were not absolutely necessary were postponed until completion of the recordings. The first week was also useful to find the appropriate walking and trotting speed in the horse walker in with which all horses maintained the same gait.

Experimental design recording week

All measurements were performed in the morning, five days in a row. The horses were taken out of the pasture and put in the barn, which was a familiar surrounding for each horse, for the measurements at rest.

In the recordings at rest, the horses were placed between cross ties and measured for ten minutes. The measurements started when the horses were having a steady heart rate. After the standing measurements the horses were brought to the walker and were walked for fifteen minutes (speed 3.2 miles/hour) and trotted for fifteen minutes (speed 7.6 miles/hour). Both devices were started simultaneously and from fifteen-minute recording, the best (high quality) five minutes data section was selected from the Polar software. The same five-minute period of data was selected from the ECG after the Polar software five-minute selection was made.

Polar recordings

A wide range of fifteen minutes was recorded to get the best five minutes (continuous) out of the software manually. The data from the Polar recorder was exported to the computer by use of an infrared device. The best five minutes in the Polar dataset were manually chosen by selecting the section with the least amount of error (mostly seen in the form of a spike or spikes). For the Polar system, Polar Protrainer 5 was available for data analysis. If there was more than five percent error, the measurements were considered useless and redone.[10]

Polar software settings
The error correction was set to three different types to gain three different sets of data:
- No correction
- Low filter power and 1 beat per minute as minimum protection zone[27]
- Moderate filter power and six beats per minute as minimum protection zone[23]

ECG-data analyses
The ECG-telemetric method is considered the gold standard[9] and all data gained by this method were inspected by myself and co-worker Monique van Leeuwen for artifacts and correct beat identification using the Impresario software. The five minute RMSSD and SD dataset derived from the ECG was compared to the Polar data of the same timeframe.

Datasets
Two HRV parameters, RR interval Standard deviation (SD) and the root mean square of successive difference (RMSSD) were used to compare the results from the two systems: Polar versus digital ECG. The Polar datasets of RMSSD and SD were gained by running the three correction methods with the Polar software. These datasets were exported into an Excel file and ECG data were added. The Excel file consisted of:
- SD and RMSSD of Polar data uncorrected (Polar 1)
- SD and RMSSD of Polar data corrected, low filter (Polar 2)
- SD and RMSSD of Polar data corrected, moderate filter (Polar 3)
- SD and RMSSD of ECG data
- Above datasets available at rest, walk and trot

Statistical analysis
PASW, version 18 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. For the statistical analysis, a regression analysis was performed with a linear mixed model with random horse effect and with fixed effects: Day, gait, ECG and the gait-ECG interaction. Polar 1, Polar 2 and Polar 3 were set as covariance. The statistical methodology was determined with the help of statistician J. van den Broek, Faculty of Veterinary Medicine, University of Utrecht, Netherlands.

Two values obtained by time domain analysis were used to investigate the correlation between Polar and ECG, namely RMSSD and SD. First, scatter diagrams were used to estimate the relation between the three different Polar corrections (Polar 1, Polar 2 and Polar 3) and the ECG data. These diagrams were evaluated and the proportion of total variability explained by the relationship between Polar and ECG (coefficient of determination, R^2) was calculated and visualized with a straight line through the data points. The closer the individual dots were to this line, the stronger the linear association of the data (RMSSD respectively SDRR) between the two variables Polar and ECG.[26] Next, linear regression analysis was performed to determine which Polar and which gait represents the best significant correlation with ECG data. A dataset of three different gaits was analysed, indicating the following: gait 1 = rest, gait 2 = walk and gait 3 = trot.

Hypothesis:
H0: population correlation coefficient = 0 and H1: population correlation coefficient ≠ 0
A significance of $P < 0.05$ was used to reject H0.

The coefficient of determination is R^2, and the correlation coefficient $r = \sqrt{R^2}$ (proportion of the total variance)[26], whereby:

r = 1 perfect correlation
r = -1 negative linear correlation
r = 0 no correlation
r = + 0,86 positive correlation
Therefore, everything between 0 and 1 is a positive correlation, the higher the number, the better the correlation is between the used Polar correction and the ECG.
Assuming all five days were the same and follow the same protocol, the main effect ‘Day’ was therefore considered having no influence on the results, so day-effects were left out of the analysis.

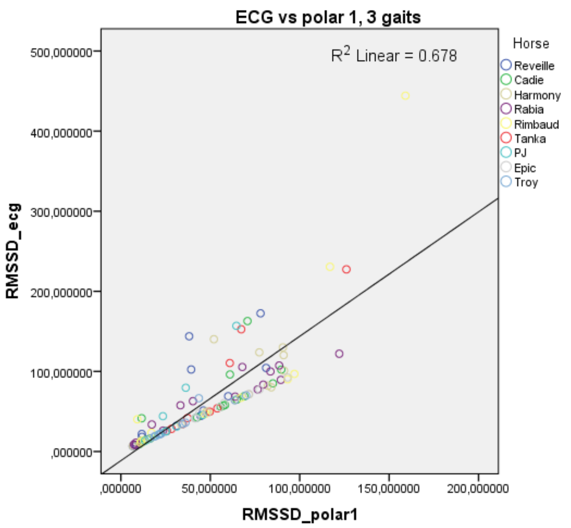
First we determined which Polar was significantly correlating to ECG data to allow the use of Polar data collection. Following that, we determined which gait gave the best and closest results, so these outcomes could be used for following studies (Chapter II and III).

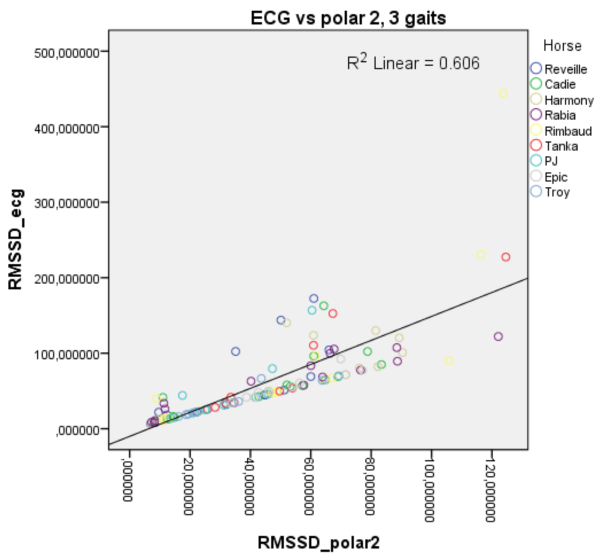
Results RMSSD

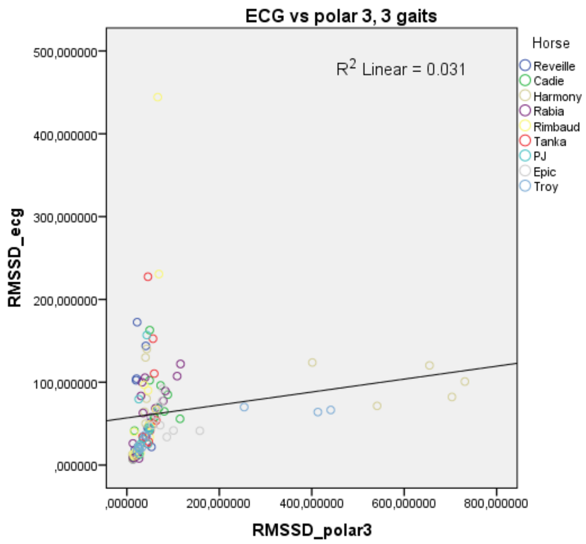
Polar 1, 2 and 3

Comparing the three gaits in all Polar correction methods, the Polar 1 filter setting (using the time domain analysis RMSSD) compared to the ECG data, correlated with an R^2 of 0.678 (r= 0.823) (see figure 1). Using the Polar 1 setting, the data required had the highest R^2 compared to the Polar 2 setting (R^2 0.606, r= 0.778) and the Polar 3 setting (R^2 0.031, r= 0.176). See figure 1. Concluding, the Polar 1 setting showed the highest correlation with ECG data. An R^2 of 0.678 means that 68% of the total variation in Polar can be explained by the linear relationship between Polar and ECG. The other 32% of the total variation in Polar cannot be explained. For the Polar 2 setting it means that 61% of the total variation in Polar can be explained by the linear relationship between Polar and ECG. The Polar 3 setting had no real correlation (only 3%) with the ECG.

Figure 1: Scatterplots representing the correlation in RMSSD for each Polar correction method compared to ECG without distinction in gaits







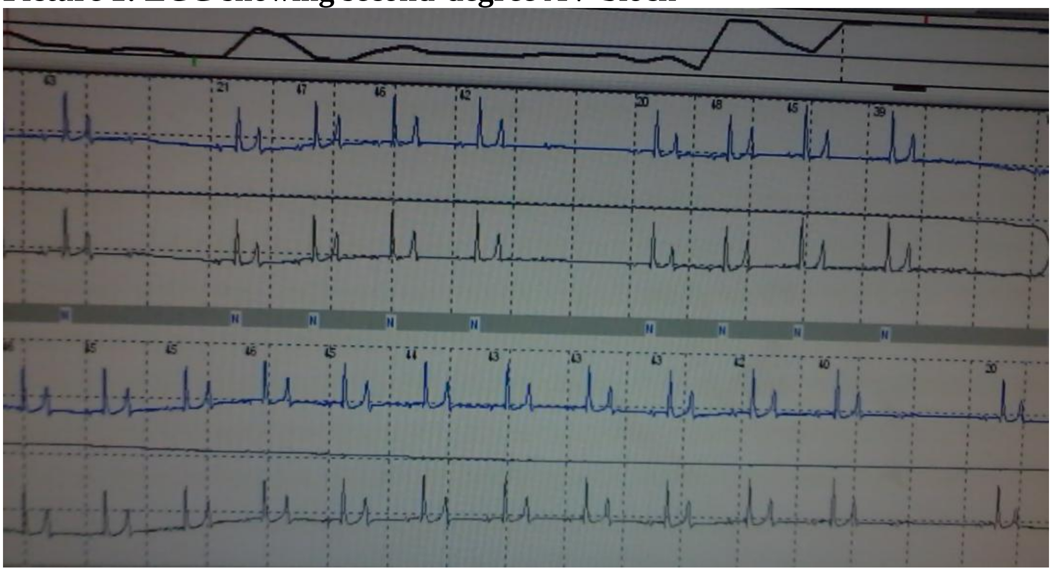
Note: scatterplots of RMSSD data of Polar (x-axis) versus ECG (y-axis). Three gaits are outlined (rest, walk, trot), for every horse separately. R^2 Linear: proportion of the total variability explained by the relationship between Polar and ECG

After this general comparison between the three Polar correction methods, each gait was investigated individually for each Polar correction method, to visualise if there was a stronger correlation between Polar and ECG in one gait in particular.

Polar 1

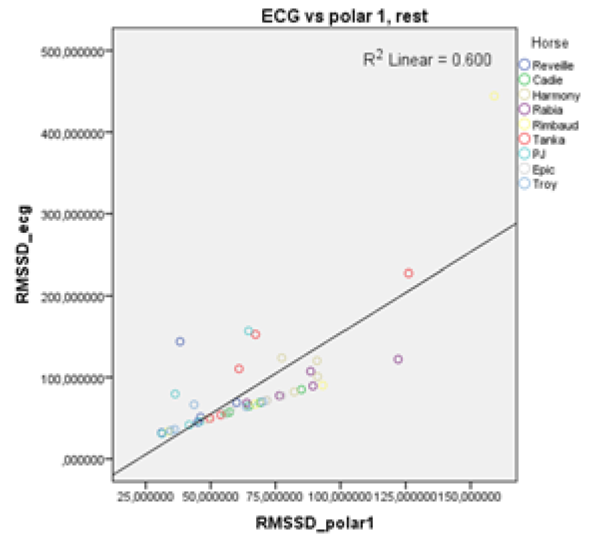
Gait 2 (walk) showed the best correlation between the RMSSD of the Polar 1 setting and ECG (R^2 0.695, r = 0.834, figure 2). An R^2 of 0.695 means that 70% of the total variation in Polar can be explained by the linear relationship between the Polar 1 setting and ECG. Therefore, this gait had a low standard error and a small 95% confidence interval, which supported a close correlation. Gait 1 (rest) also displayed a significant correlation between the RMSSD of the Polar 1 setting and ECG, although the R^2 was lower (0.600, r = 0.775) than that of gait 2 (see figure 2). Therefore, this gait also had a low standard error and a small 95% confidence interval, which supports a close correlation. Two horses were found to have second-degree AV-block at rest (shown by the electrocardiograms, picture 1) and extracting these horses from the analysis, an R^2 of 0.613 (r = 0.783) in rest is estimated (see figure 2). In both horses, the second- degree AV-block was not present during walk and trot. In conclusion, the R^2 showed a minor difference of 0.013 in favour of leaving the two horses out of the calculations. In spite of this improvement by removing the horses with second-degree AV-block, gait 2 (walk) still had the highest R^2. Gait 3 (trot) was the only gait that did not show any significant correlation between Polar 1 and ECG. In addition, the R^2 was also very low (0.213), there was a high standard error and the 95% confidence interval was wide.

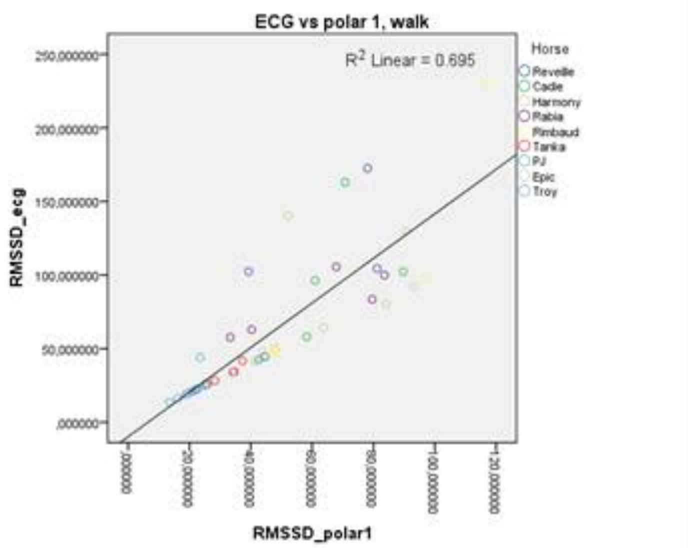
Picture 1: ECG showing second-degree AV-block

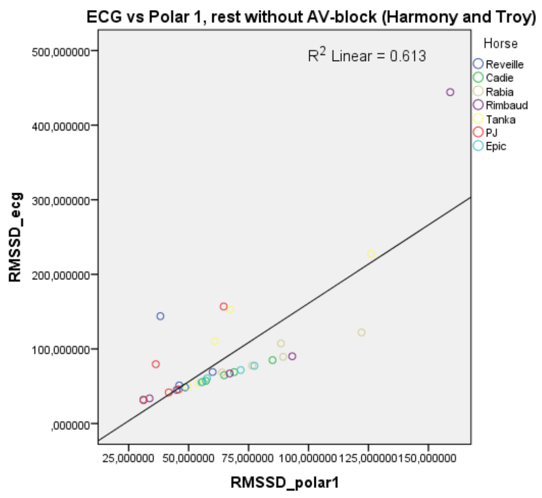


Note: second- degree AV-block were visible in two horses during rest.

Figure 2: Scatterplots representing the correlation in RMSSD for the Polar 1 setting compared to ECG, each gait separately





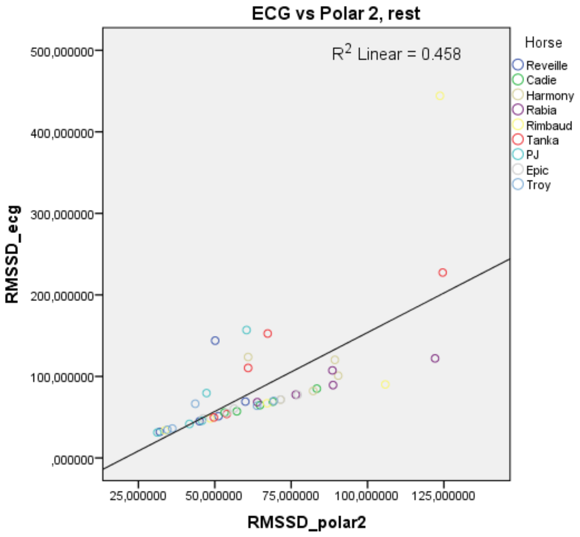


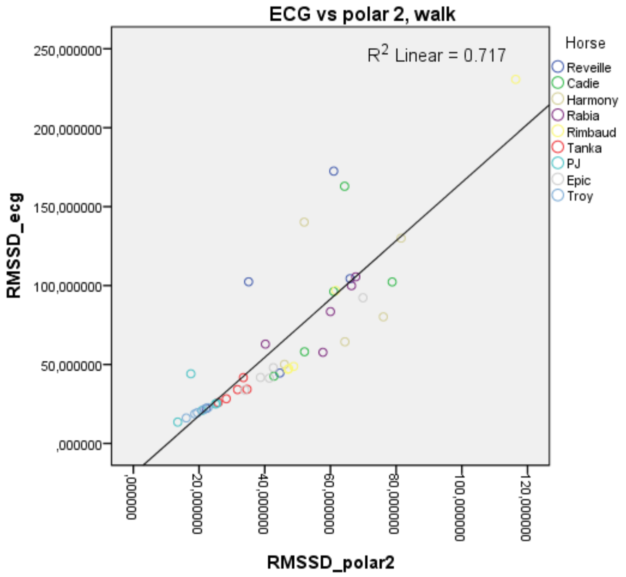
Note: scatterplots of RMSSD data of the Polar 1 setting (x-axis) versus ECG (y-axis). Two gaits are outlined (rest, walk and rest AV-block horses removed), for every horse separately. R^2 Linear: proportion of the total variability explained by the relationship between Polar and ECG

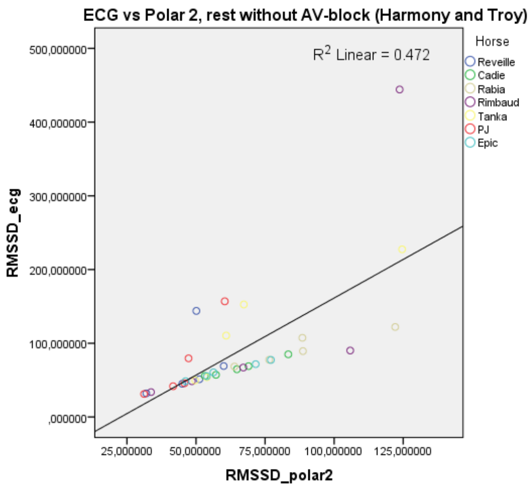
Polar 2

Gait 2 (walk) showed the best correlation between the RMSSD of the Polar 2 setting and ECG (R^2 0.717, r= 0.847, figure 3). An R^2 of 0.717 means that 72% of the total variation in Polar data can be explained by the linear relationship between the Polar 2 setting and ECG. Thereby, this gait had a low standard error and a small 95% confidence interval, which supported a close correlation. Gait 1 (rest) also displayed a significant correlation between the RMSSD of Polar 2 and ECG, although the R^2 was lower (0.458) than that of gait 2 (see figure 3). This gait also had a low standard error and a small 95% confidence interval, which supported a close correlation. Two horses were found to have second-degree AV-block at rest (shown by the electrocardiograms, figure 1) and extracting these horses out of the analysis, an R^2 of 0.472 was estimated (figure 3). In both horses, the second-degree AV-block was not present during walk and trot.
In conclusion, the R^2 shows a minor difference (0.014) in favour of leaving the two horses out of the calculations. Besides correcting for the second-degree AV-block, gait 2 (walk) still had the highest R^2. Gait 3 (trot) was the only gait that did not show any significant correlation between the Polar 2 setting and ECG. In addition, the R^2 was also very low (0.085), there was a high standard error and the 95% confidence interval was wide.

Figure 3: Scatterplots representing the correlation in RMSSD for the Polar 2 setting compared to ECG, each gait separately







Note: scatterplots of RMSSD data of the Polar 2 setting (x-axis) versus ECG (y-axis). Two gaits are outlined (rest, walk and rest without second-degree AV-block horses), for every horse separately. R^2 Linear: proportion of the total variability explained by the relationship between Polar and ECG

Polar 3

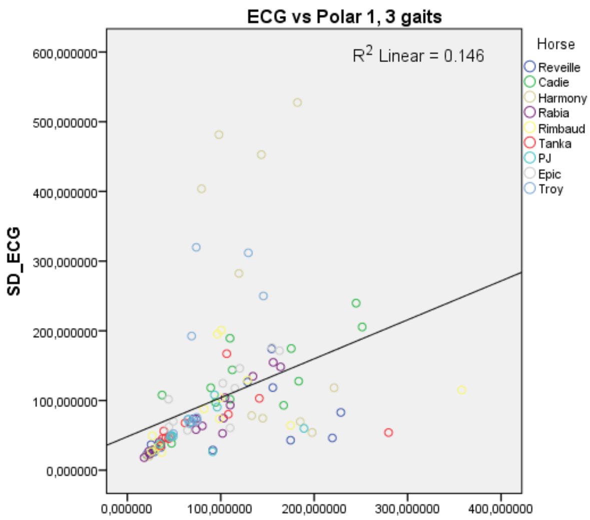
The RMSSD obtained using the Polar 3 setting had no significant correlation with the RMSSD of the ECG in any gait. Combining the absence of significance with the low R^2 leaves the Polar 3 setting out of the question for being a useful alternative for gathering RMSSD data.

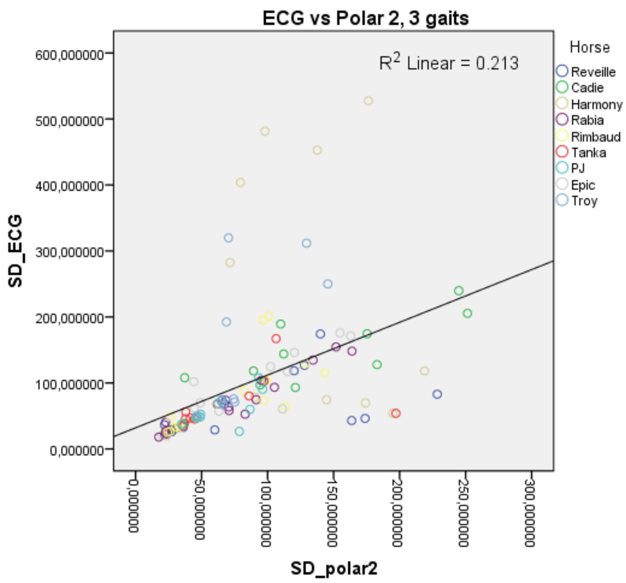
Results SD

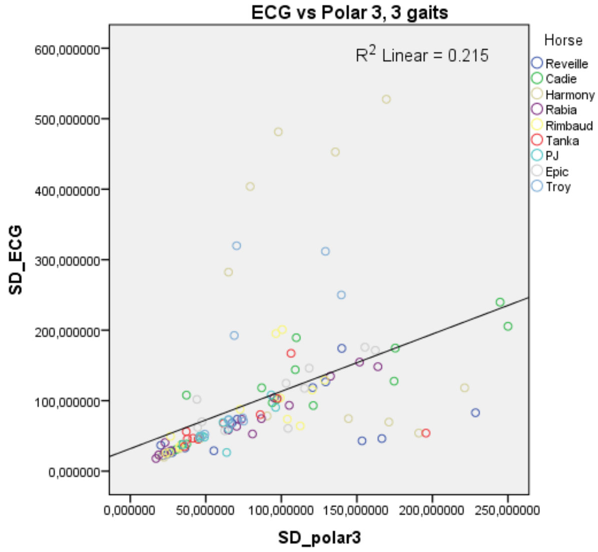
Polar 1, 2 and 3

As with the RMSSD, first the SD values of the three Polar correction methods were compared without any distinction in gaits. All the three Polar correction methods had a fairly low R^2, with 0.146 for the Polar 1 setting, 0.213 for the Polar 2 setting and 0.215 for the Polar 3 setting (figure 4). This means that only a low percentage of the Polar SD data could be explained by the ECG SD data (15-22%), concluding that there was a low correlation between the two methods. In contrast to the RMSSD scatterplots, a big spread between the horse effects was seen in the SD models. This indicated that every horse had a big effect in the comparison between Polar and ECG.

Figure 4: Scatterplots representing the correlation in SD for each Polar correction method compared to ECG without distinction in gaits







Note: scatterplots of SD data of Polar (x-axis) versus ECG (y-axis). Three gaits are outlined (rest, walk, trot), for every horse separately. R^2 Linear: proportion of the total variability explained by the relationship between Polar and ECG

After this general comparison between the three Polar correction methods, each gait was investigated individually for each Polar correction method, to visualise if there was a stronger correlation between Polar and ECG in one gait in particular.

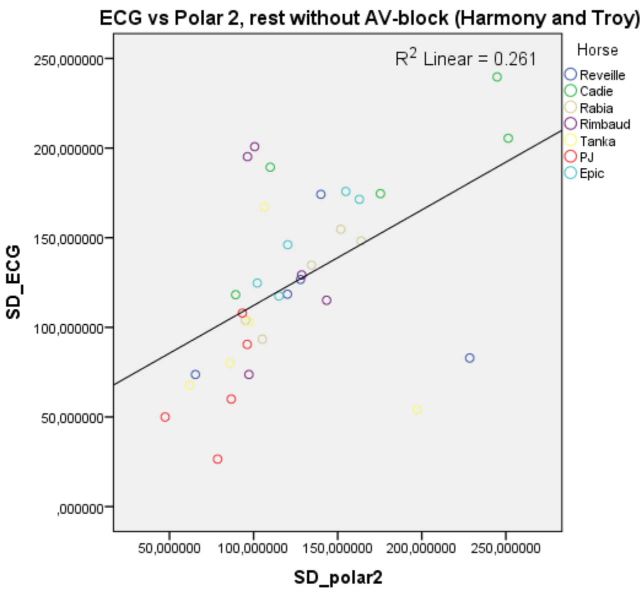
Polar 1

The SD of the Polar 1 setting had no significant correlation with the SD of the ECG in any gait. This means that the Polar SD data cannot explain the variation in the ECG SD data. Even if the correlation would have been significant, the Polar 1 setting showed a low R^2 in each gait (rest 0.00, walk 0.153 and trot 0.244), therefore only a maximum of 24% of the ECG SD data could be explained with the Polar 1 setting SD data. Even when the horses with second-degree AV-block were extracted from the analysis, the SD of the Polar 1 setting did not had a significant correlation with the SD of the ECG.

Polar 2

The SD of the Polar 2 setting only showed a significant correlation with the SD of the ECG during rest and only when the two horses with second-degree AV-block were eliminated from the data. All other gaits (walk, trot and rest with the second-degree AV-block horses) showed no significant correlation between the SD of the Polar 2 setting and the ECG. This means that in these gaits, the Polar SD data cannot explain the variation in the ECG SD data. The Polar 2 setting showed a low R^2 in each gait, with the lowest value during rest (rest 0.026, walk 0.204, trot 0.309). This means that even if the correlation between the SD of the Polar 2 setting and the SD of the ECG would have been significant, the percentage explained SD data has a maximum of 31%. The R^2 of the SD of Polar 2 at rest without the horses with second-degree AV-block was 0.261. This means that only 26% of the SD data collected with the ECG could be explained by the SD data of the Polar 2 setting.

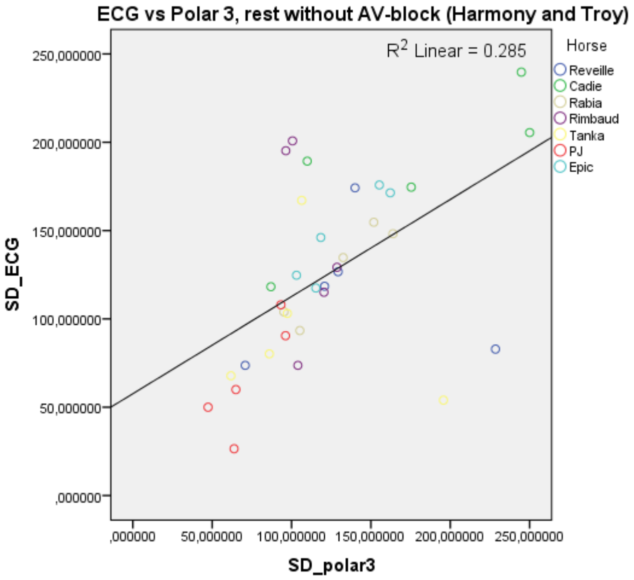
Figure 5: The correlation between the SD of the ECG and the Polar 2 setting during rest, without the horses that had second-degree AV-block



Note: scatterplot of SD data of the Polar 2 setting (x-axis) versus ECG (y-axis). Gait 'rest' is outlined for every horse separately (except the horses with second-degree AV-block). R^2 Linear: proportion of the total variability explained by the relationship between Polar and ECG

Polar 3
The SD of the Polar 3 setting had no significant correlation with the SD of the ECG in any gait, except when the two horses with second-degree AV-block were eliminated from the data. When these horses were eliminated, the R^2 was still very low (0.285, see figure 6), which means that only 28% of the SD collected with the Polar 3 setting could be explained by the SD of ECG.

Figure 6: The correlation between the SD measured from the ECG and the Polar 3 setting during rest, without the horses that had second-degree AV-block



Note: scatterplot of SD data of the Polar 3 setting (x-axis) versus ECG (y-axis). Gait 'rest' is outlined for every horse separately (except the horses with second-degree AV-block). R^2 Linear: proportion of the total variability explained by the relationship between Polar and ECG

These results showed a significant correlation between the RMSSD of both the ECG and low corrected (Polar 2) and uncorrected (Polar 1) data. This correlation was both found in rest and walk. During walk, the R^2 of the Polar 2 setting appeared to be the highest (being 0.717) compared to the R^2 of the Polar 1 setting (0.695). At rest, the R^2 of the Polar 1 (0.600) and the Polar 2 (0.458) setting were both lower compared to the R^2 of the Polar 2 setting during walk.

A significant correlation was found between the SD of both the ECG and the low corrected (Polar 2) and medium corrected (Polar 3) data at rest and only when the two horses with a second-degree AV-block were excluded. Nevertheless, there was a low correlation between the SD of the Polar 2 and Polar 3 setting and the SD of the ECG (Polar 2: r= 0.511 versus Polar 3: r= 0.534).

Chapter I: Validation of the Polar system

Discussion

Polar versus ECG data

The Polar system can be used for HRV measurements. However, a low error correction had to be manually performed in order to achieve somewhat comparable Polar data with the ECG obtained data as indicated by the R^2 of 0.717 during walk. In this study, first the five-minute segment of data had to be selected by hand. Obviously, we chose the data with the slightest error and ran the error correction after selection. The five-minute ECG obtained data was selected after evaluating the Polar results and was therefore not considered to be blind testing. The same five minutes were selected, so the results gathered were as comparable as possible. To obtain the same five minutes of recordings, both systems had to be started simultaneously. This was not always easy to accomplish, because the Polar system was not always giving reliable output, such as a frequency of over 200 when the horses were resting. Before the measurements really started, the system had to give a trustworthy frequency output and sometimes manipulations were needed (more electrode-gel, moving the electrodes, etcetera).

In the data obtained by the ECG telemetric system, some corrections had to be made manually, because the Impresario-software was not always able to select the correct R-peaks (some T-peaks were as high as R-peaks). This correction was only possible if the full electrocardiogram was recorded. However, by correcting these mistakes and subsequently running the analysis, the system did not use all the selected beats for statistics. Additionally, these corrections were made by ourselves, after just a single instruction session and our interpretation was not validated by an experienced person. In the Polar software such corrections were not possible, because an electrocardiogram was not produced. Therefore, the presence of an underlying arrhythmia would not be detected with the Polar system. In spite of these limitations, the current study showed under certain circumstances error correction with the Polar software was able to correct the data in a way that results are reasonably close to those obtained with an ECG.

Pilot study

In the first test week, most environmental disturbing factors were avoided where possible, however there were always unforeseen factors that could occur. Some measurements were recorded for a longer period of time, for example twenty minutes instead of fifteen, increasing the chance of having a steady five-minute dataset. Other factors that were not foreseen were walker-conditions, which could not always be used safely because of weather conditions, etc. Because of these issues, it was not always possible to measure every day of the week, five days in a row. When the walker was safe for use, it did not meant that the conditions were always the same; after rainfall, the ground surface was heavier than after a dry period where it was mainly dusty and firm.

The first week was also used to test different placements of electrodes, to obtain the best quality recorded data. With a bad electrode connection to the skin, so called ‘spikes’ were seen, however this was only noticed after the measurements had been performed. Some horses had to be shaved to improve better skin connection with electrodes and some had conformational issues; the shape of the thorax accounted for a worse electrode connection to the skin. Sponges and the surcingle use prevented most of these factors from affecting the results.

Horse-horse-variation
The horses also had individual variation. They were not of the same age, same breed, same gender, same training days, same herd-composition, etc. Gathering equal HRV-data, it is recommended to use a group of horses of the same age, gender, breed etcetera.

Horse walker
Another changing factor were the measurements taken in the walker. The walker had room for five horses at the same time, but there were only three Polar devices available. Therefore, three horses were measured at the same time, which meant that not every horse has one horse in front of him/her. In a study of Matsuura et al[25], they concluded that the horse that walks up front, encounters more ‘stress’ than a horse that walks in the back.[25] It is possible that this factor influenced the outcome. This can be prevented next time to place five horses in the walker at the same time, so every place is filled. However, there is always a chance that there is an argument between horses, or the fact that an Alfa-leader horse prefers to walk in front (for example). It is not certain if these factors truly influenced the data but a possible effect is difficult to rule out.

Chapter I: Validation of the Polar system

Conclusions

The hypothesis formulated for this research was: corrected HRV data collected by the Polar system correlated significantly with ECG-derived data. However, the correlations were moderately high, whereby an ‘r’ higher than 0.86 is accepted as positive correlation.[26] The question complementary to this hypothesis was what the influence of exercise would have on the results obtained with the Polar RS800cx. Regarding the hypothesis, the results indicated a significant, but moderate, correlation between the RMSSD of both the ECG and low corrected (Polar 2) and uncorrected (Polar 1) data. This correlation was found both in rest and walk. Results obtained with a moderate correction method (Polar 3) did not correlate significantly to obtained ECG results. The RMSSD of the Polar 2 setting during walk showed the highest correlation with the RMSSD of ECG (R^2 0.717, r = 0.847). During rest, the RMSSD of the Polar 1 setting showed the highest correlation with the RMSSD of the ECG (r= 0.775).

Our results indicated a significant correlation between the SD of corrected (Polar 2 and 3) data and the SD of the ECG only when the horses with AV-block were excluded from the dataset. Moreover, there was a low correlation between the SD of the Polar 2 and 3 setting and the SD of the ECG (Polar 2: 0.511 versus Polar 3: 0.534). Therefore, the SD of the Polar cannot be used interchangeably with the SD of the ECG based on the three correction methods used in the current study.

To conclude, it is recommended to use the walking gait with the Polar 2 setting (low filter power, 1 beat per minute protection zone) to obtain RMSSD data when using the Polar RS800cx device. Using ECG derived data would still be the best choice for data collection, however this was not possible in the experimental design as described in Chapter II and III.

Chapter II: The effect of exercise on HRV

Abstract

Aim of the study: to investigate the effect of a particular form of activity on HRV measurements of the next day.

Materials and Methods:

- Horses: nine (n=9) horses, being Arab or Half-Arab, mares as well as geldings. The horses have all been trained for endurance riding. These horses lived in a pasture and were privately owned. The ages ranged from four to twenty years old. Three groups were made based on training level and experience, being Elite, Midclass and Non-elite.
- Equipment: The Polar equipment used was the RS800 CX-model (Polar® Electro Oy, Kempele, Finland).
- Experimental Design: ten weeks of daily recording during walk in a horse walker. Fifteen minutes of walk were recorded from which five minutes were selected and analysed by using the Polar Protrainer 5 software. Daily activities of the horses were registered in a logbook and numbered by 1: rest, 2: walker, 3: dressage training and 4: trail conditioning.
  Data analysis: Polar Protrainer 5 was used to gather RMSSD data with error correction set on low filter power. The RMSSD of all the horses were put in an Excel file, together with the activities registered in a logbook.
- Statistical analysis: PASW, version 18 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. A one way analysis of variance (ANOVA) using a general linear model for repeated measures and Bonferroni correction was performed. Statistical analysis was performed to analyse continuous measurements (same horses, every day) and investigate the effect of different factors (activity-level, different groups) on the obtained results (HRV). RMSSD being HRV. RMSSD was transformed with Ln-correction.

Results: Elite horses had an RMSSD (3,76 ± 0,43), which was significantly higher compared to the RMSSD of midclass (3,30 ± 0,34) and non-elite horses (3,54 ± 0,32) after a resting day. The midclass horses also had a significantly lower RMSSD compared to the RMSSD of non-elite horses after a day of rest.
After walker training the elite class had an RMSSD (4,03 ± 0,36) significantly higher compared to the RMSSD of midclass horses (3,49 ± 0,37).
There was a decrease in RMSSD measured in all the horses grouped together (elite and midclass), from measurements after a walker training day (3,759 ± 0,037) compared to the RMSSD the day after trail conditioning (3,548 ± 0,057).
When comparing the RMSSD after walker training with the RMSSD after a day of rest, all classes included, the RMSSD after a day of walker training (3,759 ± 0,037) was significantly higher than the RMSSD after a day of rest (3,535 ± 0,037).
Conclusions: Various levels of activity had a significant effect on the HRV and the groups of trained horses had different HRV alterations secondary to various levels of activity.
Practical relevance: As was already explained in Chapter I, The Polar equipment is cheaper, easier to use and for sports a more suitable option compared to the ECG telemetric system. Training based on HRV will potentially give a better performance than regularly trained athletes. Perhaps training decisions based on HRV data will be useful for equine athletes as well. Before training can be based on HRV, it is useful to know how a specific form of activity influences HRV.

Chapter II: The effect of exercise on HRV

Introduction

As was mentioned previously in the general introduction, HRV can be used to validate the health of the body or the progression of a current disease in humans.[1,3] With this knowledge, athletes are using this physiological variable to fine tune their training schedule on their own HRV.[11] It is increasingly becoming a hot item in sports and more research is necessary to fully understand how HRV and sport-outcome and/or overtraining are related.[15] In the study of Kiviniemi et al[11], athletes measured their own HRV every morning and the ones that had a high HRV, were submitted to a high intensity training day, while the athletes with a low HRV performed a mild training day or took a day of rest. The athletes that trained by using HRV to determine their schedule showed a better performance than the athletes that trained by regular training schedules.[11]

Baumert et al[12] showed in another study with experienced human athletes (track and field, triathlon) during a training camp (running, cycling), that the RMSSD decreased during the training period and returned back to normal levels after recovery. The LF power, on the other hand, showed a significant increase during the training and returned back to baseline levels in the recovery period. These changes indicate a shift in the autonomic nervous system towards a more sympathetically regulated input.[12] So in case of increasing activity, HRV seems to decrease accomplished by an increase in sympathetic (LF) and a decrease in parasympathetic (HF) nervous system activity.[13] In a study of Kuwahara et al[14] with horses, the low frequency and low frequency/high frequency power ratio (sympaticovagal balance) were increased by training, while the high frequency did not change by training at all.[14] So they concluded in this article that the parasympathetic nervous system of these horses was already fully activated before training and that the training had no influence on their parasympathetic nervous system.[14]

Another study of Baumert et al[15], aimed to create an overtraining response in a couple of human athletes with a two week training camp (cycling, running), but they showed a slight overreaching (short-term overtraining) response instead of overtraining. A slight decrease was measured in the RMSSD and they concluded that the nervous system shifted towards a more sympathetic nervous system activity. Overreaching and overtraining are common problems in the athlete-world, both in recreational as in high-performance athletes. Overtraining can result in serious health problems (like being more prone to infections and depression) and should be avoided. Overreaching, on the other hand, is more physiological and resolves normally in two weeks with adequate amounts of rest.[15] Overtraining in humans is best monitored by plasma cortisol concentrations. In horses, the cortisol concentration is affected by so many different influences that it is not an useful parameter to detect overtraining.[16] It can be a useful parameter when considered in combination with other variables, but care must be taken not to ascribe changes to stress alone.[17,18]

Chapter II: The effect of exercise on HRV

Aim of the study

The aim of this study was to investigate the effect of a particular form of activity on HRV measurements of the next day. For example, a conditioning or trail conditioning activity is a more demanding exercise than a day of rest and therefore a decrease in HRV is to be expected. The question is if this effect is present on the day after the chosen exercise.

> Hypothesis 1: trail conditioning and dressage training will both have a negative effect on the HRV measurements the day after these activities; a lowering of the obtained RMSSD values compared to results obtained after a day of rest in both elite and midclass horses was expected.

Another aim was to investigate the difference in highly trained, medium trained (or less experienced) and untrained horses on the different levels of activity.

> Hypothesis 2A: elite horses will have a higher resting HRV than midclass and non-elite horses.
> Hypothesis 2B: Elite horses and midclass horses will have a higher resting HRV compared to non-elite horses.
> Hypothesis 2C: Elite horses will be less affected by heavier training levels (such as dressage and trail conditioning) and will show a higher HRV compared to midclass horses after a day of exercise.

The use of Polar was validated in Chapter I. The Polar equipment is cheaper, easier in use and for sports a more practical method compared to the ECG telemetric system, as was already explained in Chapter I. Training based on HRV will potentially give a better performance than regularly trained athletes. Perhaps training decisions based on HRV data will be useful for equine athletes as well. Before training can be based on HRV, it is useful to know how a specific form of activity influences HRV. A lower HRV is a parameter for a more dominant sympathetic nervous system influence and can indicate stress, pain or disease. The expectancy was that elite horses will have less stress, a better condition and a higher basal resting HRV compared to less experienced horses.

Chapter II: The effect of exercise on HRV

Materials and methods

Horses

In this study three groups with a total of nine (n=9) Arab/Half-Arab bred horses were used. All horses were privately owned and trained for endurance riding. In group 1 three horses between ten and twelve years of age (mean 11 ± 0.82 sd) were used. All three of Arabian breed and brought out in high level endurance competitions (100 miles). These horses were trained since they were three years of age. This group was called ‘elite’ because the horses were on peak-level training.

In group 2 there were four horses in which three had a mean age of 6 years ± 0.00 sd and were medium level endurance horses, with every horse having at least one finished 50 miles competition. The fourth horse, with an age of twenty years, has been retired for six months and completed multiple high level endurance competitions (100 miles). This second group consisted of two Arabs and two Half Arabs. These horses were trained since they were three years of age. This group was called ‘midclass’ because the horses were in medium level training.

Group 3 consisted of two horses; one was four years of age and not yet in training and the other horse was six years of age and permanently lame (diagnosed prior to this study). This group was called ‘non-elite’. In total, five mares (n=5) and four geldings (n=4) of Arabian (n=7) or Half-Arabian (n=2) breed.

Table 1: Overview of the horses used in the study

Group	Number of horses	Training level	Mean age ± SD	Gender	Comments
1 Elite	3	High-level	11 ± 0.82	2♀, 1 ⚦	-
2 Midclass	3 1 Total: 4	Medium-level	6 ± 0.00 20 9.5 ± 6.06	1♀, 2 ⚦ 1 ⚦ 1♀, 3 ⚦	- Retired for 6 months -
3 Non-elite	2	None	5 ± 1	2♀	1 horse permanently lame 1 horse not in training

Table: displays the group horses used with their mean age, SD (standard deviation) and gender. ♀ = mare, ⚦ = gelding

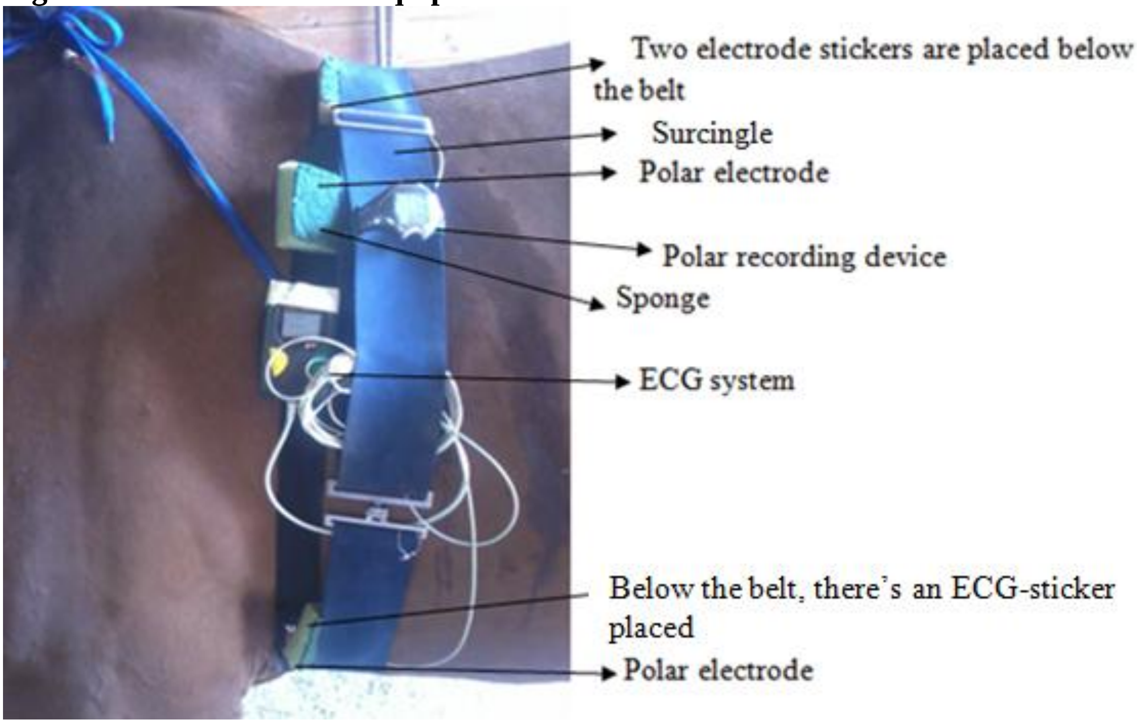
All horses were kept in a pasture during the whole year and had free access to water.
The horses in group 1 were housed in a different pasture than the horses in group 2 and 3. The horses in group 2 and 3 shared a pasture together with a few other horses, which did not participate in this study. In the dry season and winter, extra hay will be supplied. The horses in group 1 and group 2 were fed twice a day with concentrate adjusted to their needs. The horses in group 1 were getting higher fat diet (11% fat), compared to the horses in group 2 (6% fat). One horse in group 1 was getting a low carbohydrate, high fat diet (10%). All horses were trained by the same person starting at an age of three to four years.

Electrode placement and equipment

The horses were shaved at the electrode-placement locations. The skin was moistened with warm water and electrode-gel was applied to the skin. The two Polar belt-electrodes were placed at the left lower and upper portion of the thorax.

The Polar equipment used was the RS800 CX-model (Polar® Electro Oy, Kempele, Finland). Extra tape, sponges and surcingles were placed to cover the electrodes and gave them better stability to reduce movement artifact. The recording device of the Polar equipment was fixed onto the surcingle to prevent interference with devices attached to other horses (the signal of the Polar system could reach a distance of ten meters to the Polar recording device). See figure 1 for the placement of the equipment.

Figure 1: Placement of the equipment



Placing of the equipment on the horse, in this picture the ECG-telemetric system is also adjusted. For Chapter II and III, only the polar belt, sponges and surcingle were placed on the horse.

Experimental protocol

All nine horses were measured in the morning, five days in a row, for ten weeks. All measurements were performed before any form of activity/training was accomplished that day. The horses were taken out of the pasture and put in the barn to place the equipment. Three horses were measured at the same time, because there were only three Polar devices available. The horses were walked to the walker and the recording started before they started to walk. A total of fifteen minutes walking at a speed of 3.2 miles/hour was recorded.

The daily activity of each horse was registered in a logbook and exported into an Excel file. Four different activity levels were defined: day of rest, horse walker, dressage training and trail conditioning.

The day of rest meant that the horse was in the pasture during the whole day and only brought inside for feeding (concentrate). The two non-elite horses did not get any form of extra feeding and stayed in the pasture the whole day. After feeding, the horses were brought back to the pasture.

Walker activity took three hours in the morning: 1.5 hour clockwise and 1.5 hour counter-clockwise.

Dressage training was 45 minutes to an hour in a ring with a few minutes longing prior to the dressage schooling. The difficulty was adjusted to the experience of the horse.

Trail conditioning consisted of six to fourteen miles, with walking, trotting and cantering over rolling terrain. The elite horses averaged a pace of 10-14 mph whereas the midclass horses averaged a pace of 5-10 mph.

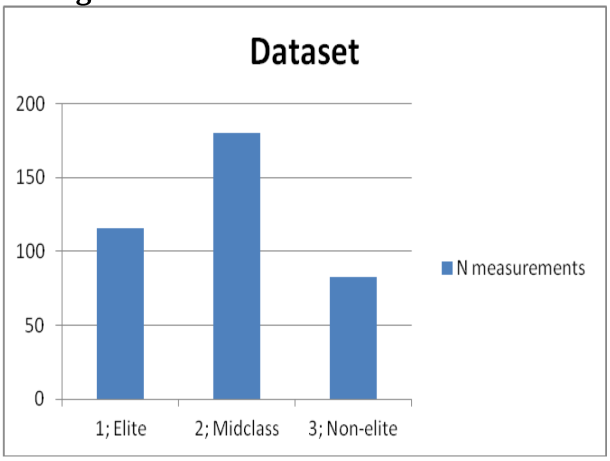
Polar recordings

A wide range of fifteen minutes was recorded to get the best five minutes (continuous) for analysis. The data from the Polar recorder was exported to the computer by use of an infrared device. The best five minutes in the Polar dataset were manually chosen by selecting the section with the least amount of error (mostly seen in the form of a spikes or spikes). The Polar Protrainer 5 software was used for analysis. If there was more than five percent error, the measurements were considered useless and redone.[10]

Datasets

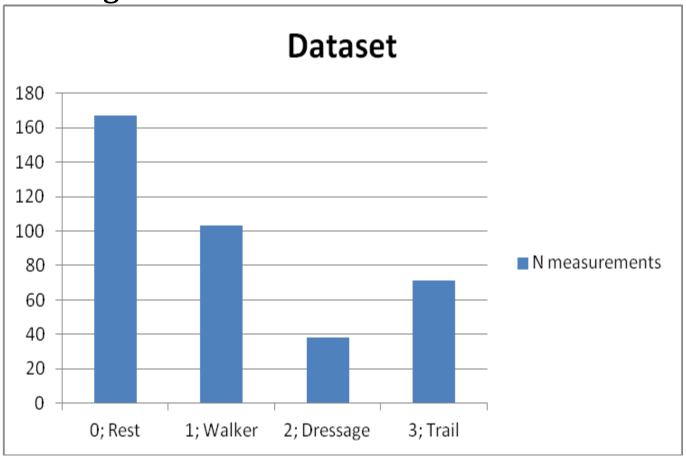
The best five-minutes section was selected and a low filter power correction with 1 beat per minute as minimum protection zone (Polar 2) was set as error correction. This error correction appeared to be valid at the walking gait, as studied in Chapter I.
The RMSSD obtained during walking gait with Polar 2 data were exported to an Excel file for each horse separately.

Histogram 1: amount of data used



Histogram: displays the amount of data grouped by class: elite, midclass, non-elite. N = number

Histogram 2: amount of data used



Histogram: displays the amount of data grouped by level of activity: rest, walker, dressage training, trail conditioning. N = number

As shown in histogram 1 and 2 above, the same number (N) of measurements were not available for each level of activity or for each class of horses (For example, not 100 measurements for each class of horses and not 100 measurements per form of activity). The non-elite was a group consisting of only two horses, compared to the midclass group where four horses were included. The most measurements were taken after a day of rest, which was mostly due to the two non-elite horses, in which no form of activity was performed.

Statistical analysis

PASW, version 18 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. A one-way analysis of variance (ANOVA) using a general linear model for repeated measures and Bonferroni correction was performed. Statistical analysis was performed to analyse continuous measurements (same horses, every day) and investigate the effect of different factors (activity-level, different groups) on the obtained results (HRV).
The outliers shown in box plots were erased from the dataset to get a more normally distributed dataset. The dataset was transformed with a Ln-correction of the RMSSD (HRV) to get a normally distributed dataset. To confirm the normal distribution, a residual analysis is performed by drawing Q-Q plots. For all statistical comparisons, a p-value <0.05 was considered significant. The RMSSD used in the study was considered as the value of the HRV. The statistical methodology was determined with the help of statistician H. Vernooij, Faculty of Veterinary Medicine, University of Utrecht, Netherlands.

Chapter II: The effect of exercise on HRV

Results

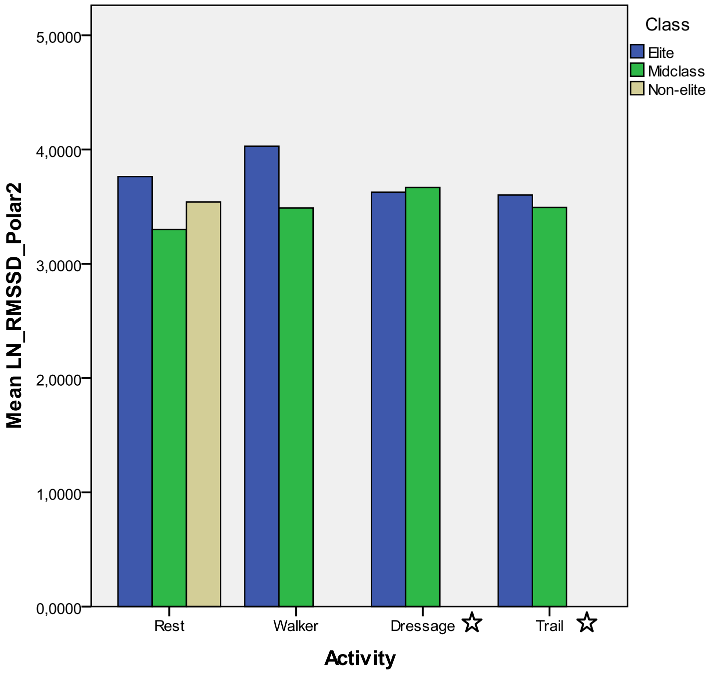
After a day of rest, the elite horses showed the highest HRV (3,76 ± 0,43 sd: mean ± standard deviation), compared to the midclass horses with an HRV of 3,30 ± 0,34 and the HRV of non-elite horses (3,54 ± 0,32). The midclass horses had the lowest HRV after a day of rest. The elite horses showed the highest HRV, which confirms hypothesis 2A. These differences appeared to significant ($P<0.05$)
After a day in the walker, the elite class horses had an HRV of 4,03 ± 0,36 which was significantly higher ($P<0.05$) compared to the HRV of midclass horses (3,49 ± 0,37). The non-elite horses did not perform any form of activity and did not have any other HRV measurements then after a resting day.
After a dressage training day, the elite class horses had a lower HRV (3,63 ± 0,49) compared to the HRV of midclass horses (3,67 ± 0,41), however the difference did not reach significance ($p>0.05$).
After a day of trail conditioning, the elite horses had an higher HRV (3,60 ± 0,47) compared to the HRV of midclass horses (3,49 ± 0,35), however the difference did not reach significance ($p>0.05$).
The results mentioned above are summarized in histogram 1 and table 1.

Table 1: Mean HRV and standard deviation shown in a table

Activity	Class	Mean	Std. Deviation	N
Rest	Elite	3,763128	,4336105	39
	Midclass	3,300717	,3425509	45
	Non-elite	3,540666	,3210536	83
	Total	3,527961	,3900410	167
Walker	Elite	4,029017	,3641197	56
	Midclass	3,488095	,3700842	47
	Total	3,782188	,4544892	103
Dressage*	Elite	3,626658	,4882249	8
	Midclass	3,668342	,4055668	30
	Total	3,659566	,4175077	38
Trail*	Elite	3,601895	,4659412	13
	Midclass	3,493595	,3510462	58
	Total	3,513425	,3732881	71
Total	Elite	3,864007	,4371448	116
	Midclass	3,473064	,3794539	180
	Non-elite	3,540666	,3210536	83
	Total	3,607524	,4224449	379

Table: mean HRV and standard deviation grouped by class and activity.
** not significant. N: amount of data used.*

Histogram 1: HRV displayed for each level of activity and for each class of horses



Histogram: on the x-axis, the four forms of activity. On the y-axis: the HRV. The three classes (elite, midclass, non-elite) displayed separately. ☆: the HRV of the midclass and elite class did not significantly differ. The HRV in the other activities were of significant importance.

A significant ($p<0.05$) increase in HRV was noticed the day after exercise rest (3,76 ± 0,43) compared to the HRV after a walker training day (4,03 ± 0,36) in elite horses (and vice versa: the decrease from walker to rest). So HRV measured the day after walker training led to an increase in HRV compared to the HRV after a day of rest.
A significant ($p<0.05$) decrease in HRV was noticed in elite horses performing walker training the day before compared to the data obtained from elite horses that performed dressage training the day before HRV recordings were made (or increase from dressage training to horse walker training). So the HRV the day after walker training (4,03 ± 0,36) was significantly higher than the HRV the day after dressage training (3,63 ± 0,49).
The decrease in HRV the day after walker training (4,03 ± 0,36) to the HRV after trail conditioning exercise (3,60 ± 0,47) in elite horses was significant as well ($p<0.05$).
These increases and decreases in HRV are displayed in figure 1.

A significant increase in HRV was noticed the day after rest (3,30 ± 0,34) compared to the HRV after a walker training day (3,49 ± 0,37) in the midclass horses ($p<0.05$). So with an increase in activity level from rest to walker, there was an increase in HRV measurements the next day.
A significant increase in HRV was noticed the day after rest (3,30 ± 0,34) compared to the HRV after a dressage training day (3,67 ± 0,41) in the midclass horses ($p<0.05$).
A significant increase in HRV was noticed the day after rest (3,30 ± 0,34) compared to the HRV after a trail conditioning day (3,49 ± 0,35) in midclass horses ($p<0.05$).
A significant increase in HRV was noticed the day after a walker training day (3,49 ± 0,37) compared to the HRV after dressage training day (3,67 ± 0,41) in midclass horses ($p<0.05$).
A significant decrease in HRV was noticed the day after a dressage training day (3,67 ± 0,41) compared to the HRV after a trail conditioning day (3,49 ± 0,35) in midclass horses ($p<0.05$).
These increases and decreases in HRV are displayed in figure 1.

Figure 1: HRV displayed for each level of activity and for each class of horses

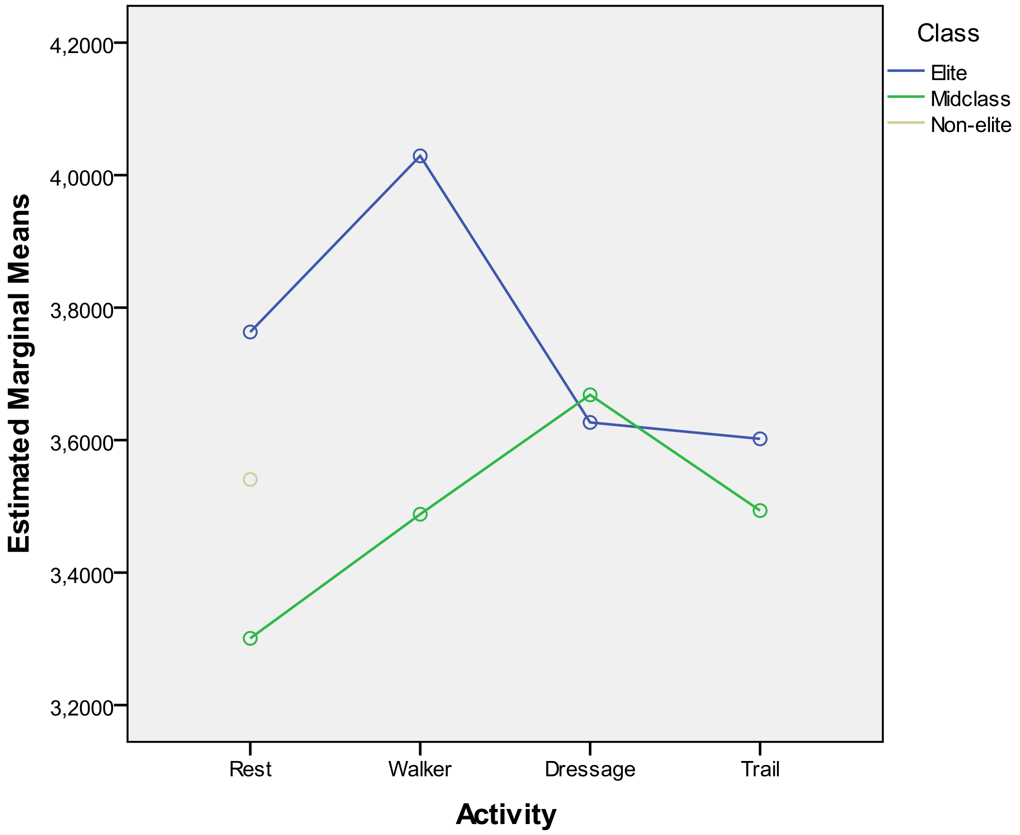


Figure: x-axis: four different activities. Y-axis: mean HRV measured the day after the described activity (estimated marginal means). Non-elite participated only in 'Rest', and is visible as a dot. Significant increases and decreases from one activity to the other is described in the text above.

Comparing the four different activities with each other, without differentiation between classes (all horses as one group), the mean of the HRV after being in the walker showed the highest outcome. The significant values were the only ones of any importance ($p<0.05$), so the increase from rest (3,53 ± 0,39: mean ± standard deviation) to walker (3,78 ± 0,45) (or decrease from walker to rest) accounted for a difference of 0,254 in HRV.
The decrease from walker (3,78 ± 0,45) to trail (3,51 ± 0,37) accounted for a difference of 0,269 in HRV.
Without making a difference in horse-classes, the HRV was significantly higher after being in the horse walker compared to the HRV after a day of rest. The same accounts for being in the horse walker compared to a trail conditioning activity the day before the HRV measurements: walker training accounted for a higher HRV. In diagram 2, the mean HRV of all horses together are outlined per activity-level.

Diagram 2: Differences in mean HRV between the activity-levels.

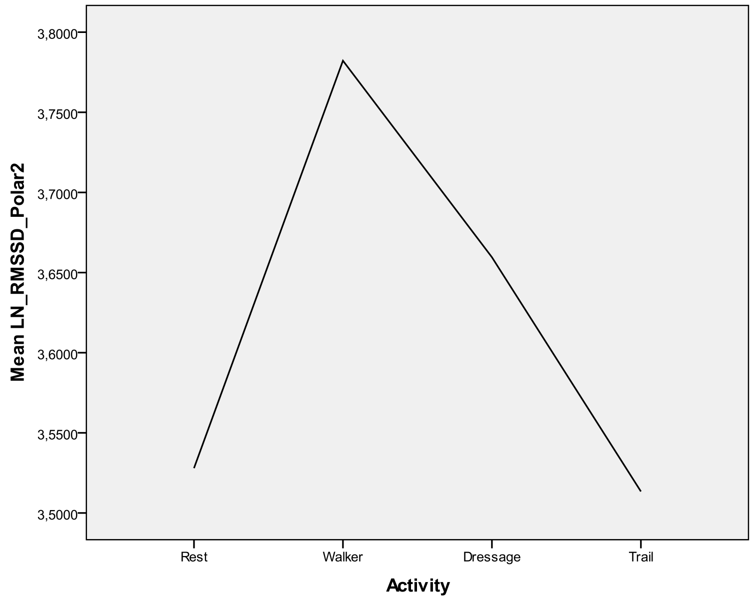


Diagram: the mean HRV is outlined per activity level, without differentiating in horse class.
On the X-axis the activities. On the Y-axis the mean HRV per activity, all horses grouped together.

The mean and standard deviation of the three groups, without taking the activity levels into account were analysed. Elite class horses had an HRV of 3,86 ± 0,44 (mean ± standard deviation), while the midclass horses had an HRV of 3,47 ± 0,38 and non-elite horses 3,54 ± 0,32 respectively. The midclass HRV was significantly lower than the HRV of elite horses ($p<0.05$). Significant difference was also shown in the elite group versus the non elite-group, in which the former case had a significantly higher HRV compared to the non-elite group ($p<0.05$). See table 2.

Table 2: significance between classes

Class *V*	*S* Class	Significance
Elite 3,86 ± 0,44	Midclass 3,47 ± 0,38	HRV Elite significant ↑ than Midclass ($p<0.05$)
Elite 3,86 ± 0,44	Non-elite 3,54 ± 0,32	HRV Elite significant ↑ than Non-elite ($p<0.05$)
Midclass 3,47 ± 0,38	Non-elite 3,54 ± 0,32	HRV Midclass not significant ↓ than Non-elite (p: 0.284)

Table: overview of the significance of class X versus class Y and vice versa. *VS* = versus

Analyzing each horse separately gave a Levene's test of equality of error variances with a $p<0.05$, in which it meant that the error variance of the dependent variable was equal, so the analysis could not be performed (even when the Ln transformed RMSSD was set back on only RMSSD values). Nothing could be said about the HRV of each horse separately. For example, no comparison in HRV between different forms of activity in each horse could be made.

Chapter II: The effect of exercise on HRV

Discussion

This study showed that there was a significant influence of the level of activity on the next day HRV measurements.

Activity-levels
The activity-level ‘walker’ suggested that it would be more ‘activity-like’ than having a resting day, but when grouping all the classes together, the results showed the opposite. Since the HRV was highest the day after walker training. At a day of rest, the horses were only taken out of the pasture for feeding, while the rest of the day, they were standing in the pasture with the rest of the herd. When they were in the walker, they walked for three hours; 1.5 hour clockwise and 1.5 hour counter-clockwise. It seemed that the walker training gave the horse more parasympathetic influence than grazing in the pasture the whole day. A possible explanation for this finding might be that the horses walked less on a resting day compared to training in the horse walker: walking activates the parasympathetic nervous system more than grazing in the pasture. Maybe standing in the pasture was less ‘safe’ than moving around in circles and in addition the sympathetic nervous system was more active. The breathing rate is also of influence on the higher HRV[2,7] and it could be that the breathing rate during constant walking is controlled better.[2] In this study the HRV was linked to the activity the day before the measurements, the activities of the few days before the HRV measurement were not taken into account. For example, a heavy trail conditioning day on Monday could still gave a decreased HRV on Wednesday. This slow recovery of HRV to normal resting values is studied in a study of Mourot et al[28] where the intensity of the training was linked to the duration of the recovery. Mild exercise accounted for a faster recovery of the HRV (minutes), while heavy exercise could accounted for a HRV on normal levels after two days.[28]

Classes
When comparing the results of the different classes, without taking the different activity-levels into account, the midclass horses showed the lowest HRV. This result was not expected. The difference in mean HRV of the midclass horses compared to the mean HRV of the non-elite horses appeared not to be significant. When examining the results obtained after a day of rest, the midclass horses had a significantly lower HRV compared to the HRV of non-elite horse. These results are contradicting to the results found in a study of Kinnunen et al[16] where the older horses (>3 years) showed a higher HRV compared to the HRV of younger horses. These contradicting results can possibly be explained by the fact that the midclass horses were still more sympathetically influenced compared to non-elite horses, due to the training that they had before the resting day(s). Another possibility could be that the midclass horses were older than the non-elite horses and had to ‘fight’ for a place in the herd, which accounted for a higher stress factor. This could be an interesting factor to keep in mind in a follow up experiment; what kind of influence has placing in a herd on HRV-levels? This is not an easy experiment, because the stability in a herd is not very predictable.
That elite horses show the highest HRV was predicted, regarding to hypothesis 2A and 2B and also support the results in the study of Kinnunen et al[16]. This result supports the suspicion that HRV becomes higher if the horse is more conditioned and becomes more experienced: the parasympathetic nervous system is more activated, the sympathetic nervous system less. These elite horses can also encounter less stress than the less trained horses, which may have something to do with self-confidence, herd composition or age. However, the strenuous exercises of the elite horses were different compared to the exercises of the midclass horses.

The elite horses had a faster pace during trail conditioning and more demanding dressage schooling, based on their experience. So another hypothesis could have been that the HRV of elite horses were lower compared to the HRV of the midclass group. In that case the outcome would not confirm the hypothesis.

Elite class results
As already explained above, the HRV of elite horses after walker training activity showed a higher HRV compared to measurements of HRV after a resting day. The decrease in HRV from walker to dressage training and the decrease in HRV from walker to trail conditioning could be explained by the increase of activity level: the decrease of HRV accounts for a more sympathetic nervous system activity.[12,13] As said earlier, it is hard to say how the activities earlier that week had an effect on the HRV measured later that week and this could still have an effect on the outcomes.

Midclass results
As for the midclass horses, an increase in HRV (similar to what was noticed in the elite group) was seen from the measurements after a day of rest compared to the HRV after a walker training day. The HRV after walker training stayed the highest compared to the other levels of activity. As already discussed above, this could be attributed to less stress in the walker, constant breathing rate, constant walking, etc. Another contradicting finding was the increase in HRV measured the day after a day of rest compared to the HRV after a dressage training day and the increase in HRV after a day of rest compared to the HRV after a trail conditioning day. Apparently, the result of the higher activity level will not contribute to a more sympathetic influence the next day. This may be due to the fact that the midclass horses are not relaxed in the pasture, the herd composition is not stable enough, etc. Furthermore, the HRV after a dressage training day showed a higher HRV compared to the HRV measured the day after a trail conditioning day. So it seemed that dressage training needs less sympathetic nervous system activity compared to trail conditioning. This differences in HRV can be explained by the fact that the trail used exists of different surfaces, plain ground and hills, different gaits and sometimes took a few hours. Dressage training, on the other hand, was in a relative safe surrounding (they could still see the herd), plain surface and had an maximum of an hour. To conclude, trail conditioning had an effect on the HRV which could be attributed to more dominated sympathetic nervous system. Dressage training seemed to affect the HRV less compared to trail conditioning.
There were some differences between the midclass group and the elite group. The elite group was housed in a special pasture with just the three elite horses housed together. The midclass horses, on the other hand, were placed in a pasture with a larger group. A bigger herd may account for more stress and sympathetically influenced horses. Dressage training or trail conditioning activity might provide more parasympathetic stimulation than being in the herd all day. For a follow up study, the same housing conditions and herd composition have to be considered.

All the values that were not expected, can be due to the fact that the correlation between Polar and ECG was not perfect (perfect = 1). The best correlation in the validation study was a moderately high ‘r’ of 0,847, while a minimum[26] of 0,86 is acquired. The Polar recordings and data analyses can still give no reliable outcomes and therefore, care must be taken to interpret the outcomes of the current study.

Equipment
The start buttons of the Polar watches (recording device) became more and more dysfunctional in the course of weeks. Before the measurements were actually started, the system had to give a trustful frequency output and sometimes manipulations were needed

(more electrode-gel, moving the electrodes, etcetera). Not only the start buttons became dysfunctional, the electrodes inserted in the Polar-belts were coming out of the belts after a few weeks of daily use and gave data with more error. The first week of comparing the two systems (see Chapter I) provided significantly similar results as the equipment deteriorated, it is possible that there were more artifacts.

Some measurements were recorded for a longer period of time, for example twenty minutes instead of fifteen, so there was more chance of having a steady five-minute dataset. Factors that were not foreseen, were walker-conditions that made it not appropriate for safe use (ditches, poles with water). So because of that, it was not always possible to measure every day of the week, five days in a row. When the walker was safe for use, that did not meant that the conditions were always the same; after rainfall, the ground was heavier than after a dry period where it was mainly dusty.
With a bad electrode connection to the skin, so called ‘spikes’ were seen and this was only noticed after the measurements. Some horses had to be shaved to get better skin connection with electrodes and some horses had thoracic conformation that seemed to correlate with worse electrode connection to the skin and more artifacts. Sponges and the surcingle prevented most of these factors from being disturbing to the results but issues could not be entirely avoided.

Horse-horse-variation
The horses used also had differences compared to each other. They were not of the same age, same breed, same gender, same training days, same herd-composition, etcetera. One elite horse was only measured for six weeks instead of ten, because the horse was participating in the endurance world championship in London. One other elite horse became lame, which was unforeseen of course. Then, one retired horse was hard to place in a group, because it was definitely not non-elite (it had accomplished many 100-mile competitions including competing at the world championships previously) and has been retired for only six months. But the horse was twenty years old and not in training anymore, so it was not really the same as the elite horses. Whether or not it was right to put the horse in the midclass group is questionable. In a study of Kinnunen[16], elite horses and previously raced horses had a significantly higher HRV than non-elite horses, so not placing the retired horse in the non-elite class seemed to be a good decision.[16] The analysis of each horse separately gave no significant outcomes, so there is no answer on the question if it was the right choice to put him in a midclass. There may also be some gender effects on HRV, but that was not the aim of this study.

Horse walker
Another changing factor was the measurements taken on the walker. The walker had space for five horses at the same time, but there were only three Polar monitors available. So three horses were measured at the same time, which meant that not every horse has one horse in front of him/her. In a study of Matsuura et al[25], it was concluded that the horse that walks up front, encounters more ‘stress’ than a horse that walks in the back.[25] It is possible that this factor influenced the outcome. This can be prevented next time to place five horses in the walker at the same time, so every place is filled. However, there is always a chance that there is an argument between horses, or the fact that an Alfa-leader horse prefers to walk up front (for example). It is not certain if these factors really influenced the data but they are difficult to rule out.

Chapter II: The effect of exercise on HRV

Conclusions

The aim of this study was to investigate the effect of a particular form of activity on HRV measurements of the next day. For example, walker training accounted for the highest HRV-measurements, compared to the other three forms of activity. This effect could be explained by more activated parasympathetic nervous system by a constant walking gait in a safe environment.
After walker training the elite class had an HRV higher compared to the HRV of midclass horses, which was expected (Hypothesis 2C). Overall, the elite horses showed a higher parasympathetic influence compared to the midclass horses, which seemed to be more affected by training level.

Trail conditioning accounted for a lower HRV compared to the HRV-measurements the day after dressage training in midclass horses. The different ground surfaces, different gaits, longer period of training, faster speed and more stressing factors will influence the sympathetic nervous system more than a stable ring-area with only 45 minutes to an hour of training.

Midclass horses did not have a higher HRV after a day of rest, compared to non-elite horses. More intensive training of the midclass horses did not account for a higher basal HRV and a more parasympathetically activated nervous system, especially compared to the non-elite horses after a day of rest.

So, various levels of activity had a significant effect on the HRV and the groups of trained horses have different HRV alterations secondary to various levels of activity.

In this study, more knowledge on the influence of different kind of activities on HRV-measurements is generated. For follow-up studies, it is advisable to use more equal groups of horses. It would be interesting to try to base the training schedule for every horse separately on their HRV to study the outcome of performance compared to regular training schedules.

Chapter III: The effect of lameness on HRV

Abstract

Aim of the study: The aim of the study was to investigate the effect of lameness on HRV after a day of rest. Hypothesis: lameness will have a negative effect on the HRV; these horses will have a lower basal HRV compared to sound horses after a resting day.
Materials and Methods:

- Horses: nine (n=9) horses, being Arab or Half-Arab, mares as well as geldings. The horses were all trained for endurance riding. The horses lived in a pasture and were privately owned. The ages ranged from four to twenty years old. Two lame horses and seven healthy (sound) horses were used in the study. One became lame and was elite-class while the other horse was permanently lame and not trained.
- Equipment: The Polar equipment used was the RS800 CX-model (Polar® Electro Oy, Kempele, Finland).
- Experimental Design: ten weeks of daily recording during walk as described in Chapter II.
  Data analysis: Polar Protrainer 5 was used to gather RMSSD data with error correction set on low filter power. The data belonging to activity 'rest' was analysed. The 'group' of lame horses contained two horses and the horse in the elite-group was lame for about three weeks.
- Statistical analysis: PASW, version 18 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. A one-way analysis of variance (ANOVA) using a general linear model for repeated measures and Bonferroni correction was performed. For all statistical comparisons, a p-value <0.05 was considered significant.

Results: The RMSSD of lame horses (3,44 ± 0,33 sd) was lower than the mean RMSSD of sound horses (3,57 ± 0,41 sd), but this difference appeared not to be significant. The elite-horse that became lame during the study described in Chapter II had a higher mean RMSSD (3,77 ± 0,30 sd) than the permanently lame non-elite horse (3,38 ± 0,29 sd).
Conclusion: No significant differences in HRV in lame versus sound horses were found in this study design. The two lame horses, on the other hand, showed a significant difference between their mean RMSSD values, where the elite horse had a higher RMSSD compared to the permanently lame horse in the non-elite group. In conclusion, the elite horse had a higher basal HRV compared to the non-elite horse.
Practical relevance: It is interesting to know how lameness affects the HRV compared to the HRV of sound horses. A future goal would be to establish if HRV can predict lameness or indicate subclinical lameness.

Chapter III: The effect of lameness on HRV

Introduction

HRV and disease
In humans that have had a myocardial infarction, for example, a decreased HRV was an indicator of a higher mortality rate.[1,9] More diseases are investigated related to changes in HRV, for example; humans with Diabetes Mellitus type 1 and a reduction in HRV are more prone to develop systemic diabetic complications.[1,9] In humans with chronic liver disease, chronic respiratory disease or heart disease a decreased HRV has a negative prognostic value as well.[1] In septic human patients, a decrease of HRV is even a predictor of the development of multiple organ dysfunction syndrome and can therefore help identify the patients at risk and give them the needed adequate therapy in time.[5]
Not only with humans, but also with horses, studies were performed where HRV is used to measure disease, discomfort and welfare-parameter.[6,10] In the study of Rietmann et al[6], horses with laminitis were treated and before and after the treatment, HRV was monitored. This study did not show any statistically significant difference between the groups (the SD was too large in HRV-parameters), however HRV might be used in the future to identify the effect of treatment; an increase in HRV may account for a decrease in pain or successful pain management.[6]

Chapter III: The effect of lameness on HRV

Aim of the study

The aim of the study was to investigate the effect of lameness on HRV after a day of rest. Hypothesis: lameness will have a negative effect on the HRV; these horses will have a lower basal HRV compared to sound horses after a day of rest.

Studies have shown that disease and stress have a negative effect on the HRV. Perhaps a lame horse will have a lower resting HRV compared to a sound horse.

Chapter III: The effect of lameness on HRV

Materials and Methods

Horses

In this study three groups with a total of nine (n=9) Arab/Half-Arab bred horses were used. All horses were privately owned and trained for endurance riding. In group 1 three horses between ten and twelve years of age (mean 11 ± 0.82 sd) were used. All three of Arabian breed and brought out in high level endurance competitions (100 miles). These horses were trained since they were three years of age. This group was called 'elite' because the horses were on peak-level training.

In group 2 there were four horses in which three had a mean age of 6 years ± 0.00 sd and were medium level endurance horses, with every horse having at least one finished 50 miles competition. The fourth horse, with an age of twenty years, has been retired for six months and completed multiple high level endurance competitions (100 miles). This second group consisted of two Arabs and two Half Arabs. These horses were trained since they were three years of age. This group was called 'midclass' because the horses were in medium level training.

Group 3 consisted of two horses; one was four years of age and not yet in training and the other horse was six years of age and permanently lame (diagnosed prior to this study). This group was called 'non-elite'. In total, five mares (n=5) and four geldings (n=4) of Arabian (n=7) or Half-Arabian (n=2) breed.

A total of 2 (n=2) lame horses and 7 (n=7) healthy (sound) horses were used. One lame horse belonged in the elite group and one lame horse in the non-elite group.

Table 1: Overview of the horses used in the study

Group	Number of horses	Training level	Mean age ± SD	Gender	Comments
1 Elite	3	High-level	11 ± 0.82	2♀, 1 ⚦	-
2 Midclass	3 1 Total: 4	Medium-level	6 ± 0.00 20 9.5 ± 6.06	1♀, 2 ⚦ 1 ⚦ 1♀, 3 ⚦	- Retired for 6 months -
3 Non-elite	2	None	5 ± 1	2♀	1 horse permanently lame 1 horse not in training

Table: displays the group horses used with their mean age, SD (standard deviation) and gender. ♀ = mare, ⚦ = gelding

All horses were kept in a pasture during the whole year and had free access to water.

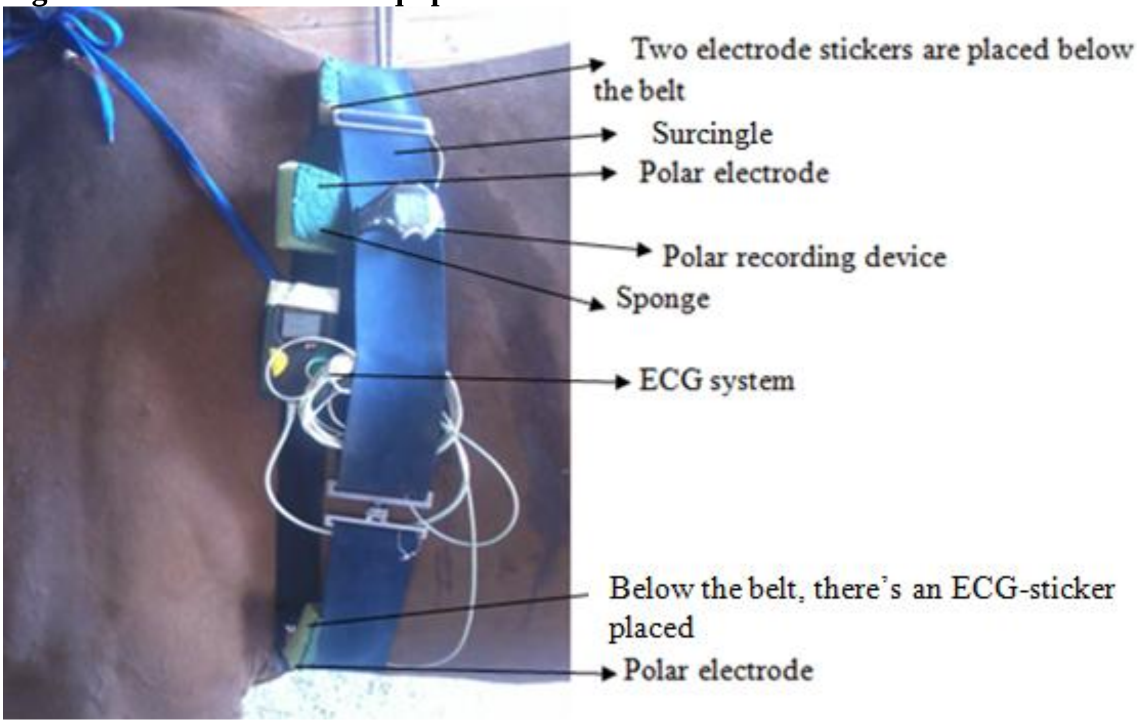
The horses in group 1 were housed in a different pasture than the horses in group 2 and 3. The horses in group 2 and 3 shared a pasture together with a few other horses, which did not participate in this study. In the dry season and winter, extra hay will be supplied. The horses in group 1 and group 2 were fed twice a day with concentrate adjusted to their needs. The horses in group 1 were getting higher fat diet (11% fat), compared to the horses in group 2 (6% fat). One horse in group 1 was getting a low carbohydrate, high fat diet (10%). All horses were trained by the same person starting at an age of three to four years.

Electrode placement and equipment

The horses were shaved at the electrode-placement locations. The skin was moistened with warm water and electrode-gel was applied to the skin. The two Polar belt-electrodes were placed at the left lower and upper portion of the thorax.

The Polar equipment used was the RS800 CX-model (Polar® Electro Oy, Kempele, Finland). Extra tape, sponges and surcingles were placed to cover the electrodes and gave them better stability to reduce movement artifact. The recording device of the Polar equipment was fixed onto the surcingle to prevent interference with devices attached to other horses (the signal of the Polar system could reach a distance of ten meters to the Polar recording device). See figure 1 for the placement of the equipment.

Figure 1: Placement of the equipment



Placing of the equipment on the horse, in this picture the ECG-telemetric system is also adjusted. For Chapter II and III, only the polar belt, sponges and surcingle were placed on the horse.

Experimental protocol

All nine horses were measured in the morning, five days in a row, for ten weeks. All measurements were performed before any form of activity/training was accomplished that day. The horses were taken out of the pasture and put in the barn to place the equipment. Three horses were measured at the same time, because there were only three Polar devices available. The horses were walked to the walker and the recording started before they started to walk. A total of fifteen minutes walking at a speed of 3.2 miles/hour was recorded.

The lame horses did not get any form of activity, only days of rest (whole day in the pasture), which were registered in a logbook. The healthy horses in group 1 and 2 were also used in the study, but only the recordings after a resting day were used in this analysis. The day of rest meant that the horse was in the pasture during the whole day and was only brought inside for feeding. The two non-elite horses did not get any form of extra feeding and stayed in the pasture the whole day. After feeding, the horses were brought back to the pasture.

Polar recordings

A wide range of fifteen minutes was recorded to get the best five minutes (continuous) out of the software manually. The data from the Polar recorder was exported to the computer by use of an infrared device. The best five minutes in the Polar dataset was manually chosen by selecting the section with the least amount of error (mostly seen in the form of a spike or spikes). The Polar Protrainer 5 software was used for data analysis. If there was more than five percent error, the measurements were considered useless and redone.[10]

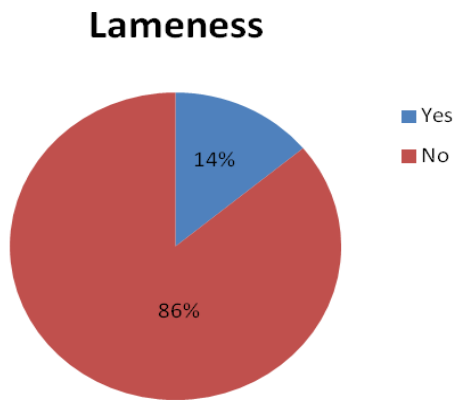
Datasets

The best suitable five minutes were selected and a low filter power correction with 1 beat per minute as minimum protection zone (Polar 2) was set as error correction. This error correction appeared to be valid at the walking gait, as studied in Chapter I.

The RMSSD (HRV) at a walking gait with Polar 2 data was exported into an Excel file for each horse separately. The data belonging to the activity ‘rest’ was analysed. The activities ‘walker’, ‘dressage training’ and ‘trail conditioning’ were not used in this study (these have been used in Chapter II).

The lame horse group contained two horses: one from the elite-group was lame for three weeks and the other lame horse was from the non-elite group and permanently lame. See diagram 1 for the amount of data used of lame horses and sound horses.

Diagram 1: Data used of lame versus sound horses



Note: data obtained from lame horses accounted for 14 percent of the total amount of gathered data, after days of rest (no data after days of activities). Yes: data of lame horses. No: data of sound horses after day of rest

Statistical analysis

PASW, version 18 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. A one-way analysis of variance (ANOVA) using a general linear model for repeated measures and Bonferroni correction was performed. Statistical analysis was performed to analyse continuous measurements (same horses, every day) and investigate the effect of different factors (activity-level, different groups) on the obtained results (HRV).

The outliers shown in box plots were erased from the dataset to get a more normally distributed dataset. The dataset was transformed with a Ln-correction of the RMSSD (HRV) to get a normally distributed dataset. To confirm the normal distribution, a residual analysis is performed by drawing Q-Q plots. For all statistical comparisons, a p-value <0.05 was considered significant. The RMSSD used in the study was considered as the value of the HRV. The statistical methodology was determined with the help of statistician H. Vernooij, Faculty of Veterinary Medicine, University of Utrecht, Netherlands.

Chapter III: The effect of lameness on HRV

Results

The HRV of lame horses (3,44 ± 0,33 sd) was lower than the mean HRV of sound horses (3,57 ± 0,41 sd). The difference of HRV between the two groups was 0,13: a difference that appeared not to be significant. The amount of data used in lame horses was 51, which accounted for the permanently lame horse and the elite horse that became lame, and was set on activity 'rest' for a couple of weeks. The dataset of sound horses with activity 'rest' was 116, including elite, midclass and the non-elite sound horse. See table 1.

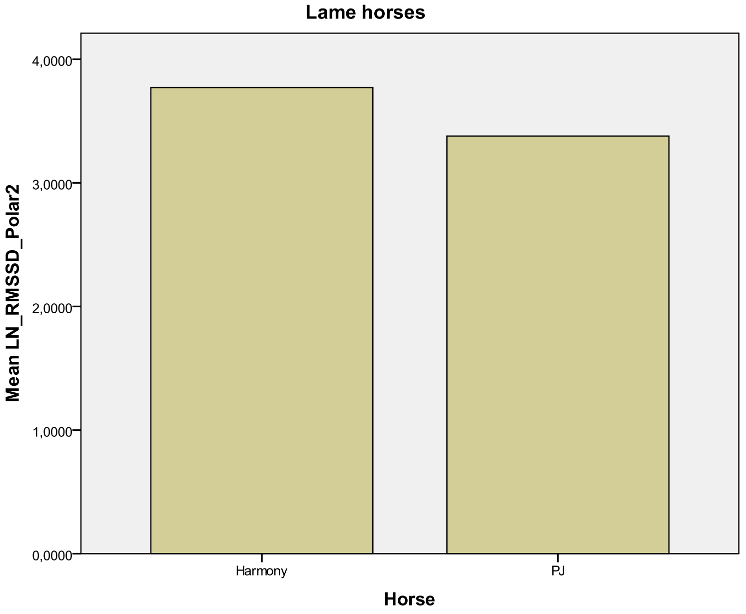
Table 1: HRV of healthy versus lame horses

Lame	Mean	Std. Deviation	N
normal	3,566436	,4097451	116
lame	3,440450	,3281511	51
Total	3,527961	,3900410	167

The table displays the mean HRV and standard deviations of two groups: sound horses (n=7) and lame horses (n=2). Normal = sound. N= number of datasets used. SD= Standard deviation. *Values not significant

The two lame horses were of different groups, namely elite and non-elite. Harmony, the elite-horse that became lame during the study described in Chapter II had a higher mean HRV (3,77 ± 0,30 sd) than PJ, the permanently lame non-elite horse (3,38 ± 0,29 sd). The difference in HRV between the two horses was 0,39, which appeared to be significant. The data is displayed in histogram 1.

Histogram 1: HRV of the two lame horses



Note: The histogram displays the data of table 2. The difference between the two horses is visualized.

Analyzing each horse separately gave a Levene's test of equality of error variances with a $p<0.05$, in which it meant that the error variance of the dependent variable was equal, so the analysis could not be performed (even when the Ln transformed RMSSD was set back on only RMSSD values). Nothing could be said about the HRV of each horse separately. For example, HRV data of the elite horse Harmony in weeks where he was sound cannot be compared to the HRV in weeks where he was lame.

Chapter III: The effect of lameness on HRV

Discussion

When only looking at one activity-level, for instance ‘rest’, the elite class showed the highest HRV, something that was expected from a highly trained group (Chapter II). On the other hand, in a study of Kuwahara et al[14], they stated that the parasympathetic nervous system is already fully activated before training and that training has no influence on the parameters of the parasympathetic nervous system.[14] RMSSD accounts mostly for the parasympathetic nervous system[9,10], so that would mean that the non-elite group will never get such a high HRV as the elite group. However, the non-elite group consisted only of two horses of which 50% was lame. Lameness may account for a lower HRV, as studied by Rietmann et al[6] and this horse (PJ) may lower the mean HRV of group 3.[6] Also, in the current study and the study of Rietmann et al[6], no significant differences in HRV are obtained from lame horses versus sound horses. In the current study, a very low amount of subjects (especially the lame subjects) was used.

A better study design needs to be chosen in future experiments to examine the effect of lameness on HRV, for example inclusion of only permanently lame horses with the same background.

There were some differences in the recording data of the lame horses. The elite horse was lame for three weeks, while the other horse was lame during the total amount of the ten weeks recording. When comparing the two lame horses with each other, the elite horse had during the ‘lame period’ still a significantly higher HRV than the non-elite permanently lame horse. This could be explained by the fact that the elite horse was better trained, so maybe a higher resting HRV was present based on training level. Besides that, the pain level was not quantified for both horses. It could be that the elite horse had less pain and/or stress and therefore a higher HRV compared to the non-elite horse, or the non-elite horse showed a lower HRV because of other factors than lameness (thinking of composition of the herd with all forms of stress included, see discussion Chapter II).

Conclusions

No significant differences in HRV in lame versus sound horses were found in this study design, so it cannot be stated that lameness induces an effect on sympaticovagal balance. The two lame horses, on the other hand, showed a significant difference between their RMSSD (HRV), because the elite horse had a higher HRV compared to the permanently lame horse in the non-elite group.

It is interesting to know how lameness affects the HRV compared to the HRV of sound horses. A future goal would be to establish if HRV can predict lameness or indicate subclinical lameness. Another interesting factor would be to gather data of horses that become lame during a period of recordings and see if HRV might predict the lameness.

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