Assessment of the heart rate variability in endurance horses before and after official competition

Picture: logo WEG 2010

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Chapter one: Introduction

Endurance is an equestrian discipline in which the horse and the rider try to finish a certain distance in the least amount of time and with the horse still in a fit condition after the competition. To ensure that the horses stay healthy, there are several veterinary checks during and directly after the ride. It takes several years of training before a horse is fit enough to compete at the highest level, which is a 100 mile (160 km) distance in one day. Some of them will never get to this level, despite the large amount of training. But how can you tell in advance the difference between horses that will do well and horses that will fail in competition? The answer is not that simple and the ability of the horse to perform during such a long distance test, depends on a lot of factors. A few of these factors that contribute are training, nutrition, orthopaedic condition, mental strength and the cardiovascular system. The ability of the cardiovascular system to cope with a stressor such as an endurance competition will be subject of this research. This parameter can be measured with the use of heart rate variability (HRV).

Cardiovascular function is regulated by the autonomic nervous system and HRV is a way to describe this regulation by determining the variability in R-R intervals. An R-R interval is the time between two R-peaks of the QRS-complex (Gehrke, Baldwin et al. 2011, Dukes ,2004, Lombardi, Stein 2011). The autonomic nervous system is subdivided into the sympathetic and parasympathetic nervous system. They have a contrary effect on the heart rate and contractility. The vagus nerve, the operator of the parasympathetic nervous system, provides a continuous restraint on the heart action at rest and predominates at rest, while sympathetic tone increases with increasing physical activity. Most of the time, a rise in resting heart rate is caused by an increase in sympathetic tone, but it can also be influenced by a sudden drop in vagal tone. The response of the heart to the decrease in vagal tone is faster and allows a better beat-by-beat regulation of the heart rate and contractility than the response to an increase in sympathetic tone (von Borell, Langbein et al. 2007, Dukes ,2004, Lombardi, Stein 2011). This sharp two element autonomic regulation of the heart results in a small (milliseconds) beat-to-beat variation in heart rate called heart rate variability (Yamamoto, Hughson et al. 1991, Lombardi, Stein 2011). The amount of variability of this non-constant heart rate, thus a high HRV, is a sign of healthy cardiac function and allows a better response to changing environmental requirements (von Borell, Langbein et al. 2007, Thayer, Hahn et al. 1997).

There are various ways to present HRV, one category of which is the time domain indices. The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996), further referred to as The Task Force of Cardiology, recommends SDRR and RMSSD as indices for time domain HRV assessment. SDRR stands for the standard deviation of the RR intervals and is a good estimate of overall variability in heart rate. It is the simplest variable to calculate and determine HRV and it reflects all the cyclic components responsible for the HRV in the recording period. SDRR gives thus an extensive and complete presentation of HRV. RMSSD is an abbreviation for the root mean square of successive differences and gives a more short-term view on HRV than SDRR. It is one of the most commonly used measures and has the best statistical properties compared to the other time domain indices. To obtain reliable results, recording times of both RMSSD and SDRR values should be standardized (Task Force of the European Society of Cardiology the North American Society of Pacing and Electrophysiology 1996). A reduction in either RMSSD or SDRR reflects a shift in autonomic balance towards a more sympathetic dominance. In contrast, an increase in these indices reflects a shift towards a more parasympathetic dominance. RMSSD is a particularly good parameter to reflect vagal activity and is the primary time domain parameter used to estimate HRV (Visser, van Reenen et al. 2002, von Borell, Langbein et al. 2007, Gehrke, Baldwin et al. 2011).

In this research report, both RMSSD and SDRR are used as parameters to reflect the HRV of endurance horses. In chapter two, the validation of the Polar heart rate monitor is described in a one week pilot study. This validation is used to justify the use of the Polar heart rate monitor. In chapter three, this heart rate monitor is used to collect data of endurance horses in competition.

Chapter two: validation

Reasons for performing study: Despite its frequent use to measure heart rate variability (HRV), the reliability of the Polar RS800cx has not yet been proved for this use in equine research. It is a popular device to obtain HRV data in equine research, because of its simplicity, time efficiency and low cost compared to the gold standard, a Holter ECG. Nevertheless, the reliability and accuracy of data acquired with a Polar device is uncertain, because it can only store R-R interval data and does not show the underlying electrocardiogram. Furthermore, there is disagreement on whether corrected or uncorrected Polar data correlates best to ECG derived data.

Hypothesis: Corrected HRV data collected with the Polar system correlate significantly to ECG-derived HRV data. The question complementary to this hypothesis is what the influence of exercise is on the validity of the results obtained with the Polar RS800cx.

Methods: Two time domain indices (RMSSD and SDRR) were used to analyze the correlation between simultaneous measurements of the Polar RS800cx and Spacelab Holter ECG. Data was obtained during rest, walk and trot and three different automatic correction methods were used. With the linear mixed model analysis, it was determined which Polar correction method and which gait produced data that resulted in the best correlation with the ECG data.

Results: A moderate significant correlation was found between the RMSSD of the Polar and ECG during rest and walk, both with uncorrected data and a low correction method. The SDRR showed no good significant correlation between the Polar and ECG.

Conclusions: The walking-gait with a low correction method (Polar 2) was recommended to collected RMSSD data with the Polar RS800cx. The SDRR obtained with this Polar device should not be used in equine research.

Introduction

Already in the late ‘80’s, there was a fascination for the use of heart rate monitors for equine research purposes. The two most frequently used monitors in veterinary and human research are the Polar heart rate monitors and the Holter electrocardiographic (ECG). The latter is commonly seen as a gold standard for heart rate variability (HRV) data acquiring. When measuring heart rate with an ECG, each QRS complex is detected and the R-R intervals are determined (Task Force of the European Society of Cardiology the North American Society of Pacing and Electrophysiology 1996). These QRS complexes can be visualized on a computer, the position of the R-R intervals can be manually verified and artifacts can be corrected. According to von Borell et al. (2007) artifacts can appear because of misidentification of R-peaks, ectopic beats, poor conduction between the electrodes and the skin, physical activity, malfunctioning equipment, noises from muscle action potentials and environmental electromagnetic inference. These artifacts must be identified and corrected to obtain valid data (von Borell, Langbein et al. 2007).

Polar heart rate monitors are popular because of their simplicity, time efficiency and low cost compared to a Holter ECG (Wallen, Hasson et al. 2012). The Polar heart rate monitors detect the R-peaks during recording and store the R-R interval data in digital form (von Borell, Langbein et al. 2007). These data can be visualized on a computer, but the underlying ECG is not visible, nor can it be verified or corrected. There is an automatic correction tool in the Polar software to correct artifacts in the R-R interval data. However, because the Polar heart rate monitor records only inter-beats intervals (IBI) and shows no ECG, it is difficult to identify errors in the data (von Borell, Langbein et al. 2007, Wallen, Hasson et al. 2012). Therefore, the reliability and accuracy of data acquired with a Polar device is questionable.

In human research, results of the comparison between the Holter ECG and Polar are conflicting. Radespiel-Tröger et al. (2003) found good agreement between an ECG and a Polar system for time domain analysis, while Nunan et al. (2008) found a poor agreement. In the former study and in the study of Weippert et al. (2010), it was found, that the Polar system was a reliable method to collect R-R intervals but both studies used a different Polar device and Polar software than was used in this current study. On the other hand, in the research of Wallen et al. (2012), the same Polar RS800 with Polar ProTrainer 5 software was used as in the current research and had good to excellent agreement with the ECG acquired data from human male subjects on all HRV measures studied (SDRR, RMSSD and frequency domain analysis). For women, especially woman over 60 years of age, the correlation was much weaker. They also found that the PPT 5 software could not satisfactorily identify and correct all artifacts without visual inspection (Wallen, Hasson et al. 2012).

To our knowledge, the utility of the Polar RS800cx for equine HRV research purposes has not yet been proved, despite the frequent use of Polar devices in equine research (Rietmann, Stauffacher et al. 2004, Cottin, Medigue et al. 2005, Kinnunen, Laukkanen et al. 2006, Visser, van Reenen et al. 2002, Cottin, Barrey et al. 2006, Munsters, Visser et al. 2012, Schmidt, Biau et al. 2010, Schmidt, Aurich et al. 2010, Schmidt, Hodl et al. 2010, Munsters, de Gooijer et al. 2012, Nagy, Bodó et al. 2009, Werhahn, Hessel et al. 2012, Becker-Birck, Schmidt et al. 2012). Nevertheless, Polar Electro Oy states that “*the Science version of the RS800CX is dedicated to the measurement of resting heart rate and heart rate variability (HRV) in research applications”* (Polar Electro Oy, Finland). Because of this discrepancy between the

Polar company and the scientific field, this one week pilot study was performed to validate the use of the Polar RS800cx in equine research.

In an equine study of Sloet van Oldruitenborgh-Oosterbaan et al. (1988), the reliability of three different heart rate monitors was tested in a comparison with a telemetry system under three different circumstances. One of these monitors was the Horse Tester®, a device of Polar Electro, Finland. In this study, a high correlation (0.997) during exercise in a treadmill (walk, trot, gallop) was found between the heart rates given by the Polar device and telemetry (Sloet van Oldruitenborgh-Oosterbaan, van den Hoven et al. 1988). After publication of this article in 1988, as far as the author knows, only one other equine validation study for a Polar system was published. Parker et al. (2009) found no significant differences in time domain indices (mean inter-beats intervals (IBI), mean heart rate (HR), RMSSD and pNN50) between the Polar S810i and ECG data recorded simultaneously on horses standing still and loose in a stable. There was a closer agreement between the uncorrected Polar data and the ECG in standing horses and between the corrected Polar data and the ECG when they were loose in the stall. There was less agreement between the Polar data and ECG in the field condition, where only mean IBI for both corrected and uncorrected data, and the RMSSD for corrected Polar data had no significant differences with the ECG. The corrected Polar data had a closer agreement with the ECG than the uncorrected data (Parker, Goodwin et al. 2009, Jonckheer-Sheehy, Vinke et al. 2012). In the article, caution is suggested when using the Polar system without an ECG, but the results were promising.

Different Polar devices have been validated for use in various animal species, including the pig (Marchant-Forde, Marlin et al. 2004), dairy-cow (Hopster, Blokhuis 1994) and dog (Jonckheer-Sheehy, Vinke et al. 2012). In these studies, there were no significant differences found between results generated with a Polar device and an ECG. Marchant-Forde et al. (2004) found that in pigs, corrected Polar derived data related better to the ECG data than uncorrected Polar derived data. Hopster and Blokhuis (1994) found a better correlation between results obtained with a Polar device and results obtained with an ECG when cows were standing still than while walking. The research of Jonckheer-Sheehy et al. (2012) was the only one that used the same Polar RS800cx as used in the current research.

The aim of this study was to validate the use of the Polar RS800cx for HRV assessment in the equine species. Therefore, Polar data was collected simultaneously with the Spacelab Holter ECG. In previous research there was a discussion about the use of corrected versus uncorrected Polar data. Most studies conclude that corrected Polar data gives the best correlation with ECG data (Marchant-Forde, Marlin et al. 2004, Parker, Goodwin et al. 2009, Jonckheer-Sheehy, Vinke et al. 2012). Following this, the hypothesis is: corrected HRV data collected by the Polar system correlate significantly to ECG-derived HRV data. The question complementary on this hypothesis is what the influence of exercise is on the validity of the results obtained with the Polar RS800cx.

Materials and methods

Horses

7 Arabian and 2 Anglo-Arabian endurance horses were used, ranging between 3 and 20 years of age (8,7 ± 5). Both mares (n=5) and geldings (n=4) were used. Of these horses, three were top-level, three were medium-level, one was retired but former top-level and two were not in training (one of these two was permanently lame). Top-level is defined by completion of at least one 100 mile competition. Medium-level is defined by participation in endurance competition but not at top-level yet (i.e. not having entered a 100 mile ride), with a completion of at least one 50 mile competition. All horses were healthy and no arrhythmias were found. Two horses had second degree atrioventricular heart block at rest, confirmed by the ECG. This heart block disappeared during walk and trot. All the animals were at least two years stationed at the farm and were housed in groups in big pastures. All horses were used to handling and the exerciser. Before the start of the study, there was one trial week in which the researchers and the horses could get familiar with the devices and the procedure. All the horses had unlimited hay and water in their pasture. The study took place in the summer of 2012.

Equipment

Heart rate variability was measured continuously, using a Polar RS800cx (Polar Electro Oy, Kempele, Finland) and a Spacelab Holter ECG (Del Mar Reynolds Medical, Hertford, UK). The Polar belt was attached to the thorax with the two electrodes on the left lateral thorax wall, one just below the withers and one behind the front leg. The Polar belt was stabilized with an elastic girth on top of it, on which the storage device was attached. Warm water was used to moisten the skin to improve conduction between the electrode and skin (Cottin, Medigue et al. 2005, Visser, van Reenen et al. 2002, Cottin, Barrey et al. 2006). In addition, electrode gel was placed between the skin and the electrode to maximize conduction (Parker, Goodwin et al. 2009). The Spacelab Holter ECG electrodes were also placed at the left lateral thorax wall, two just below the withers and one behind the front leg. The storage device was attached to the elastic girth. Underneath the elastic girth on top of both the Polar and the Spacelab Holter ECG electrodes, sponges were placed to give more pressure on the electrodes and thereby facilitate better conduction. Whenever the Polar device was not measuring correctly before the measurement started (for example showing an incorrect very high heart rate), the measurement was not started until a correct recording was obtained. Conduction could often be improved by reapplying the equipment, using more water or electrode gel between the skin and the electrodes, or by relocating the sponges. The exerciser that was used in this pilot study was a round five horse model. The horses could move freely between two gates. The walking speed was set at 3.2 miles/h and the trotting speed at 7.6 miles/h.

Experimental procedures

HRV was first measured while the horses stood inside the barn on cross ties for 10 minutes. After this measurement, the horses were taken outside to the exerciser. In the exerciser, the HRV measurement was started again, after which the horses walked for 15 minutes and trotted for 15 minutes. During the measurements, the horses were observed closely and if there was an obvious external influence, the measurement period would start over (Physick-Sheard, Marlin et al. 2000). The recordings always took place in the morning, to standardize the protocol and avoid the influence of diurnal rhythms (von Borell, Langbein et al. 2007).

Time domain indices

As recommended by the Task Force of Cardiology, SDRR and RMSSD were used as measures of time domain HRV (Task Force of the European Society of Cardiology the North American Society of Pacing and Electrophysiology 1996). The RMSSD is defined as the root mean square of successive differences. It can be obtained by first calculating the difference between each RR interval in the five minute period, after which these values are squared and summed. Finally, this value is averaged and the square root of this total is obtained to display the RMSSD value (von Borell, Langbein et al. 2007, Gehrke, Baldwin et al. 2011). SDRR stands for the standard deviation of the RR intervals and is a good estimate of overall variability in heart rate.

Polar data processing & system settings

The Polar RS800cx detects the R-peaks of the heart rate during recording and stores these R-R intervals in digital form in a storage device (von Borell, Langbein et al. 2007). All the data collected on the Polar storage device was downloaded at the end of each day onto a laptop computer via infrared. Polar ProTrainer 5 software (Polar Electro Oy, Kempele, Finland) was used to obtain a graphic view of the R-R intervals. For each measurement, the 5 best minutes were selected by visual inspection. Reason to exclude (parts) of the data included the presence of more than 5% obviously false data (excessive artifacts, spikes or a high heart rate based on artifacts) (Visser, van Reenen et al. 2002, Wallen, Hasson et al. 2012, von Borell, Langbein et al. 2007). The Task Force of Cardiology (1996) states that the duration of the recordings should be standardized and they advise the use of short-term 5 minute recordings (Task Force of the European Society of Cardiology the North American Society of Pacing and Electrophysiology 1996).

The Polar ProTrainer 5 (PPT 5) software has automatic error correction options and calculates the time and frequency domain indices of HRV (Wallen, Hasson et al. 2012). Three different settings were chosen in the PPT 5 software to correct the raw data for errors and artifacts. Polar 1 is the setting without error correction, which displays the measured R-R intervals as registered by the storage device. Polar 2 is the setting with a very low filter power and a minimum protection zone of 1 beat per minute. This setting was advised by the Polar company when the aim of this equine research was explained (Polar Electro Nederland 2012). The last setting was one with a moderate filter power and a minimum protection zone of 6 beats per minute and is referred to as Polar 3. This is the standard setting in the PPT5 program and is generally used in human research (Wallen, Hasson et al. 2012, Nunan, Jakovljevic et al. 2008). As previously described, the best five minutes were chosen based on visual evaluation, followed by automatic correction by these three different PPT5 settings. The time domain indices RMSSD and SDRR were calculated by the Polar ProTrainer 5 software and documented in Microsoft Excel 2007.

ECG data processing

The data of the Spacelab Holter ECG were stored on a laptop computer after each recording with use of a card reader. All these data transferred from the laptop to a desktop computer with use of the card reader at once and analyzed with the Impressario software (Del Mar Reynolds Medical, Hertford, UK) on this computer. The Impressario software identifies the R-peaks in the ECG and marks them. These marks and the electrocardiogram (ECG) were visually inspected and corrected manually. The software calculated the time domain indices of HRV from this corrected ECG, using the same 5 minute part that was chosen from the Polar

ProTrainer 5 analyzed data. The time domain indices SDRR and RMSSD were documented in Microsoft Excel 2007.

Statistical analysis

The corrected Polar and ECG data were statistically analyzed with SPSS Statistics 18. The statistical methods were determined with acknowledgement of a statistician of Utrecht University, NL (J. van den Broek). First, scatter diagrams were used to estimate the relation between the three different Polar corrections (Polar 1, Polar 2 and Polar 3) and the Spacelab data. These diagrams were evaluated and the proportion of the 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 are to this line, the stronger the linear association of the data (RMSSD respectively SDRR) between the two variables Polar and ECG (Petrie, Watson 2006). The correlation coefficient is estimated by r, which is $\sqrt{R^2}$. A correlation coefficient equal to one means there is a perfect correlation between the two tests (Polar and ECG), a correlation coefficient equal to zero means there is no correlation at all between the two tests.

The individual horse effect was determined by using different colours in the scatter diagrams to distinguish each horse. After that, a linear mixed model analysis with random horse effect and with fixed effects day, gait, the Polar values RMSSD and SD and the Polar-gait interaction was performed to estimate if the linear association was significant. Assuming all five days where equal and followed the same protocol, the main effect ‘day’ is therefore considered as having no significant influence on the result. Therefore, day-effects were eliminated from the analysis. The linear mixed model analysis was used to determine which Polar correction method (Polar 1, Polar 2 and Polar 3) and which gait (gait 1: rest, gait 2: walk, gait 3: trot) obtained data that resulted in the highest significant correlation with the ECG data. The null hypothesis states that the population correlation coefficient is equal to zero, so there is no correlation between data obtained with a Polar or ECG. The alternative hypothesis, H1, states that the population correlation coefficient is not equal to zero, so there is a correlation between Polar derived data and ECG derived data. When $P < 0.05$, the null hypothesis was rejected.

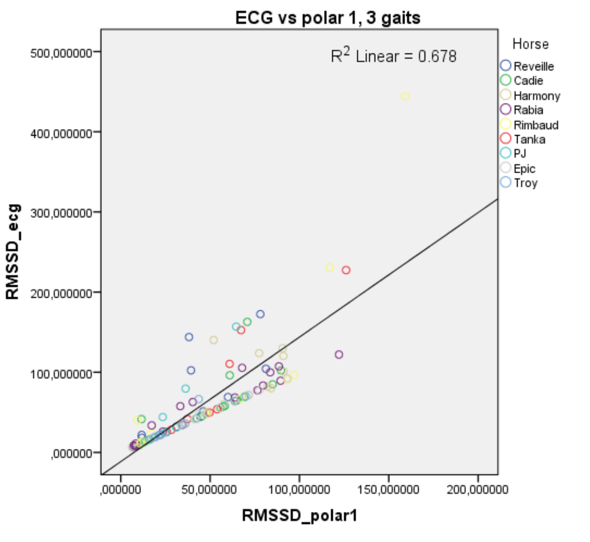
Results

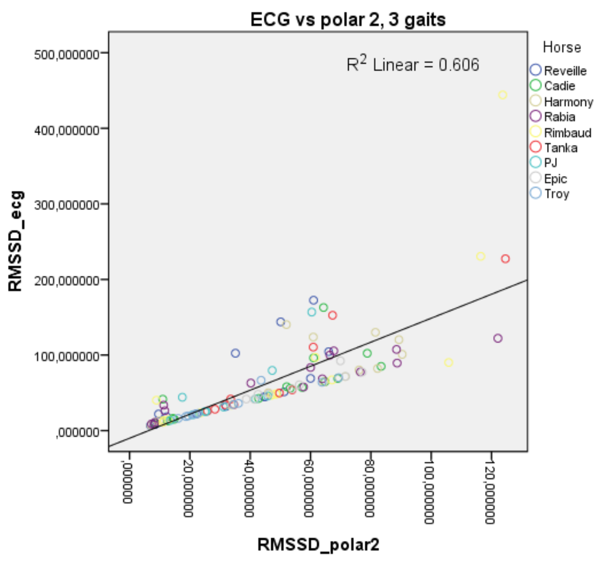
It was first determined which Polar correction method produces HRV data significantly correlating to the ECG data, to allow the use of Polar for data collection. Following that, the gait which gives the best correlation between the two tests was determined, so these outcomes can be used in following studies (chapter 3).

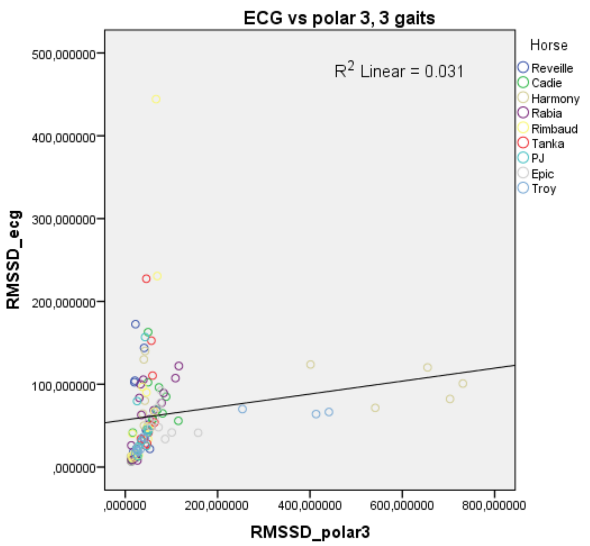
Results RMSSD

Polar 1, 2 and 3
First, the three gaits in all Polar correction methods were compared. The Polar 1 filter setting, using the time domain analysis RMSSD compared to the ECG data, correlates with an R^2 of 0.678 (r = 0.823) (figure 1). Using the Polar 1 filter setting, the acquired data had the highest R^2 compared to the Polar 2 setting (R^2 0.606, r = 0.778) and Polar 3 setting (R^2 0.031, r = 0.176) (figure 1). Therefore, Polar 1 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 can not be explained. For the Polar 2 filter 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 filter 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







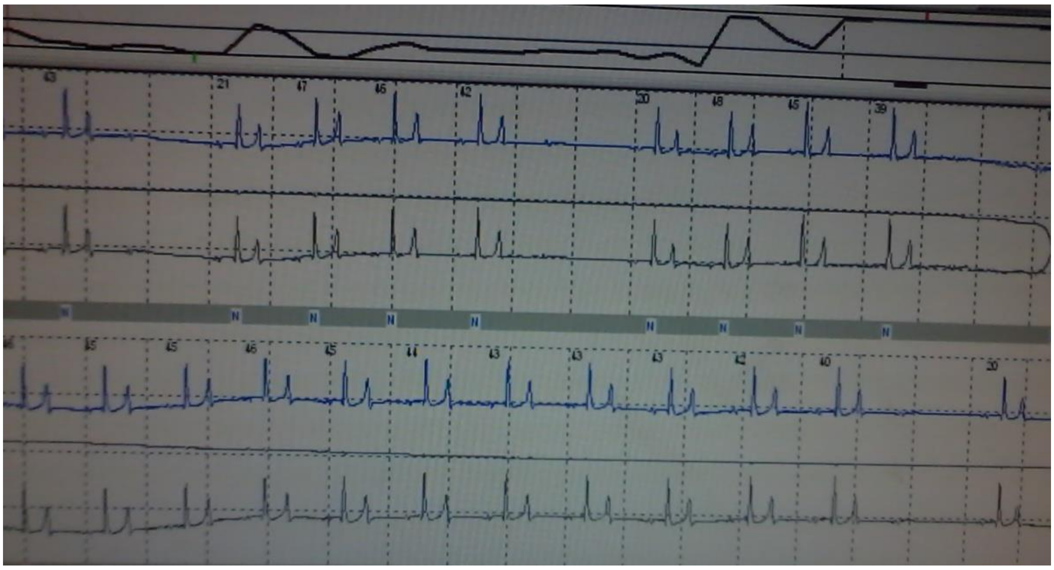
Note: scatterplots of RMSSD data of Polar (x-axis) versus ECG (y-axis). Three *gaits are outlined (rest, walk, trot), for every horse separately. R^2 Linear: proportion of the total variability explained by the relationship between Polar and ECG*

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

Polar 1

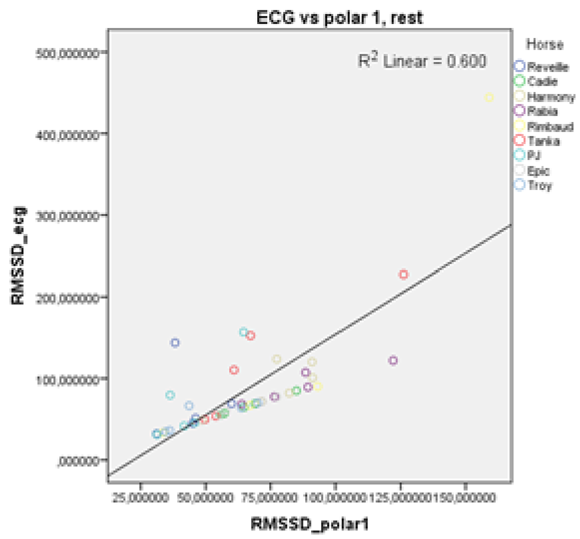
Gait 2 (walk) showed the best significant correlation between the RMSSD of the Polar 1 filter setting and ECG (R^2 0.695, r = 0.834, figure 2). An R^2 of 0.695 means that 70% of the total variation in Polar can be explained by the linear relationship between Polar 1 and ECG. Therefore, this gait had a low standard error and a small 95% confidence interval, which supports a close correlation. Gait 1 (rest) also displayed a significant correlation between the RMSSD of Polar 1 and ECG, although the R^2 was lower (0.600, r = 0.775) than that of gait 2 (figure 2). Therefore, this gait also had a low standard error and a small 95% confidence interval, which support a close correlation. Two horses were found to have second degree AV blocks at rest (shown by the electrocardiograms, picture 1) and extracting these horses out of the analysis, an R^2 of 0.613 (r = 0.783) in rest was estimated (figure 2). In both horses, the AV block was not present during walk or trot and the R^2 showed a minor difference 0.013 in favor of leaving the two horses out of the calculations. Besides correcting for the 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 very low (0.213), there was a high standard error and the 95% confidence interval was wide.

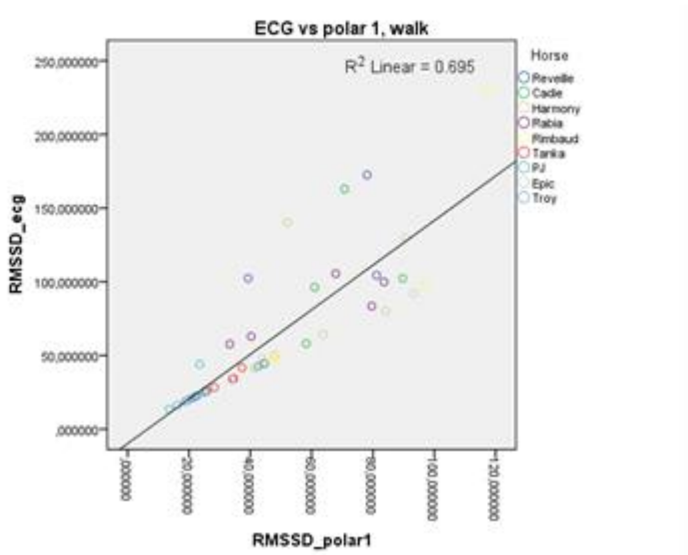
Picture 1: ECG showing second degree AV-blocks

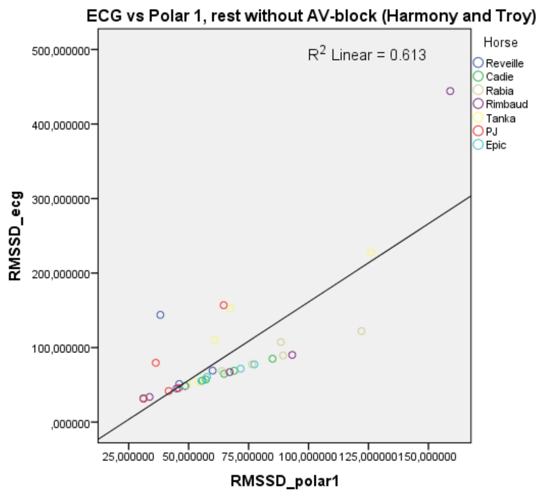


Note: second degree AV-blocks were visible in two horses during rest.

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







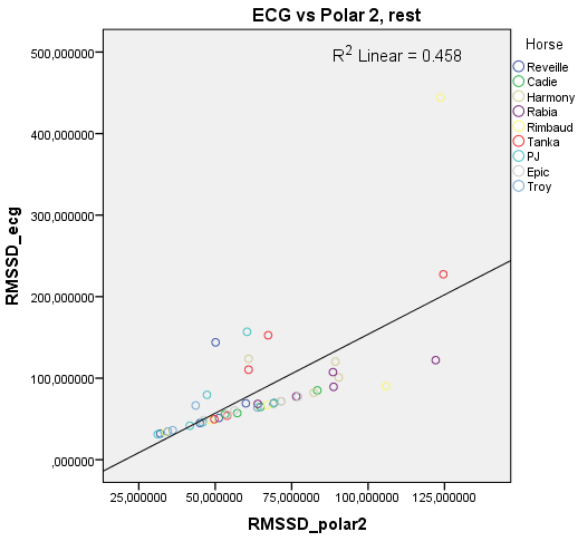
Note: scatterplots of RMSSD data of Polar 1(x-axis) versus ECG (y-axis). Two gaits are outlined (rest, walk and rest with AV block horses removed), for every horse separately. R^2 Linear: proportion of the total variability explained by the relationship between Polar and ECG

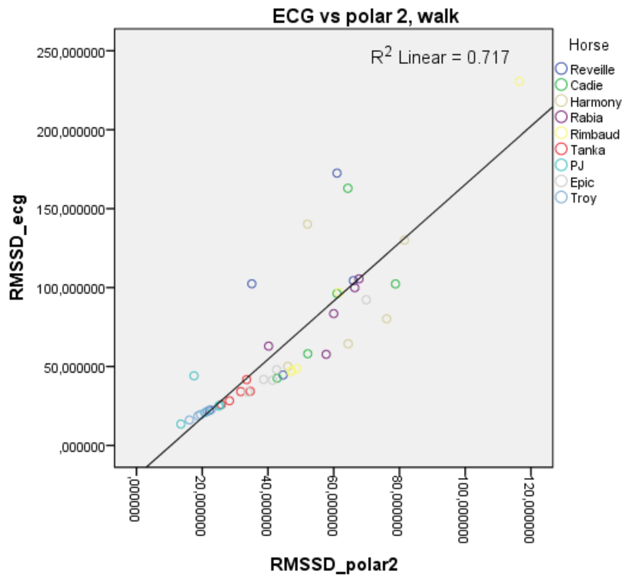
Polar 2

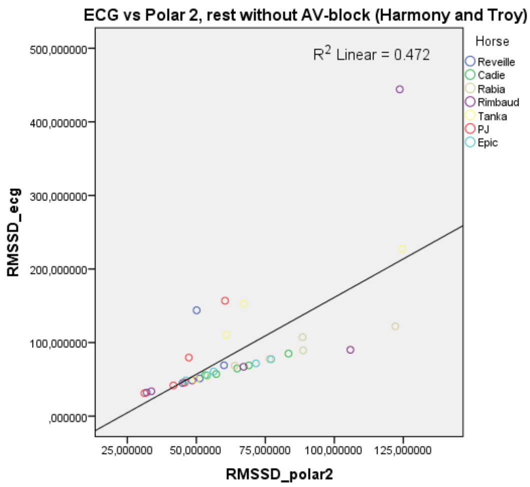
Gait 2 (walk) showed the best significant correlation between the RMSSD of the Polar 2 filter setting and ECG (R^2 0.717, r = 0.847, figure 3). An R^2 of 0.717 means that 72% of the total variation in Polar can be explained by the linear relationship between Polar 2 and ECG. Therefore, this gait had a low standard error and a small 95% confidence interval, which support 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 (figure 3). Therefore, this gait also had a low standard error and a small 95% confidence interval, which support a close correlation. Two horses were found to have second degree AV block at rest (shown by the electrocardiograms) and extracting these horses out of the analysis, an R^2 of 0.472 in rest was estimated (figure 3). In both horses, the AV block was not present during walk or trot and the R^2 showed a minor difference 0.014 in favor of leaving these two horses out of the calculations. Besides correcting for the 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

2 and ECG. Also, the R^2 was 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 Polar 2 compared to ECG, each gait separately







Note: scatterplots of RMSSD data of Polar 2 (x-axis) versus ECG (y-axis). Two gaits are outlined (rest, walk and rest wit 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 3

The RMSSD of the Polar 3 filter setting had no significant correlation with the RMSSD of the ECG in any gait. Considering the absence of significance and the low R^2 leaves Polar 3 out of the question for being a useful alternative for ECG measurements.

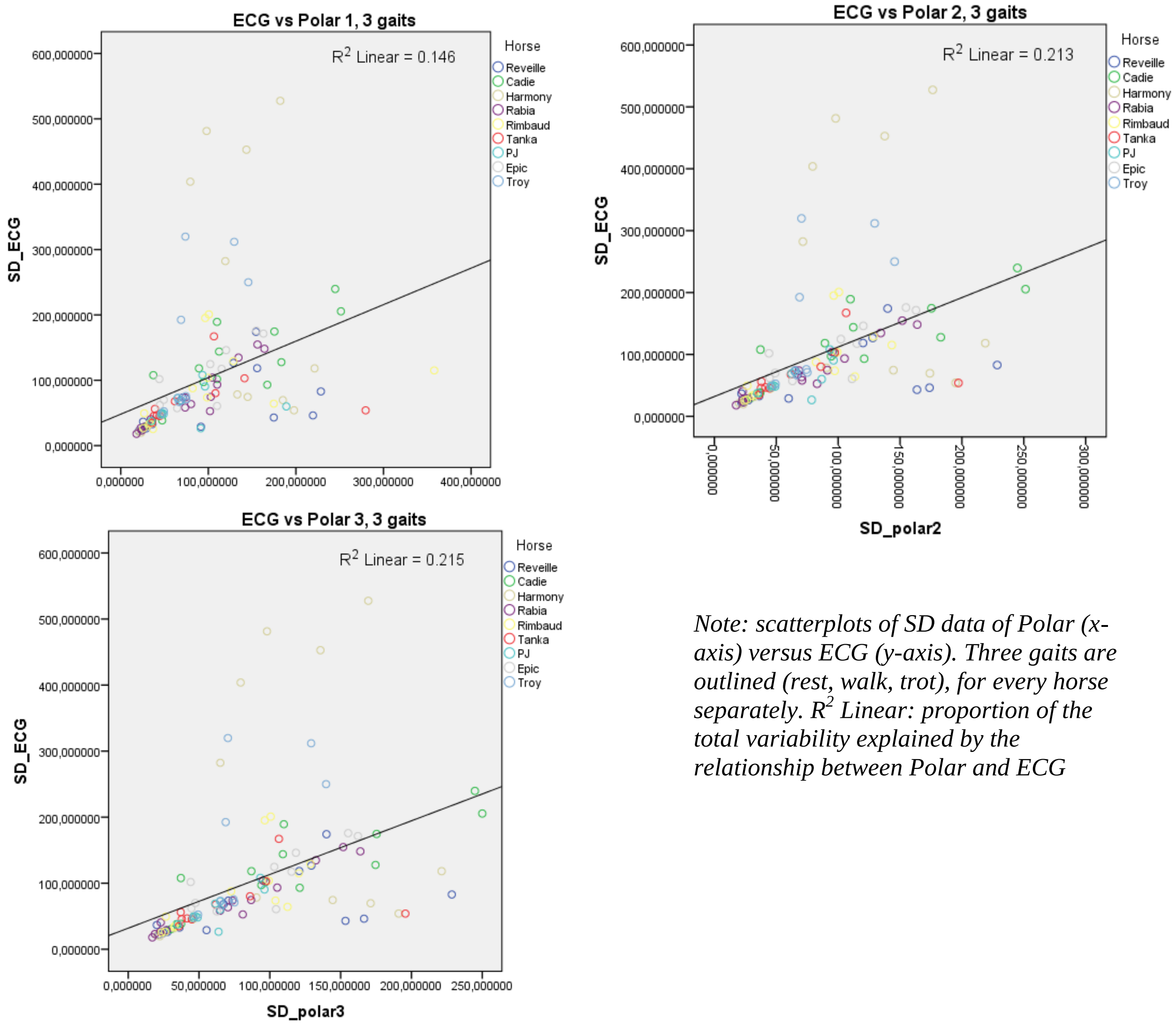
Results SD

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 Polar 1, 0.213 for Polar 2 and 0.215 for Polar 3 (figure 4). This means that only a low percentage of the Polar SD data can 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 indicates 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 with the 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

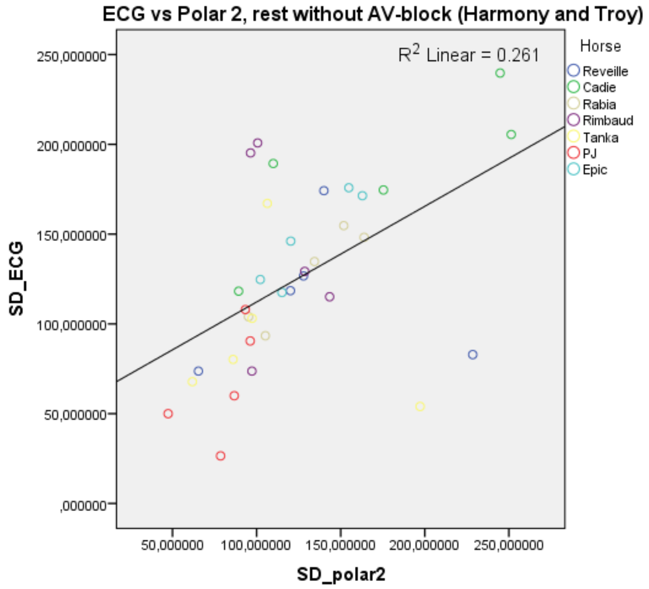
After this general comparison between the three Polar correction methods, each gait was investigated individually for each Polar correction method, to visualize if there was a stronger correlation between Polar and ECG in one gait in particular.

Polar 1

The SD of the Polar 1 filter 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, Polar 1 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 SD data. Even when the horses with second degree AV block were extracted from the analysis, the SD of Polar 1 still did not have a significant correlation with the SD of the ECG.

Polar 2
The SD of the Polar 2 filter setting showed only 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 AV-block horses) showed no significant correlation between the SD of Polar 2 and the ECG. This means that in these gaits, the Polar SD data cannot explain the variation in the ECG SD data. Polar 2 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 Polar 2 and the SD of the ECG would be significant, the percentage explained SD data is maximum 31%. The R^2 of the SD of Polar 2 in rest without the horses that showed second degree AV block was 0.261. This means that only 26% of the SD collected with the ECG can be explained by the SD of Polar 2.

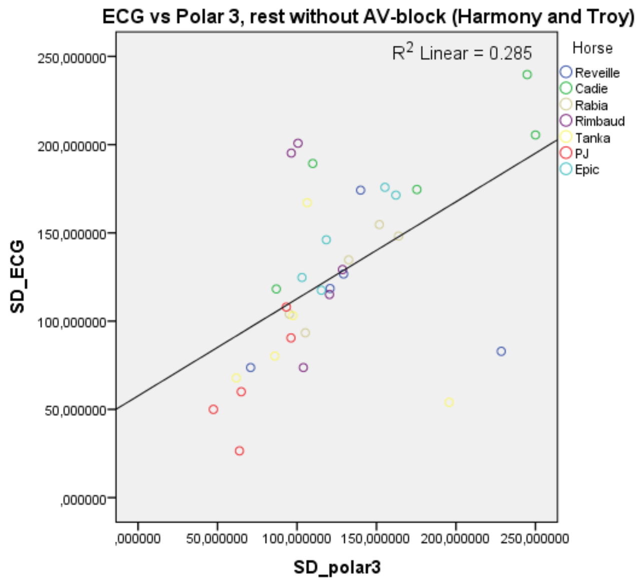
Figure 5: The correlation between the SD of the ECG and Polar 2 during rest, wit the horses that have a second degree AVblock removed



Note: scatterplot of SD data of Polar 2 (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 filter 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, figure 6), which means that only 28% of the SD collected with the Polar 3 can be explained by the SD of ECG.

Figure 6: The correlation between the SD of the ECG and Polar 3 during rest, with the horses that have second degree AV block removed



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

The results indicate a significant 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. During walk, the R^2 of the Polar 2 setting appeared to be the highest (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 corrected (Polar 2 and 3) data and the SD of the ECG in rest, but only when the horses with a second degree AV block were excluded from the dataset. 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).

Discussion

The outcome of this study only partly corresponds to previous equine research. Sloet van Oldruitenborgh-Oosterbaan et al. (1988), found a high correlation (0.997) between the heart rates obtained with a Polar device and telemetry during exercise on a treadmill (walk, trot, gallop). However, in this research only heart rates were examined, which gives a less accurate and detailed determination of the regulation of the autonomic nervous system than HRV (von Borell, Langbein et al. 2007). The measurement of HR is less sensitive to small deviations than HRV measurement. This could explain the better correlation found between the HR measured with Polar and ECG in comparison with the moderate correlation in HRV found in this study.

Nevertheless, Parker et al. (2009) found no significant differences in time domain indices (mean inter-beats intervals (IBI), mean heart rate (HR), RMSSD and pNN50) between the Polar S810i and ECG data recorded simultaneously on horses standing still and loose in a stable. There was less agreement between the Polar data and ECG in the field condition, where only mean IBI for both corrected and uncorrected data, and the RMSSD for corrected Polar data had no significant differences with the ECG. The same was found in our pilot study, were a better agreement was found between Polar and ECG while the horses were resting or walking. Trotting, compared to the 'field condition' in the study of Parker et al. (2009) had a weak correlation between the used time domain indices of Polar and ECG. The latter study did not use the SDRR as time domain indices, thus no comparison can be made at this point. Furthermore, equal to our pilot study, Parker et al. (2009) found a tighter relation between the uncorrected Polar data and the ECG data of standing horses and between the corrected Polar data and the ECG data when they were loose in the stable. In our pilot study, also a better correlation was found between corrected Polar data and the ECG data when the horses were walking, which could be comparable to horses loose in a stable.

In another study, Wallen et al. (2012) used the same Polar RS800 with Polar ProTrainer 5 software as used in the current research, and found good to excellent agreement with ECG acquired data for men on all evaluated HRV measures (SDRR and RMSSD). However, they also found that the PPT 5 software could not satisfactorily identify and correct all artifacts without visual inspection. In our pilot study, two different automatic correction methods of the PPT5 software were used and compared to no correction of the data at all. The findings of Wallen et al. (2012) correspond with the current research, where the corrected Polar data in most cases had a lower significant correlation coefficient than the uncorrected Polar data. In our research only during walk, the highest significant correlation coefficient was found with a low correction method (Polar 2) instead of uncorrected (Polar 1) RMSSD data. If the PPT5 software does not identify and correct all artifacts, this could mean that a higher correlation coefficient could have been found when the Polar heart rate monitor was used with a different correction program.

Based on this pilot study, there was a low correlation between the SDRR of the Polar RS800cx and the Holter ECG. Therefore, the SDRR of the Polar cannot be used interchangeably with the SDRR of the ECG based on the three correction methods used in the current study. SDRR is a good estimate of overall variability in heart rate and reflects all the cyclic components responsible for the HRV in the recording period. It gives an extensive and complete presentation of HRV, and therefore it is regretful that there was such a low correlation found between the Polar and ECG on this indices. However, RMSSD is one of the most commonly used measures for HRV and has the best statistical properties compared to the other time domain indices. It is a particularly good parameter to reflect vagal activity. A moderate correlation was found between the RMSSD obtained with the Polar RS800cx and the ECG, and this indices is a good parameter to reflect HRV (Task Force of the European Society of Cardiology the North American Society of Pacing and Electrophysiology 1996, (Visser, van Reenen et al. 2002, von Borell, Langbein et al. 2007, Gehrke, Baldwin et al. 2011).

There are various reasons why not all data obtained a high significant correlation between Polar and ECG. The best explanation why the Polar did not correlate perfectly with the ECG is the presence of artifacts which were incorrectly identified by the software. According to von Borell et al. (2007), artifacts can appear because of various reasons, mentioned in the introduction of this research. One of these reasons is a poor conduction between the electrodes and the skin. Also, the Polar device can double count the heart rate at each heart beat, because both T and R-waves are interpreted as R-waves (Polar Electro Oy, Finland). This error appears because the T-waves of horses can be very pronounced, and therefore can imitate the R-wave (von Borell, Langbein et al. 2007). Therefore, it is possible that sometimes T-waves were marked instead of R-waves, or that double counting occurred. This could explain the moderate correlation found between the data obtained with the Polar and the ECG.

Before the start of the study, there was one trial week in which the researchers and the horses could get familiar with the devices and the procedure. All horses were already conditioned to handling and the exerciser. In this trial week, various methods of installation of the Polar and ECG monitors were tried to create the least amount of artifacts. The size of the T-waves can be reduced by changing the site of the electrodes in some cases (von Borell, Langbein et al. 2007). Finally, the procedure described in the materials and methods section was found to be the best suited. During this trial, the speed of the exerciser was also determined by finding a speed in which all horses could comfortably walk or trot. Whenever a horse was standing still or trotting while he should be walking, or walking or cantering while he should be trotting, the speed was adjusted. After a few days, the most comfortable speed for all horses was set at a 3.2 miles/h walk and a 7.6 miles/h trot. This fixed speed was used to standardize the research. However, this could have influenced the results, since all horses were forced to walk or trot in this fixed speed instead of their own preferred pace.

Second, environmental factors could have influenced the amount of artifacts. These factors were kept as stable as possible with the least possible stimuli that could distract the horses. During the measurements, the horses were observed closely and if there was an obvious external influence, the measurement period would start over (Physick-Sheard, Marlin et al. 2000). This way, data with the least possible amount of noise was obtained. This method is not completely trustworthy, because all observations were subjective. Therefore, it is possible that environmental factors and thereby mental stress of the horses could have influenced the outcome of the study and that a higher correlation between Polar and ECG would have been found in a laboratory setting. In the study of Parker et al. (2009), less agreement between Polar data and ECG data was also found in the field condition compared to the stable. Nevertheless, the statement of the Polar company regarding the Polar RS800cx (Polar Electro Oy, Finland), does not distinguish between the application of the device in laboratory and field studies. Taking this into consideration, the Polar RS800cx should be compatible to be used in a field study.

Because not all measurements were taken on the same day, weather conditions were variable. Since an open air exerciser was used, the footing was dependent on the weather (dusty, heavy, puddles). Both weather and footing could have influenced the collected data and the mental state of the horses.

HRV is influenced by a variety of factors, like sex, breed, age, diurnal rhythms, respiration, fitness level, posture and physical activity. In scientific research, it is therefore important to standardize or control these factors (von Borell, Langbein et al. 2007). Variations in HRV should be detected by both Polar and ECG in the same amount, but there is no knowledge available to what extent the Polar device is able to do so. Because the results of this pilot study had to be used in various equine endurance studies, all horses were endurance Arabian or Anglo-Arabian. The diurnal rhythms were taken into account, and measurements were only performed in the morning. Furthermore, physical activity was divided into rest, walk and trot and these groups were separated in the data analysis. The sex of the horses was equally divided, with 5 mares and 4 geldings. There were three different fitness levels, but no further notice was given to separate the data of these three groups or the different sexes. Also, there was a big difference in age, ranging between 3 and 20 years of age (8,7 ± 5). In a research of Wallen et al. (2012), the same Polar RS800 with Polar ProTrainer 5 software, as used in the current research, had a good to excellent agreement with the ECG acquired data for men on all HRV measures. For women, especially woman over 60 years of age, the correlation between Polar and ECG was much weaker. This indicates that the differences in sex, age and possibly the fitness level could have more effect on the Polar system versus the ECG system and therefore could have influenced the data. However, scatterplots were made to evaluate the data and to avoid this error. In these scatterplots, except for two horses with a second degree AV block at rest, there was no evidence that individual horses were obscuring the data. Furtheremore, there was a rather large data set of 45 files per gait (9 horses, 5 days), which increased the reliability of the study.

A disadvantage of the Polar RS800cx device is that the R-R data only can be observed after the measurement, when the data is downloaded into a computer. It is impossible to see the cardiac electrical activity (i.e. the ECG). Whenever the Polar device was not measuring the heart rate correctly, the measurement was not started until an accurate recording was obtained. However, during the measurement, no judgement could be made about the value of the obtained data. This means that there is a delay in evaluation of the data, and therefore false data is excluded from the study but cannot be replaced by repeating the measurement. Additionally, it is impossible to know if some false data is included in the analysis. For example, if arrhythmia were present. On the computer, the Polar data was judged by visual inspection of the researchers. Based on previous research, the 5 best minutes were selected (Visser, van Reenen et al. 2002, Wallen, Hasson et al. 2012, von Borell, Langbein et al. 2007, Task Force of the European Society of Cardiology the North American Society of Pacing and Electrophysiology 1996). Because this selection was performed manually by only one researcher based on visual inspection, a certain amount of subjectivity cannot be ruled out. The ECG data was visually inspected and beat labelling was corrected by the researchers. Before using the Impressario software, the researchers received one sole verbal instruction and for using the PPT5 software only a guideline was present. Although the researchers worked very thoroughly, the lack of in-depth knowledge and experience could have contributed to the risk of error occurence in the ECG data interpretation.

Of the ECG data, the same 5 minute section that was chosen based on the Polar ProTrainer 5 software was analyzed. However, the Polar and ECG readings both had to be started manually which was impossible to perform on exactly the same time. On the computer, both Polar and ECG display the time alongside the R-R interval graphic respectively electrocardiogram. Nevertheless, the time on the Polar and ECG measuring devices could only be adjusted in whole minutes. Therefore, there could be a small time shift in Polar and ECG R-R interval results, which could have influenced the amount of correlation found between the two methods.

Conclusion

The hypothesis formulated for this research was: corrected HRV data collected by the Polar system correlate significantly with ECG-derived HRV data. However, the correlations were moderately high. The question complementary to this hypothesis was what the influence of exercise is on the validity of the results obtained with the Polar RS800cx. The results indicate a significant, but moderate correlation between the RMSSD of both the ECG and minor 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) were not significantly correlated with the obtained ECG results. The RMSSD of Polar 2 during walk showed the highest correlation with the RMSSD of ECG (R^2 0.717, r = 0.847). During rest, the RMSSD of Polar 1 showed the highest correlation with the RMSSD of the ECG (R^2 0.600, r = 0.775).

Our results indicate a significant correlation between the SD of corrected (Polar 2 and 3) data and the SD of the ECG only when the horses with second degree AV block were excluded from the data set. However, the correlation between the SD of the Polar 2 and 3 and the SD of the ECG (Polar 2: R^2 0.261 versus Polar 3: R^2 0.285) were very low. 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.

From our results, the walking-gait with Polar 2 (low filter power, 1 beat per minute protection zone) to obtain RMSSD data with the Polar RS800cx device yields HRV data closest to the ECG. Using ECG derived recordings would still be the best choice for data collection, however this was not possible in the experimental design as described in chapter three.

Chapter three: Competition

Summary

Reasons for performing study: Cardiovascular function is regulated by the autonomic nervous system and heart rate variability (HRV) is a way to assess this regulation. A high HRV is a sign of healthy cardiac function and allows a better response to changing environmental requirements. The goal of the current research was to determine if HRV, measured the day before and directly after a competition, can predict the performance level during that endurance competition.

Aim of the study: The expectation was that elite endurance horses have higher HRV than non-elite or disqualified horses at endurance events because of a better fitness level. Therefore, there will be examined to what extent HRV measured before an endurance competition can predict the outcome of the ride and to what extent HRV measured after the competition can be related to the outcome of the ride.

Methods: Measurements were taken the day before and immediately after the finish of two separate endurance competitions with the Polar RS800cx. Data obtained both before and after riding 100, 75 and 50 mile distances at these events were used. RMSSD values were calculated and analyzed with one-way ANOVA and logistic regression analysis.

Results: RMSSD measured the day before and immediately after the competition did not result in any significant differences between the groups (elite, non-elite and disqualified) nor a significant probability to finish in a certain group. The HRV, and therefore the difference in dominance between the sympathetic and parasympathetic nervous system, had no predictable value for the outcome of a competition in our study.

Conclusions: No statements can be made about the HRV in different groups of endurance horses. There was no distinction found between the HRV of elite, non-elite and disqualified horses. Following this research, RMSSD as a measurement of HRV, cannot be used to provide information on the performance level of the horses included in this study at a given point in time.

Introduction

Heart rate variability is used in different fields of human and veterinary research. In 1965, the first clinical use of HRV was recognized when researchers noticed that a drop in HRV correlated with fetal distress before change in heart rate was detected (Task Force of the European Society of Cardiology the North American Society of Pacing and Electrophysiology 1996, Saul 1990). Also, a decrease in HRV was found useful for risk assessment and prediction of adverse outcomes in several clinical settings in humans, such as acute myocardial infarction and congestive heart failure (Lombardi, Stein 2011, Radespiel-Tröger, Rauh et al. 2003, Task Force of the European Society of Cardiology the North American Society of Pacing and Electrophysiology 1996, Pieper, Hammill 1995). Since then, there have been various studies in equine research evaluating HRV, including its use to assess pain, acute and chronic stress, various pathological conditions (like laminitis), problems according to management or training regimes and overtraining. In most of these studies a significant reduction in HRV was found under more stressful circumstances (Matsuura, Tanaka et al. 2010, Kinnunen, Laukkanen et al. 2006, Rietmann, Stauffacher et al. 2004, von Borell, Langbein et al. 2007, Visser, van Reenen et al. 2002, Rietmann, Stuart et al. 2004, Cottin, Medigue et al. 2005). The majority of equine exercise studies evaluating HRV were performed on non-endurance horses, such as Standardbred or Thoroughbred horses, with a maximum sample size of 10 horses (Cottin, Medigue et al. 2005, Kinnunen, Laukkanen et al. 2006, Physick-Sheard, Marlin et al. 2000, Cottin, Barrey et al. 2006).

Kinnunen et al. (2006) showed that intense exercise decreased the HRV of trotters the day after the training. Another finding was that elite horses and previously raced horses had a significantly higher HRV in all cases during three different training periods compared to the non-elites and the ones that had not raced yet. In this study, the training periods were precompetition, basic and competition periods and HRV was measured with the Polar S810, 3-5 times a week during 12 months. The goal was to detect the effect of exercise intensity on the HRV the following day (Kinnunen, Laukkanen et al. 2006). A similar equine study by Voss et al. (2002) demonstrated a decrease in SDRR, measured with an ECG, when proceeding from light to heavier workloads in an aqua treadmill. This finding of a decrease in HRV whith increasing workloads was confirmed by Thayer et al. (1997), which also used an ECG to obtain the data. Decreased HRV, supported by a decreased mean RR interval, RMSSD and SDRR, was also found during repeated exercise and in the rest periods in between (Cottin, Barrey et al. 2006) and when longing a horse in comparison to rest (Becker-Birck, Schmidt et al. 2012, Schmidt, Aurich et al. 2010). These last three research projects described all used the Polar S810 to obtain HRV data. In all the research projects mentioned above, HRV was used to estimate the impact of exercise.

Although it is known that high HRV allows a better adaptation to a changing environment (Thayer, Hahn et al. 1997), it has not yet been used to predict fitness and thereby outcome in equine competition as far as the authors know. Nevertheless, studies in human research have shown that increased (baseline) HRV can be related to improved performance in endurance running and a better adaptation to endurance training. (Buchheit, Chivot et al. 2010, Vesterinen, Hakkinen et al. 2011). On the other hand, low HRV seems to predict a poor adaptation to endurance training (Vesterinen, Hakkinen et al. 2011). The HRV in all these cases was measured before, during and after the training period. During the training period, the HRV was measured at rest and during and after exercise. HRV was even suggested to be an indicative measurement for overtraining in human athletes (Baumert, Brechtel et al. 2006, Tian, He et al. 2012).

The goal of the current research was to determine if HRV, measured the day before and directly after a competition, can predict the performance level during that endurance competition. Following this step, even training schedules could possibly be based on this measurement and assist in bringing the horse to the highest possible level and reducing the risk of overtraining (Baumert, Brechtel et al. 2006, Kiviniemi, Hautala et al. 2007, Tian, He et al. 2012). The hypothesis was that elite endurance horses have a higher HRV than non-elite or disqualified horses at endurance events because of a better fitness level.

Materials and methods

Horses

Data was collected at two different AERCcompetition rides in the USA, the Vermont Moonlight ride and the Biltmore AERC National Championship Endurance. The breed, age and sex of the participating horses was variable and no background information was known, although the majority of the horses were Arabians or Arabian crosses. All horses were at least five years old at the time of the ride, since this is one of the AERC competition requirements.[1] Nothing is known about training, nourishment and housing of the horses. At both competitions, almost all horses were transported to the rides over variable distances. The horses arrived at least half a day to one week before the competition. Most participating horses were placed in small grazing paddocks alongside the trailer, alone or with one or two other horses. Some were restrained with a high-tie, a long elastic rope to a high pole secured to the trailer, alongside the trailer. All horses were participating in the 50, 75 or 100 mile distance.

Field circumstances

The Vermont Moonlight ride was held on the 21st of July 2012.. The competition included the 100, 75 and 50 mile distances. The Vermont Moonlight ride was held near West Windsor, Vermont. This area is situated in east central Vermont, just west of the Connecticut River and the New Hampshire border. The 100, 75 and 50 mile competitions were held simultaneously on the same trail. In addition to the endurance horses, an ultramarathon was simultaneously held on the course, so that the horses had to share the trail with 350 human athletes running the 100 miles. The geographical characteristics of this trail are woodlands, rolling meadows and small streams, with significant elevation changes over the entire course.[2] Vermont has a humid continental climate. On the 21st of July, the temperature was 21 to 29 degrees Celsius in daytime with a minimum of 13 degrees Celsius at night. No rain fell and the windspeed was 3 km/h.[3]

The Biltmore AERC National Championship Endurance was held on the 20th and 22nd of September 2012.The competition included only the 100 and 50 mile distances. The Biltmore AERC National Championship Endurance was situated on the grounds of George Vanderbilt's historic Biltmore Estate in Asheville, North Carolina. The ride follows the French Broad River and leads over rolling hills and low mountains.[3] North Carolina has a humid subtropical climate. On the 20th of September, when the 100 mile competition was held, the temperature was 19 to 23 degrees Celsius in daytime with a minimum of 14 degrees Celsius at night. No rain fell and the windspeed was 7 km/h. On the 22nd of September, during the 50 mile competition, the temperature was 21 to 27 degrees Celsius in daytime with a minimum of 13 degrees at night. No rain fell and the windspeed was 11 km/h.[4]

[1] AMERICAN ENDURANCE RIDE CONFERENCE, 2011. AERC Rules and Regulations.

[2] USA GOVERNMENT, 2013-last update, West Windsor, Vermont. http://www.westwindsorvt.govoffice2.com/ (april, 2013).

[3] BILTMORE EQUESTRIAN CENTER, 2012-last update, AERC national championship. http://www.aercatbiltmore.com/ (april, 2013).

[4] WEATHER.ORG, 2013-last update, Weather History. http://weather.org/weatherorg_records_and_averages.htm (april, 2013).

Both the Vermont and Biltmore competitions were national endurance rides on the East Coast of the United States of America. The geographical, climate and weather conditions were found to be very similar.

Equipment

The Polar RS800cx with PPT 5 software (Polar Electro Oy, Kempele, Finland) was used to measure heart rate variability continuously. The attachment of the Polar belt was the same as described in chapter two. The only exception was that in this field study, no warm water was available. Therefore, cold water was used to moisten the skin to improve the contact between electrode and skin (Munsters, Visser et al. 2012). After that, electrode gel was placed between the skin and the electrode to maximize the conduction (Parker, Goodwin et al. 2009, Hopster, Blokhuis 1994).

Experimental procedures

The aim of the research was explained to the riders while installing the Polar device. The recordings were taken both 'before' and 'after' the competition. The recordings before the ride were obtained right after the veterinary check-in just outside the veterinary area. These measurements were all in the afternoon on the day before the ride. The time of recording after the finish could vary between late afternoon and early morning, depending on the time of finishing for the individual competitor. These recordings took place at the crewing area of the horse. The recordings of the horses that were disqualified were taken as soon as possible at the treatment barn or at the vet gate where disqualification occurred.

In the subsequent part of this research the words 'before' and 'after' the competition will be used to refer to these two measurement periods. All recordings lasted 7 to 10 minutes, depending on the willingness of the rider to participate. Especially after the ride, several riders were reluctant to participate in the study, instead of taking care of their horses and go to sleep. During the recording, the horses were eating or grazing while standing in one spot. Whenever there was an obvious external influence or the horse reacted to something, the recording time was prolonged. If the Polar device was not measuring correctly before the measurement started (for example showing a very high heart rate that was not accurate), the measurement was not started until a correct recording was obtained after reapplying the equipment.

Data processing

As described in chapter two, all the data collected on the Polar storage device was downloaded into a laptop computer via infrared at the end of each day. Polar ProTrainer 5 software was used to obtain a graphic view of the R-R intervals. Of each measurement, the 5 best minutes were selected by visual inspection. The reason to exclude (parts) of the data was the presence of more than 5% obviously false data (excessive artefacts, spikes or a high heart rate based on artefacts) (Visser, van Reenen et al. 2002, Wallen, Hasson et al. 2012, von Borell, Langbein et al. 2007). Only the RMSSD was used as a time domain variable because this was the only variable that had a significant correlation and a moderate correlation coefficient compared to the ECG (chapter two). The participating horses were standing quietly while eating or grazing, which is highly comparable with a resting state. Therefore, the Polar ProTriainer 5 setting without any correction (Polar 1) was used because this setting had the highest R^2 in rest (0,600) compared to the ECG (chapter two). The RMSSD values were calculated by the PPT5 software and documented in Microsoft Excel 2007.

Sample size
A total of 218 horses were starting in the competitions. Each horse was assigned to one of three groups, either the elite, non-elite or disqualified group. Horses were considered elite if they won or finished one of the competition rides within 10% of the winning time. Horses were considered non-elite if they completed the ride, but not within 10% of the winning time. Horses were considered disqualified if one of the ride veterinarians disqualified them from the competition.

The percentage participation depended on the willingness of the riders. To improve the motivation to participate, the aim of the study was announced on the websites of the rides. Of the total of 218 horses, 157 participated in the study (table 1). This resulted in 224 Polar data files, of which 107 were collected before and 117 were collected after the competition. This was 90-97 percent of the starting 100 mile horses, 100 percent of the starting 75 mile horses and 55-60 percent of the starting 50 mile horses. Of these 157 files, an average of 23 percent (13-36% depending on distance and ride) was excluded based on more than 5 percent obvious false data at visual inspection of the Polar files (Visser, van Reenen et al. 2002, Wallen, Hasson et al. 2012, von Borell, Langbein et al. 2007).

Table 1: Number of horses participating in the HRV research

Competition	# horses starting (# disqualified)	# horses participating in the study	# horses measured before the ride	# horses measured after the ride	# excluded data files (%)
Vermont 100	30 (6)	29	21	26	10 (21%)
Vermont 75	6 (0)	6	6	4	2 (20%)
Vermont 50	38 (5)	21	11	12	4 (17%)
Biltmore 100	48 (17)	43	36	32	9 (13%)
Biltmore 50	96 (23)	58	33	43	27 (36%)
Total of all competition	218 (51)	157	107	117	52 (23%)

Note: the number of horses starting and participating in the research of all 5 competitions are shown, with underneath the total amount of horses starting and participating. The last column displays the amount data files excluded based on more than 5 percent obvious false data at visual inspection of the Polar files.

Statistical analysis
The visually inspected, uncorrected Polar obtained RMSSD data was statistically analyzed with SPSS statistics 18. The statistical methods were determined with acknowledgement of a statistician of Utrecht University, NL (H. Vernooij). The RMSSD values obtained 'before' and 'after' the competition were used as input for the statistical analysis. To prevent any false conclusions, each competition was evaluated separately, because the events and circumstances were thought to be very similar but not entirely interchangeable. For the analysis, first the one-way analysis of variance (one-way ANOVA) and subsequently the logistic regression analysis was performed. A combination of these two analyses was used because the one-way ANOVA can only explain the difference between group variances, but does not assign a predicted value to the mean RMSSD of the different groups (elite, non-elite and disqualified). The logistic regression analysis is therefore used as a predicted measure to evaluate the chance that a certain horse belongs to a certain group.

First, box plots were made in SPSS statistics 18 to evaluate the distribution of the data set and to identify possible outliers (visualized with dots) and extreme values (visualized with asterisks). Following the criteria of the one-way ANOVA, the extreme values were excluded from the analysis. After this, the one-way ANOVA, with ‘group’ as factor, ‘RMSSD’ as dependent variable and the post hoc Bonferroni test, was performed. The variable ‘group’ is explained by the elite, non-elite and disqualified status of the different participating horses. The reason to perform the one-way ANOVA was to determine if the population group means differ, thus to verify if there is a difference between the mean RMSSD in the elite, non-elite and disqualified group (Petrie, Watson 2006). One of the assumptions of the one-way ANOVA is that the group variances are equal. Whenever the assumption ‘equal group variances’ is not satisfied, identified with Levene’s test of homogeneity of variances, a log transformation will be used to equalize the group variances.[5] In the one-way ANOVA, the H0 states that there is no difference between the mean RMSSD of the elite, non-elite and disqualified group. The alternative hypothesis, H1, on the other hand states that at least one of these group means is dissimilar. With the post hoc Bonferroni test, the dissimilarity can be analyzed to determine exactly which group means differ significantly from each other (Petrie, Watson 2006). In both the one-way ANOVA and the post hoc Bonferroni test, the null hypothesis is rejected if $P < 0,05$.

After the one-way ANOVA, the logistic regression analysis was performed. The logistic regression analysis gives a more complete answer to the aim of this study, namely to find out to what extent elite horses have a higher HRV compared to non-elite horses or horses that were disqualified. There will be examined to what extent HRV measured before an endurance competition can predict the outcome of the ride and to what extent HRV measured after the competition can be related to the outcome of the ride. In the logistic regression analysis, the dependent variable ‘group’ was divided in elite, non-elite and disqualified horses. RMSSD was used as a covariate. Only two groups can be compared at the same time, therefore the analysis was performed three times for every distance. The outcome of the analysis predicts the chance (odds) to be part of the elite, non-elite or disqualified group based on the RMSSD. Hereby, the null hypothesis states that the odds ratio is zero, thus RMSSD cannot predict the group to which a certain horse belongs (elite, non-elite or disqualified). The alternative, H1, hypothesis states that the odds ratio is not equal to zero, thus RMSSD can predict the group to which a certain horse belongs. The null hypothesis is rejected if $P < 0,05$. The odds ratio itself indicates the magnitude and direction of the probability to belong to one group versus the other if RMSSD increases by one unit. The percentage of change is the odds ratio minus one. Thus, if the odds ratio is one or close to one, this indicates an equal chance to belong to each group and thus no difference between the groups. When the odds ratio is higher than one, there is a positive effect. When the odds ratio is between zero and one, there is a negative effect. Finally, the Nagelkerke R^2 displays the quality of the model and is comparable to the R^2 of linear regression analysis. The R^2 gives the proportion of the total variance in one group (elite, non-elite or disqualified) that can be explained by the RMSSD. Even if there is a significant odds ratio, the R^2 displays the quality of the model (Sieben 2002, Petrie, Watson 2006).

[5] MEDCALC.ORG, 2013, One-way analysis of variance, http://www.medcalc.org/manual/one-way_analysis_of_variance.php (december, 2012).

Results

Time points of data sampling

The recordings of the finished horses were taken between 8 minutes and 75 minutes after completion, of which 90% was within 30 minutes and 70% within 15 minutes after completion (18 ± 16 min). The recordings of the disqualified horses were taken between 8 minutes and 4 hours after disqualification, of which 90% was within 90 minutes after disqualification (63 ± 48 min). This delay in collecting the data of the disqualified group was due to the time the rider gets to prepare his horse before he presents his horse to the veterinarians for re-examination and the time used to transport the horse to the treatment barn. In the Vermont 75, no horses were disqualified so this data could not be obtained. In the Vermont 50, the disqualified group was only measured after the ride and in the Biltmore 50, the elite group was only measured after the ride. Therefore, several groups are missing in the underlying analyses.

Results of RMSSD data collected before competition

First, a one-way ANOVA was performed on the RMSSD data obtained before the competition. In the Vermont 100, no equal group variances were identified with Levene's test of homogeneity of variances. Therefore, a log transformation was used to equalize the group variances (table 2). In all the five different distances, no significant difference was found between the mean RMSSD of the elite, non-elite and disqualified group (table 2). This means there was no indication that RMSSD was higher or lower in a particular group compared with the others and thus there was no difference in the sympathetic/parasympathetic balance between elite, non-elite and disqualified horses based on this HRV variable. Most of the standard deviations were very high (table 2).

Table 2: Difference in mean RMSSD between elite, none-elite and disqualified groups

		Group mean RMSSD		
Distance	**Sig.**	**Elite (N; SD)**	**Non-elite (N; SD)**	**Disqualified (N; SD)**
Vermont 100**	0,186	88,47 (3; 54,60)*	55,70 (14; 25,00)*	124,33 (3; 117,65)*
Vermont 75	0,561	64,00 (3;18,43)	78,70 (1; 0,00)	***
Vermont 50	0,984	62,95 (2; 9,55)	62,40 (8; 36,38)	***
Biltmore 100	0,165	51,78 (5; 23,99)	47,84 (14; 12,32)	68,91 (8; 37,82)
Biltmore 50	0,685	***	112,72 (18; 64,68)	95,83 (3, 73,57)

*Note: one-way ANOVA on RMSSD data obtained before the competition to differentiate between elite, non-elite and disqualified horses.The group mean RMSSD are presented (group size (N) and standard deviation of the RMSSD (SD)). A significant (sig.) difference between groups is found when P<0,05. * Values before log-transformation, ** Distances with log-transformation (when no equal group variances were identified with Levene's test of homogeneity of variances, a log transformation was performed to equalize the variances) *** No data available for this group*

Although there were no significant differences between the mean RMSSD of the three groups found with the one-way ANOVA, the logistic regression analysis was performed to estimate if the RMSSD before the ride can predict the group to which a horse belongs. However, no significant odds ratios were found with this analysis (table 3). This means no proof was found that the RMSSD obtained before the ride can predict the group (elite, none-elite or disqualified) in which a certain horse belongs. In conclusion, RMSSD, and therefore the difference in dominance between the sympathetic and parasympathetic nervous system, had no predictable value for the outcome of a competition. Even if the odds ratio would be significant, there were very low Nagelkerke R^2 values (table 3), meaning that the model is of low quality.

Table 3: Predictability of RMSSD values on outcome of competition

Distance	Group comparison	Odds Ratio	Significance	Nagelkerke R^2
Vermont 100	elite (0) – non-elite (1)	0,972	0,144	0,210
	elite (0) – disqualified (1)	1,006	0,579	0,072
	non-elite (0) – disqualified (1)	1,020	0,163	0,283
Vermont 75	elite (0) – non-elite (1)	0,000	0,994	1,000
	elite (0) – disqualified (1)	***	***	***
	non-elite (0) – disqualified (1)	***	***	***
Vermont 50	elite (0) – non-elite (1)	0,999	0,982	0,000
	elite (0) – disqualified (1)	***	***	***
	non-elite (0) – disqualified (1)	***	***	***
Biltmore 100	elite (0) – non-elite (1)	1,008	0,735	0,009
	elite (0) – disqualified (1)	0,393	0,310	0,177
	non-elite (0) – disqualified (1)	1,019	0,143	0,149
Biltmore 50	elite (0) – non-elite (1)	***	***	***
	elite (0) – disqualified (1)	***	***	***
	non-elite (0) – disqualified (1)	0,995	0,669	0,016

*Note: logistic regression analysis on RMSSD data obtained before the competition to predict the group (elite, non-elite or disqualified) to which a horse belongs.The odds ratio indicates the magnitude and direction of the probability to belong to one group versus the other if the RMSSD increases by one unit. The percentage of chance is the odds ratio minus one. The Nagelkerke R^2 displays the quality of the model and is comparable to the R^2 of lineair regression analysis. A significant odds ratio is found when $P<0,05$. *** No data available for this group*

Results of RMSSD data collected after competition

Comparable to the previous part of this section, first a one-way ANOVA was performed on the RMSSD data collected after the competition. In the Biltmore 100, no equal group variances were identified with Levene's test of homogeneity of variances. Therefore, a log transformation was used to equalize the group variances (table 4). In four of the five different competition distances, no significant difference was found between the mean RMSSD of the elite, non-elite and disqualified group (table 4). This means there is no indication that the RMSSD is higher or lower in a particular group compared to the others, i.e. there was no difference in the sympathetic/parasympathetic balance between elite, non-elite and disqualified horses. As in the one-way ANOVA performed on the RMSSD data obtained before the competition, most standard deviations were rather high (table 4).

In the Vermont 100 a significant difference ($P = 0,009$) was found in the mean RMSSD of the elite, non-elite and disqualified group. This indicates that at least one of the group RMSSD means was dissimilar (table 4). For that reason a post hoc Bonferroni test was performed. With this test, there was a significant difference found in mean RMSSD between the elite and non-elite group ($P = 0,044$) and between the elite and disqualified group (P =

0,008). The mean RMSSD of the elite group was the lowest (14,80) followed by the non-elite group (46,50) and the disqualified group (60,00) (table 4). RMSSD is one of the time domain indices that reflects HRV. This means that the elite group had the lowest HRV compared to the non-elite and disqualified group. There was no significant difference between the mean RMSSD of the disqualified and non-elite group. The outcome of the post hoc test indicates a shift in autonomic balance towards a more sympathetic dominance in the elite group and a shift towards a more parasympathetic dominance in the non-elite and disqualified group.

Table 4: Difference in mean RMSSD between elite, none-elite and disqualified groups

		Mean		
Distance	**Sig.**	**Elite (N; SD)**	**Non-elite (N; SD)**	**Disqualified (N; SD)**
Vermont 100	0,009	14,80 (2; 3,11)	46,50 (10; 14,65)	60,00 (5; 16,52)
Vermont 75	0,587	54,47 (3; 30,46)	31,90 (1; 0,00)	***
Vermont 50	0,513	34,85 (2; 3,46)	40,94 (5; 12,47)	29,55 (2; 12,66)
Biltmore 100**	0,333	49,40 (5; 18,72)*	47,53 (13; 26,47)*	59,87 (10; 70,16)*
Biltmore 50	0,682	24,40 (1; 0,00)	63,86 (17; 50,77)	58,45 (10; 30,13)

*Note: one-way ANOVA on RMSSD data obtained after the competition to differentiate between elite, non-elite and disqualified horses.The group mean RMSSD are presented (group size (N) and standard deviation of the RMSSD (SD)). A significant (sig.) difference between groups is found when P<0,05. * Values before log-transformation, ** Distances with log-transformation (when no equal group variances were identified with Levene's test of homogeneity of variances, a log transformation was performed to equalize the variances) *** No data available for this group*

Following, the logistic regression analysis was performed to estimate if the RMSSD measured after the competition can predict the group to which a horse belongs. With this analysis, no significant odds ratios were found (table 5). This means no proof was found that the RMSSD obtained after the ride can predict the group (elite, none-elite or disqualified) in which a certain horse belongs. Concluding, the RMSSD, and therefore the difference in dominance between the sympathetic and parasympathetic nervous system, could not predict the outcome of a competition. As in the logistic regression analysis performed with RMSSD data obtained before the ride, the Nagelkerke R^2 values were very low. This means that even if the odds ratio would have been significant, the model is of low quality.

Table 5: Predictability of RMSSD values on outcome of competition

Distance	Group comparison	Odds Ratio	Significance	Nagelkerke R^2
Vermont 100	elite (0) – non-elite (1)	1,000	0,996	0,000
	elite (0) – disqualified (1)	1,024	0,402	0,110
	non-elite (0) – disqualified (1)	1,076	0,100	0,265
Vermont 75	elite (0) – non-elite (1)	0,864	0,554	0,373
	elite (0) – disqualified (1)	***	***	***
	non-elite (0) – disqualified (1)	***	***	***
Vermont 50	elite (0) –non-elite (1)	1,068	0,477	0,202
	elite (0) – disqualified (1)	0,888	0,482	0,183
	non-elite (0) – disqualified (1)	0,913	0,282	0,331
Biltmore 100	elite (0) – non-elite (1)	0,997	0,879	0,002
	elite (0) – disqualified (1)	1,014	0,370	0,113
	non-elite (0) – disqualified (1)	1,016	0,167	0,146
Biltmore 50	elite (0) – non-elite (1)	1,108	0,338	0,261
	elite (0) – disqualified (1)	1,146	0,365	0,407
	non-elite (0) – disqualified (1)	0,997	0,752	0,005

*Note: logistic regression analysis on RMSSD data obtained before the competition to predict the group (elite, non-elite or disqualified) to which a horse belongs.The odds ratio indicates the magnitude and direction of the probability to belong to one group versus the other if the RMSSD increases by one unit. This percentage of chance is the odds ratio minus one. The Nagelkerke R^2 displays the quality of the model and is comparable to the R^2 of lineair regression analysis. A significant odds ratio is found when $P<0,05$. *** No data available for this group*

Discussion

Based on previous research, there was expected that the elite group would have the highest HRV compared to the other groups. However, the findings in the current study were the opposite of what was expected. In the results of this research, there was only a significant difference found in one distance, namely the Vermont 100 RMSSD values obtained after the competition. The elite group had the lowest HRV compared to the non-elite and disqualified group. This indicates a shift in autonomic balance towards a more sympathetic dominance in the elite group and a shift towards a more parasympathetic dominance in the non-elite and disqualified group. This could implicate that the elite group had less capacity to modulate the autonomic nerve system after the ride, and maybe experienced more stress than the other groups. In most (equine) studies, a significant reduction in HRV is found under more stressful circumstances (Matsuura, Tanaka et al. 2010, Kinnunen, Laukkanen et al. 2006, Rietmann, Stauffacher et al. 2004, von Borell, Langbein et al. 2007, Visser, van Reenen et al. 2002, Rietmann, Stuart et al. 2004, Cottin, Medigue et al. 2005).

A reason that the obtained findings did not agree with previous research could be the fact that the horses in the elite group put in more effort than the horses in the other groups. Especially in the Vermont 100, the elite group finished within 13 hours and 30 minutes (13 hours and one minute ± 27 minutes). The non-elite group finished within 19 hours and 57 minutes (16 hours and 30 minutes ± 113 minutes). Concluding, the elite group finished on average 3 hours faster than the non-elite group. The disqualified group did not even complete the ride, thus had to put in the smallest effort. This could explain the lower HRV found in the elite group after the competition. On the other hand, if this assumption is true, it is remarkable that this only occurred in the Vermont 100 and not in the Biltmore 100 or any of the shorter distances.

Another reason that the results did not agree with previous research could be the small data set. In the Vermont 100 RMSSD values obtained after the competition, there were 17 data files available for analysis. In the entire study, there were a total of 124 data files. Of these 17 files, only 2 files were from horses in the elite group, 10 from the non-elite group and 5 from the disqualified group (table 4). Since there was only a significant difference found between the elite group and the other two groups, this is only based on two files. Furthermore, the standard deviations in this distance were also fairly high. Considering this portion of the data represents such a small part of the complete data set, it is believed that no valuable meaning can be given to these outcomes. Also, in the logistic regression analysis, no statistically significant outcomes were found, so there was no evidence that the RMSSD measured before or after the competition can be correlated to the outcome of the competition.

The gold standard for measuring heart rate variability is the electrocardiograph (ECG) (Task Force of the European Society of Cardiology the North American Society of Pacing and Electrophysiology 1996, Wallen, Hasson et al. 2012). Nevertheless, the Polar RS800cx device was used to perform the HRV measurements, because of its simplicity, time efficiency and low cost compared to a Holter ECG (Wallen, Hasson et al. 2012). Furthermore, in chapter two was found that there was a moderate correlation between RMSSD data collected with the Polar RS800cx and RMSSD data collected with the Holter ECG. In chapter three, only the RMSSD was used as a time domain indices to formulate conclusions about HRV in endurance horses. This is one of the time domain indices recommended by the Task Force of Cardiology

(1996). However, because only one parameter was used to make a statement about the HRV in different groups of endurance horses, no comparison could be made about different indices.

The decision to use time domain indices instead of frequency domain indices had valid practical and scientific reasons. With frequency domain indices, a more precise validation of the changes in autonomic balance is possible. On the other hand, because the higher precision, a higher standard in input data is required, meaning that the data should be absolutely free of artefacts. This makes frequency domain indices almost exclusively usable under controlled conditions, which was not applicable in this field study. Time domain indices are less influenced by the conditions (Pumprla, Howorka et al. 2002). Furthermore, the appropriate frequency limits that should be used to calculate frequency domain indices are uncertain in equine research, although Kuwahara et al. (1996) and von Borell et al. (2007) suggest certain frequency bands to use. Two of the time domain indices that are strongly correlated with frequency domain indices are the SDRR and RMSSD (Pumprla, Howorka et al. 2002). Based on the outcome of chapter two, only RMSSD was used in this current study. Because the frequency domain indices of the Polar device are not validated for equine research and these indices are more sensitive to artifacts, which are difficult to estimate with the Polar device (Parker, Goodwin et al. 2009), it was determined to solely rely on the time domain indices for the current study.

Taking our results into consideration, the reason that no significant difference was found between the three groups (elite, non-elite and disqualified) could be various. First, it is possible that there is no difference present between elite, non-elite and disqualified horses in endurance competitions. Although, in previous research, there was a significantly higher HRV found in elite trotters versus non-elite trotters (Kinnunen, Laukkanen et al. 2006). This study used the Polar S810 to obtain HRV data. Also, in human research, an increased baseline HRV was found to be related to improved performance in endurance running and a better adaptation to endurance training (Buchheit, Chivot et al. 2010, Vesterinen, Hakkinen et al. 2011). Trained human athletes had a significantly higher HRV compared to healthy untrained subjects and trained athletes suffering from overtraining syndrome (Baumert, Brechtel et al. 2006, Mourot, Bouhaddi et al. 2004). Low HRV seemed to predict a poor adaptation to endurance training (Vesterinen, Hakkinen et al. 2011) and thus probably a lesser result in competition.

Furthermore, there is no known history of the participating horses. A horse participating in the 50 mile event could be a veteran competing in 100 mile races for years or it could be a horse competing its first 50. Also, the goal of the rider is unknown. Was the goal to finish, or to win? These variables all could influence the research group into which the horse was placed, but where he might not have belonged.

This research did not take all variables in account, which could have influenced the outcome. For example, no information was available on the breed, sex or age of the participating horses. Visser et al. (2002) found no explicit difference in HRV between different ages in horses, but that research only used horses between 9 and 22 months of age. In this current study, the spread in age was a lot wider with a range between probably 5 and 18 years of age. However, other studies found that sex and age indeed do influence HRV (von Borell, Langbein et al. 2007, Baumert, Brechtel et al. 2006). Another factor that can influence HRV is the diurnal rhythm (von Borell, Langbein et al. 2007, Gehrke, Baldwin et al. 2011). This could have influenced the data collected after the competition, since there was a wide spread in the time when data was collected. However, the data collected before the competition had the same outcome, and this data was collected in the same timeframe for every horse.

Another factor that might have influenced the results is the amount of time after finishing before the recordings were taken. Especially in the disqualified group, there was a delay in collecting the data. Becker-Birck et al. (2012) states that determination of HRV 30 minutes or later after training will not detect differences between groups. In this study, horses were lunged with different neck positions and R-R intervals were recorded continuously from 60 minutes before to 60 minutes after lunging. Values returned to normal within 30 minutes after exercise. This seems true unless horses have been exposed to extreme workloads or challenges (Becker-Birck, Schmidt et al. 2012). The 50 to 100 mile competition rides used in this research can be seen as more than a normal training and possibly even as extreme challenges for the horses. Therefore, the data recorded 30 minutes or more after the competition is still acknowledged as useful to make a statement about the HRV and thus the shift in parasympathetic/sympathetic balance of the different groups. Furthermore, some studies even state that the HRV can reflect on the level of the previous day activities in horses (Kinnunen, Laukkanen et al. 2006).

Further, an influence that could have affected the data is psychological stress before starting the competition. The majority of the participating horses were trailered towards the competition over various distances. All of them arrived at least half a day before the ride to be in time for the veterinary pre-competition check-up. However, some of them arrived a couple of days before the ride, and others just came out of the trailer when we performed the measurements before the ride. Although in various research, no significant HRV change was found after road transport, there was an elevated salivary cortisol found in these studies which also indicates a stress response (Schmidt, Biau et al. 2010, Schmidt, Hodl et al. 2010). The influence of transportation can therefore not completely be ruled out. Also the housing in the basecamp of the competitions was very different than most of the participating horses probably experience at home. Most horses had small paddocks and were alone, or with one or two other horses. A small number of horses were bound with a long elastic rope to a high pole secured to the trailer (a high-tie). In the study of Werhahn et al. (2012), a decrease in SD and RMSSD was found when horses were only held in a stable without outlet. Since most endurance horses in the United States of America are held in pastures, and the housing at the basecamp was fairly small and new, this could have influenced our results. Especially the results from horses measured before the competition. On the other hand, especially the horses participating in the longer distances (75 and 100 mile) probably are used to stay temporarily in a small paddock and traveling to competitions. This means there could also be no great influence of this stress response on the data.

The last discussion points are based on the materials and methods used for this research. The current study is a field study, which implies that the environmental factors are not fully controlled. The measurements before the ride were taken just outside the veterinary area to make sure every rider would see the researchers and would have a chance to participate. The measurements after the ride were taken in the treatment barn or at the crewing area of the horse. The horses were observed during the measurements and whenever there was an obvious stimulus, the measurement would be prolonged. Because the research was dependent upon the participation of the rider, there was a limit to this. A disadvantage of the Polar RS800cx device is that the data only can be observed after the measurement, when the data is downloaded into a computer. This means that there is a delay in evaluation of the data, and therefore false data is excluded from the study but cannot be replaced by repeating the measurement. Therefore a lot of the valuable data was lost due to more than 5% artefacts (table 1, 23% of the total data) (Visser, van Reenen et al. 2002, Wallen, Hasson et al. 2012, von Borell, Langbein et al. 2007).

The Polar data was judged by visual inspection of the researchers. For each measurement, the best five minutes were selected. Because this selection was performed manually by only one researcher based on visual inspection, a certain amount of subjectivity cannot be ruled out. A further remark should be made about the Polar RS800cx equipment. Because the same Polar belts were used in daily research, the equipment was used extensively (3 belts were used several times per day for 3 months). The quality of the belts was starting to decrease quickly, and therefore the belts were unpleasant to work with. The electrodes came loose from the belts and the start button of the storage device was difficult to use. Therefore, especially in the Biltmore 50 mile event, which was the last competition, the equipment produced a lot of false data, which resulted in a high exclusion rate (36%, table 1). Throughout the whole study, the same criteria were used to exclude the data. However, it cannot be ruled out that there was more false data that was not explicitly visible. Furthermore, most standard deviations found in the statistical analyses were rather high (table 2 and 4), which could explain the lack of significant difference found in between groups.

Although the Polar RS800cx system was validated with use of a Spacelab Holter ECG (chapter two), the R^2 in rest was only 0,600. During walk, there was a higher R^2 (0,717). However, it was chosen to measure HRV on grazing horses, which is thought to be most similar to a resting state. Expectations were that riders would be more willing to participate if the research minimized the impact on the competition and on the horse (especially with the post-ride measurements, when the last riders finished in the middle of the night after almost 20 hours of riding). Also, this procedure was easier to standardize because all horses were willing to eat or graze and stand still. The horse and owner could participate in the study while standing in their own crewing space. If walk had been chosen, the speed would differ dependent on the rider’s walking speed. Also, most horses try to graze or eat which disturbs the standardized walk.

Nevertheless, it would be interesting to repeat the research with a Holter ECG to compare the results. In human research, it was found that endurance training based on daily HRV measurements could efficiently improve cardiorespiratory fitness and improve the results in a maximal exercise test compared to common training methods (Kiviniemi, Hautala et al. 2007). Furthermore, HRV seems to be an indicative measurement for overtraining in human athletes (Baumert, Brechtel et al. 2006, Tian, He et al. 2012). These outcomes indicate that HRV measurements could be a powerful method to use in training schedules and to achieve better competition results. The question if this is also true in the horse physiology would be an interesting step in future equine research.

Conclusion

The aim of this study was to determine if elite horses have a higher HRV compared to non-elite horses or horses that get disqualified at endurance events. Therefore, the main questions in this research were: to what extent HRV measured before the ride can predict the outcome of the ride and to what extent HRV measured after the ride can be related to the outcome of the ride.

To answer the first question, data was analyzed by both a one-way ANOVA and a logistic regression analysis. Neither statistical methods demonstrated a significant difference between groups, nor a significant probability to finish in a certain group. In conclusion, HRV obtained by measuring RMSSD before the ride could not be used to predict the outcome of the ride. Regarding the second question, the same methods of analysis were used. Although there was a significant difference found in one distance (Vermont 100), the logistic regression analysis did not show any significant probability to finish in a certain group. In conclusion, HRV obtained by measuring RMSSD using the polar system after the ride had no predictive value to the outcome of the ride.

Both before and after an endurance competition the HRV, as measured with the RMSSD using the Polar RS800cx, and therefore the difference in dominance between the sympathetic and parasympathetic nervous system, had no predictable value for the outcome of a competition. There was no difference in the HRV between elite, non-elite and disqualified horses. Therefore, based on this research, no statements can be made about the HRV in different groups of endurance horses. Following this research, RMSSD as a measurement of HRV, cannot be used to provide information on the performance level of the horses included in this study at a given point in time.

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