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Comparison of resting heart rate variability of race horses and pleasure horses and the influence of behavioural type

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1) **INTRODUCTION**

Horses nowadays are a major part of life and have several different functions in human society for example, used for work, sport or simply acting as family pets. These functions all have an effect on the horse, both psychologically and physically, possibly acting as stressors. Stress can be defined as physiological, homeostatic and behavioural responses in the horse resulting from interactions with environmental stimuli.

HRV became popular to assess autonomic stress and pain reactions especially in non-verbally communicating animals using HRV analysis based on easy-to-use automatic RR-detectors. (Stucke et al, 2015). The autonomic nervous system response to athletic training and rehabilitative exercise programs after various disease states is thought to be a conditioning phenomenon, HRV data should be useful in understanding the chronological aspects of training and the time to optimal conditioning as it relates to the autonomic influences on the heart (Task, 1996).

In this thesis we will focus on using heart rate variability as a way of measuring the effect of training and behaviourally expressed stress. This is an excellent non-invasive method which will cause no stress to the animals throughout taking the measurements.

The heart rate at rest can be highly variable and can give us a variety of information on the health and happiness of our horses. Generally the greater the heart rate variability the healthier your heart muscle is. High heart rate variability is associated with dominance of the parasympathetic nervous system which is the side of the autonomic nervous system which promotes rest, digestion, sleep and recovery. In this thesis we will look at the resting heart rate variability of race horses and compare to pleasure horses. We will find out from the owners whether these horses are nervous or calm horses in their everyday lives and will see if there is a difference in the results between these two types of horses. Animal Welfare has become prominent in society in recent years so finding out how physical and psychical stress affects the cardiovascular system and general health of our horses is of vital importance.

1) LITERATURE REVIEW

2.1) What is heart rate variability?

The cardiovascular system is mostly controlled by autonomic regulation through the activity of sympathetic and parasympathetic pathways of the autonomic nervous system. Analysis of HRV permits insight into this control mechanism (Aubert et al, 2003). HRV was defined as the standard deviation of the normal R-R intervals. Even when an individual appears to maintain a steady heart rate, there is always some beat-to-beat fluctuation in cycle-length due to changes in the internal and external environment in horses (Kinnunen et al, 2006).

The parasympathetic influence on heart rate is mediated via release of acetylcholine by the vagus nerve. The sympathetic influence on heart rate is mediated by release of epinephrine and norepinephrine. The RR interval variations present during resting conditions represent a fine tuning of the beat-to-beat control mechanism. Vagal afferent stimulation leads to reflex excitation of vagal efferent activity and inhibition of sympathetic efferent activity. The opposite reflex effects are mediated by the stimulation of sympathetic afferent. Efferent vagal activity also appears to be under "tonic" restraint by cardiac afferent sympathetic activity. Efferent sympathetic and vagal activities directed to the sinus node are characterized by discharge largely synchronous with each cardiac cycle which can be modulated by central (e.g. vasomotor and respiratory tenters) and peripheral (e.g. oscillation in arterial pressure and respiratory movements) oscillator. These oscillators generate rhythmic fluctuations in efferent neural discharge which manifest as short- and long-term oscillation in the heart period (Heart, 1996). Normal heartbeat and blood pressure vary secondary to respiration (respiratory sinus arrhythmia), in response to physical, environmental, mental and multiple other factors and is characterised by a circadian variation (Aubert et al, 2003). The primary mechanisms of respiratory sinus arrhythmia (RSA) are understood to be the modulation of cardiac vagal efferent activity by the central respiratory drive and the lung inflation reflex, and the degree of RSA increases with cardiac vagal activity. RSA benefits the pulmonary gas exchange and may improve the energy efficiency of pulmonary circulation by saving heartbeats.

As many commercial devices now provide an automated measurement of HRV, the human cardiologist has been provided with a seemingly simple tool for both research and clinical studies in humans. (Heart, 1996). However not many methods of measuring heart rate variability are available for use in horses. Devices such as garmin and the polar method are used. Many studies have been completed that suggest the polar method does not give accurate HRV results in horses. It was stated that polar is not good for the detection of abnormal beats. However, in horses at rest there is a similarity between polar and better ECG recording systems, therefore it is very suitable for us to use here. (Vendrig et al, 2016).

In humans it is also very important in sports science. In the paper (Aubert et al, 2003) they investigate the importance of exercise training and its relation to the control and regulation of the cardiovascular function by the ANS and can exercise training be used to retard the advance of coronary and other heart diseases and can heart rate variability be used as a predictor or as a marker of the progression of cardiovascular disease. (Aubert et al, 2003). It can also give us information on things like myocardial infarction, myocardial dysfunction, diabetic neuropathy, tetraplegia and many other disorders (Heart, 1996). These are just some examples of how important heart rate variability is in sport and medicine for humans. In horses it is also a very important parameter and is used as an investigative method in many of the papers we review here, for example, it has been used to monitor cardiovascular responses to training in horses and to determine the possibility of over training horses (Kinnunen et al, 2006), to determine the effects of show jumping on the stress levels of horses (Covalesky et al, 1992) and to determine stress levels in response to hot iron branding (Erber et al, 2012).

2.2) Heart Rate Variability Analysis

We use two different types of heart rate variability analysis. These are the time domain analysis and the frequency domain analysis. Time-domain indices only allow describing the net effects of interaction between the sympathetic nervous system and the parasympathetic nervous system (Stucke et al, 2015). These indices are the mean RR, SDNN, mean HR, STD HR, RMSSD, NN50, Pnn50, RR triangular index and TINN. Mean RR is the mean of the RR intervals. SDNN is the standard deviation of IBIs of a single 5-min period (SDNN, ms). It is a good predictor of overall variability present at the time of recording. SDNN reflects all the cyclic components responsible for variability in the period of recording. It is influenced by both the sympathetic and parasympathetic nervous system. The total variance of HRV increases with the length of analyzed recording (Von Borell et al, 2007). RMSSD (root mean square of successive differences) is used to estimate the high frequency beat-to-beat variations that represent vagal regulatory activity. The short term components of HRV assess beat-to-beat variations. These include the NN50 and pNN50. The NN50 is calculated from successive RR differences. This is the number of successive intervals differing more than 50ms or the corresponding relative amount. From here the Pnn50 is calculated from the proportion of beats differing by 50ms (NN50/total number of IBIs). These highly correlate with the RMSSD. They too are also good estimators of vagal activity and high frequency variations in heart rate. The RMSSD method is preferred to pNN50 andNN50 because it has better statistical properties. When using geometrical analysis, recordings of at least 20 minutes (but preferably 24 hours) should be used to ensure the correct performance of the geometrical methods (Von Borell et al, 2007). The HRV triangular index measurement is the integral of the density distribution (ie. the number of all NN intervals) divided by the maximum of the density distribution. This is used to estimate of overall HRV. The triangular interpolation of NN interval histogram (TINN) is the baseline width of the distribution measured as a base of a triangle approximating the NN interval distribution (the minimum square difference is used to find such a triangle) (Von Borell et al, 2007).

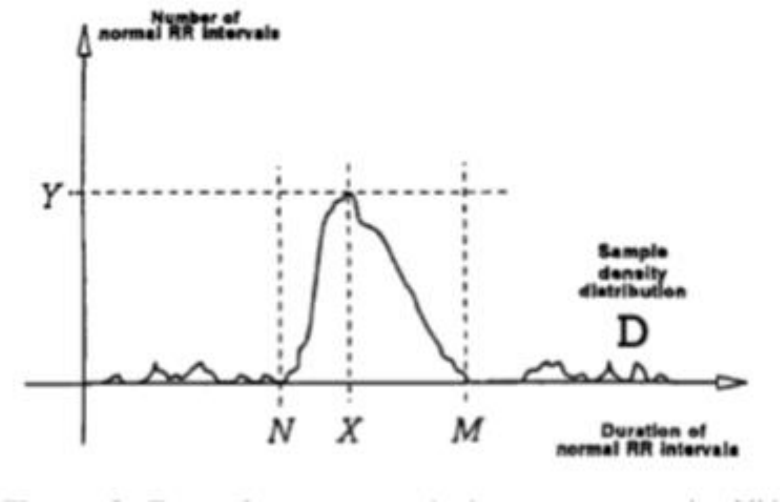


Figure 1-To perform geometrical measures on the NN interval histogram the sample density distribution D is constucted which assigns the number of equally long NN intervals to each value of their lengths. The most frequent NN interval X is established. That is y=D(x) is the maximum of the sample density distribution D. The HRV triangular index is the value obtained by dividing the area integral of D by the

maximum Y. When constructing the distribution D with a discrete scale on the horizontal axis, the value is obtained by the formula: HRV index =(Total number of all NN intervals)/Y. For the computation of the TINN measure, the values N and M are established on the time axis and a multilinear function q constructed such that q(t)=0 for t ≤ N and t≥M and q(X)=Y. The TINN measure is expressed in milliseconds and given by the formula TINN=M-N.

The poincare plot is a graphical representation of the correlation of successive RR intervals. Qualitative and quantitative analysis is then performed on these graphs. We fit an ellipse to the plot to allow us to interpret the shape of the plot. The ellipse is then oriented in accordance with the line of identity. Standard deviation of the point's perpendicular to the line of identity describes short term variability. This is denoted as SD1. SD2 describes long term variability.

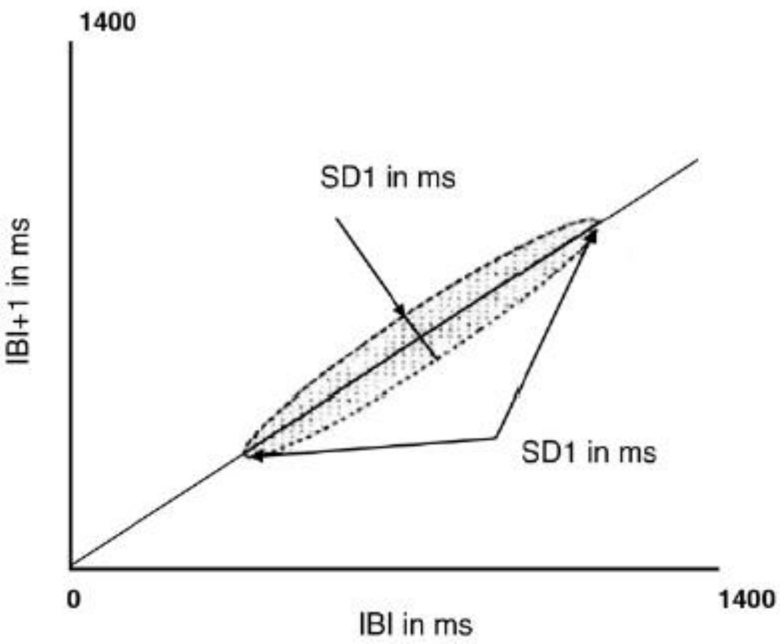


Figure 2-Quantitative analysis of Poincaré plot. SD1 is the SD of instantaneous IBI variability measured from axis 1. SD2 is the Standard Deviation of long-term continuous IBI variability measured from axis 2.

Frequency domain analysis was also done. This type of analysis gives a more specific overview of the sympaticovagal balance. The kubios software uses a cubic spline interpolation method. A heart rate variability spectrum was calculated here with FFT based welches periodogram method and with the AR method. Absolute powers of VLS, LF and HF, LF and HF band powers in normalized units, the LF/HF power ratio and peak frequencies for each band are obtained. The high frequency (HF) band: (0.15–0.4 Hz in humans) represents vagal activity or parasympathetic activity and can be abolished by vagal blockade, for example with atropine or scopolamine. The low frequency (LF) band (0.04–0.15 Hz) and the very low frequency (VLF) band (≤0.04 Hz) are associated with sympathetic or sympathetic plus vagal activity, and their physiological meaning has been much debated. These represent both parasympathetic and sympathetic activity and therefore can also be abolished by sympathetic blockade, for example with beta-blocker propranolol or atenolol. The VLF band occurs in short-time measurements, but should only be interpreted in long-term recordings (24 h). The VLF variations in heart rate are thought to be due to peripheral vasomotor tone, thermoregulation and the rennin–angiotensin-system (Marchant-Forde, 2003). The maximum central frequency of the low frequency band mirrors baroreceptor reflex related changes in autonomic nervous system control. Therefore, when using the LF/HF ratio as a measure of sympathovagal balance, one has to appreciate that this measure may also be influenced by other physiological functions like thermoregulation or myogenic activity of vessels (Von Borell et al, 2007). During Spectral analysis of HRV by Fast Fourier Transofmation the distribution of the power and the central frequency of LF and HF are not fixed but may vary in relation to changes in autonomic modulations of heart period. The heart rate should not change during the recording. Ectopic beats, arrhythmic events, missing data and noise effects may alter the estimation of the PSD of HRV. Proper interpolation (or linear regression or similar algorithms) on preceding successive beats on HRV signal or on its autocorrelation function may decrease this error (Task, 1996). Preferentially short-term recordings which are free of ectopy, missing data and noise, should be used. In some circumstances, however, acceptance of only ectopic-free short-term recordings, may introduce significant selection bias. In such cases, proper interpolation should be used and the possibility of the results being influenced by ectopy should be considered (Kamath et Fallen, 1995).

Time-Domain Variable	Approximate Frequency-Domain Correlate
SDNN	Total power
HRV triangular index	Total power
TINN	Total power
SDANN	ULF
SDNN index	Mean of 5 minute total power
RMSSD	HF
SDSD	HF
NN50 count	HF
pNN50	HF
Differential index	HF
Logarithmic index	HF

Figure 3- Approximate correspondence of time domain and frequency domain methods

2.3) What is stress?

Stress may be defined as the homeostatic, physiological, and behavioural responses detectable in an animal as a result of interactions with environmental stressors. Stress is associated with activation of the hypothalamopituitary-adrenocortical system. When an animal perceives an event as stressful a generalized physiological scheme ensues. Corticotrophin-releasing factor is secreted from the hypothalamus, causing the release of the tropic hormone, adrenocorticotropic hormone (ACTH), from the anterior pituitary. ACTH acts at the level of the adrenal cortex causing a release of corticosteroid hormones such as corticosterone and cortisol into the general circulation. The major effect of this increase in corticosteroids is a reduction in inflammation and an increase in glucose metabolism. Increased plasma cortisol concentrations have been used as an indicator of the activation of the hypothalamo-pituitaryadrenocortical system in humans and horses. In horses, cortisol accounts for almost 90% of the circulating corticoids (Covalesky et al, 1992).

The most immediate stress response is an increase in adreno-medullary and sympathetic nervous activity, leading to a release of epinephrine and rise in heart rate. In addition, heart rate variability (HRV; short-term fluctuations in HR) is an indicator for the response of the autonomic nervous system to stress and reflects the oscillatory antagonistic influence of the sympathetic and parasympathetic branch of the autonomic nervous system on the sinus node of the heart. (Lewinski et al. 2013)

Chronic stress in horses and other species has been implicated in many deleterious conditions including increased aggressive behaviour, decreased growth and reproductive capability; inhibited synthesis of immunoglobulins, and increased risks of gastric ulceration and colic/diarrhoea.(Covalesky et al, 1992) Therefore stress will affect a horses overall performance in sport and in their daily lives. Signs of stress can be subtle so it is important to keep an eye on your horse's behaviour as their health and performance can suffer so much. Simply, your horse being a little restless in his stable could be a sign. It can also be very simple things that cause stress in your horse's life such as a change in feed or a change in routine. (Covalesky et al, 1992)

Although the polar method is seen as a non-invasive method and less stressful to the animal than taking blood samples would be it is still a stress in itself. The device must be kept attached to the horse. (Kinnunen et al, 2006) tells us that two electrodes are placed on wet skin on the left lateral thorax wall and fixed thoroughly with a stable girth. The electrodes are connected to a storage device, fixed onto the girth. This is something that the horse is not used to and may be perceived as a stressor. Also the measurements are taken over a certain period of time which may be long.

2.4) Adaptation to psychical stress

In the study done by (Covalesky et al, 1992), the correlation of behaviour to both heart rate and plasma cortisol that was found describes an intimate relationship between behaviour and adrenocortical hormones (Covalesky et al, 1992). (Becker-Birck et al, 2013) also discovered that sympathoadrenal activity was not only induced by physical activity of the animals but also by emotional factors in the horses before and most likely also during the competition. This is an indicator of anticipatory stress. (Becker-Birck et al, 2013). (Kedzierski et al, 2013) stated that the measurement of cortisol release rate was used also for the estimation of the horse's performance. (Mircean et al) stated that during the same type of physical effort, serum cortisol levels were higher in horses with a low performance level than in well trained ones. In the other study, (Marc et al) showed that plasma cortisol response to standardized exercise tests can be a useful tool for evaluation of performance during the training process. (Kedzierski et al, 2013).

2.5) Public performance vs. home

In another study,(Lewinski et al. 2013), they analysed HR, HRV and cortisol release as physiological stress parameters in horses and their riders during a public equestrian performance and during a rehearsal that differed from the public performance only by the absence of spectators (Lewinski et al. 2013).

In a similar study done by (Becker-Birck et al, 2013) cortisol was measured as well as heart rate. RR interval was recorded before, during, and after riding, and heart rate and HRV variables were calculated (Becker-Birck et al, 2013). In the study done by (Lewinski et al. 2013), they found that the physiological response of the riders, but not their horses, differed between a public performance and a non-public rehearsal. While increases in HR are mainly caused by physical activity, decreases in HRV also indicate a stress response (Lewinski et al. 2013). (Becker-Birck et al, 2013) found that Heart rate was higher in horses participating in show jumping compared with dressage for day 1 of the competitions. The difference in heart rate most likely reflects different physical demands in dressage and show jumping, whereas the less pronounced changes in HRV and lack of significant HRV differences between groups may indicate that the presentations were not perceived as major stressors by the horses (Becker-Birck et al, 2013). The same was found by (Lewinski et al. 2013), that the presence of spectators may cause a more pronounced sympathetic and/or decreased parasympathetic activity in riders than in the horses (Lewinski et al. 2013).

In a study done by (Peeters et al, 2013) they showed that there was no correlation between the stress levels of the rider and her own horse. This is in agreement with the idea of (Lewinski et al. 2013), that during the presentation, but not the performance, it is possible that the riders might have been influenced by the expectations of the spectators, increased demands of their own superiors and trainers, or indeed an expectation of the riders themselves that their horses might be more difficult in a performance. All of these factors may have been perceived as potential psychological stressor by the riders.

2.6) Adaptation to stress/pain

In a study done to compare the stress response and tissue reaction related to branding and microchip implantation in foals by analysing behaviour and physiological stress variables they found that because habituation to humans affects an animal's stress response, experiments in adult horses may only partially be comparable to studies in foals. In adult horses, branding induced more pronounced behavioural changes than microchip implantation, but cortisol release did not increase in both groups. In foals, fixation for branding or microchip implantation induced an immediate behavioural response and a transient increase in HR which did not occur in adult horses. Although familiar with humans, the foals were less accustomed to handling than adult horses. The response to fixation might therefore have masked differences in the response to branding and microchip implantation, but in this case the response to the actual identification would be less pronounced than the response to fixation alone. This proves that the older animals with more experience with handling are calmer animals and don't perceive the same things as stressors that young animals would.
(Erber at al, 2012)

2.7) Animals adapt to stressors in their environment

In a study done by (Kinnunen et al, 2006) they found that elite horses as well as previously raced horses had significantly higher HRVs in all training periods compared to the non-elites or those that had not raced yet. Previously raced horses had been under regular training for notably longer periods than the horses that had not yet raced. The difference in resting HRV between previously raced and unraced horses may also be explained by the higher level of emotionality of unraced horses, due to the fact that they were not acclimatised to the changing environmental factors at the same magnitude as previously raced horses. (Kinnunen et al, 2006).

HRV, reflecting cardiovascular control exerted by both parasympathetic and sympathetic nervous system, has been used to evaluate modifications of autonomic functions due to acute exercise or training. (Mourot et al, 2004). In humans physical training has been shown to increase HRV in young and in old healthy subjects but also in patients with heart disease and that endurance athletic activity, such as running or cycling, may have a substantially favourable effect on the cardiac autonomic profile (Sztajzel et al, 2008). Sometimes this can be taken too far and individuals can be over exercised.

An overtraining state (OT), originating in a ‘training/competition/recovery imbalance may happen quite often in high level performance sports. Moreover, training as well as non-training stressors are thought to make this OT state worse with large individual variations.

It is well known that over-training causes hormonal imbalance in athletes. Due to these hormonal changes, over-training will lead to an autonomic imbalance. In which way that the ANS changes (parasympathetic versus sympathetic) is still controversial. (Aubert et al, 2003). There are several studies detecting HRV in horses in which HRV has been reported to reflect the influence of different psychological factors on the autonomic nervous system. Horses have a high aerobic capacity and during periods of training at high intensities are prone to overreaching (Tyler et al, 1998). Over reaching is an acute condition where you perform over your limits while training. This can then lead to over training, a chronic condition. A clinical syndrome of overtraining in horses appears to be similar to that of human athletes, making comparative physiological studies relevant.

In the study done by (Kinnunen et al, 2006), horses that had raced previously had significantly higher HRV during all training periods compared to those that had not raced yet. They found that intense exercise decreased HRV of the trotters during recovery. At the onset of exercise, heart rate is increased by a reduction in vagal tone and a temporary increase in sympathetic tone. Prolonged physical activity is associated with a continued withdrawal of vagal activity and an attenuation of sympathetic nervous system tone. The lowest HRV was recorded in the competition period, which is physically and mentally the most stressful training period for horses. This shows that stressful conditions can result in a decrease in HRV (Kinnunen et al, 2006).

In the paper written by (Aubert et al, 2003) they stated that heavy training shifted the cardiac autonomic balance of the sympathetic over the parasympathetic drive. Night-time results of HRV parameters proved a good tool to estimate cumulated physical fatigue. Therefore, they concluded that HRV could be valuable for optimising individual training profiles. This is in agreement with (Kinnunen et al, 2006) statement that HRV recording may be a useful marker to monitor recovery and possible overreaching/overtraining in equine performance; and HRV has potential to be used as an easy-to-use and affordable technique to monitor recovery. (Kinnunen et al, 2006)

2) MATERIALS AND METHODS

During our study we examined two separate groups of horses. The first group was a group of 6 thoroughbred horses from Alag Training Racecourse Stables. These measurements were taken in 2015. These horses are kept in a box and fed meadow hay and concentrates. They do not get left out in the paddock to graze. The horses are healthy and all undergo the same training and the same type of management. They undergo regular race training of high intensity daily and frequently visit the racecourse and participate in races. They are always mixing with other horses and crowds of new people at these race events and during trainings. The measurements were taken in the morning before they started their training for the day so that this training did not interfere with the results. A history was taken and a clinical examination performed to evaluate the general health status of the animals. A questionnaire was also taken by the rider and trainer to find out about the horse's behavioural type (nervous or calm). The horses are listed in the following table:

Horse name	Breed	age	Gender	Horse type	Health Status	Training
Race Horse 1	thoroughbred	3	M	Calm	Healthy	Race
Race Horse 2	thoroughbred	3	M	Nervous	Healthy	Race
Race Horse 3	thoroughbred	4	M	Calm	Healthy	Race
Race Horse4	thoroughbred	4	S	Calm	Healthy	Race
Race Horse5	thoroughbred	3	S	Calm	Healthy	Race
Race Horse 6	thoroughbred	4	S	Calm	Healthy	Race

Table 1: Race horses

The second group of the study was a group of 6 pleasure horse from private stables in Hungary. The measurements were taken in 2016. The horses are pleasure horses or older retired sport horses, all under the same management. They are stabled in a box at night and let out to a paddock during the day. They are fed similar meadow hay and concentrates as the previous group of thoroughbred horses except, at the race course the concentrate ratio is tripled compared to the pleasure horses. These horses have a low level of training in comparison to the race horses. They are used for pleasure riding and this would not be every day. They are therefore kept in a quiet and calm environment with the presence of these 6 horses alone. There is no introduction of new horses. The measurements were again taken in the morning before they were left out to the paddock. Some of the horses have some on-going chronic disorders. The horses are listed in the following tables.

Name	Age	Gender	Type	Training	Health status
PH1	19	G	Calm	No performance training, exercise(2-3*week)	Chronic lameness, mild RAO
PH2	12	S	Nervous	Retired competing m level dressage, exercise 3*week	IAD
PH3	12	G	Nervous	No performance training. Exercise 2*week	Chronic lameness(arthritis)
PH4	23	G	Calm	Competing show jumper (120-130). Recent train 3*week	Low grade RAO, Podotrochleosis
PH5	19	M	Calm	Always pleasure 3*week	
PH6	27	S	nervous	Retired dressage. No training now	Poor Body condition, Cushings

Table 2: Pleasure Horses

The heart rate variability was measured using Polar S810 heart rate monitor. This is a non-invasive method. The horses were tested in the morning, before training. Two electrodes were placed on wet skin on the left lateral thorax wall and fixed firmly in place with a stable girth. One electrode was below the withers and the other just caudal to the foreleg. Electrode gel was also placed between the electrode and the skin. Sponges were placed between girth and the electrodes also, to provide more pressure on the electrodes. This was all to maximise the conduction to ensure accurate results. The electrodes were connected to a storage device, fixed onto the girth. Intervals between R peaks were stored continuously in msecs. This was performed on the horses when they were in complete rest, while standing. They were not allowed to eat or do anything else that would disturb the measurements but could freely move themselves. 5 minute segments of data was retrieved and an average of two of the 5 minute segments were used as the results. To analyse our results, we used the kubios heart rate variability software version 2.2. We also performed a statistical analysis on our data using the statistical T-Test in microsoft excel to compare the differences between the race and pleasure horses.

3) <u>Results</u>

	Units	PH1	PH2	PH3	PH4	PH5	PH6
Mean RR*	(ms)	1440.65	1592.75	1335.15	1502.95	1221.4	1412.6
STD RR (SDNN)	(ms)	97.65	73.45	174.55	64.95	107.25	108.6
Mean HR*	(1/min)	41.845	37.76	46.01	40	49.51	42.8
STD HR	(1/min)	2.945	1.77	6.665	1.825	4.42	3.455
RMSSD	(ms)	88.85	34.7	84.2	70.75	55.2	61.95
NN50	(count)	111.5	26	36.5	100.5	65	52.5
pNN50	(%)	53.85	13.95	16.25	50.8	26.45	24.35
RR triangular index		20.8	8.8985	8.864	13.743	19.4485	13.2255
TINN	(ms)	0	0	40	0	20	0
VLF (0−0.04 Hz)	ms2)	4270	891.5	25500.5	2588.5	4470.5	3907.5
LF (0.04−0.15 Hz)	(n.u.)	37.25	77.2	80.05	41.6	80.5	69.9
HF (0.15−0.4 Hz)	(n.u.)	62.75	22.8	19.95	58.4	19.5	30.1
Total	(ms2)	7723.5	1531.5	26488.5	4449.5	7343	6639.5
LF/HF	(ms2)	0.6	3.3895	4.529	0.732	4.2055	4.2455
SD1	(ms)	63	36.9	29.85	50.15	39.1	43.9
SD2	(ms)	123.1	97.25	243.75	76.9	146.5	147.3
nervous score		Calm	nervous	nervous	Calm	calm	nervous
sgm5mins	ms)	Average	average	average	average	average	average

Table 3: results of 6 pleasure horses

	Unit	RH1	RH2	RH3	RH 4	RH5	RH6
Mean RR*	ms)	1469.25	1469.6	1662.25	1856.8	1661.65	1515.55
STD RR (SDNN)	(ms)	105.5	248.4	180.35	160.1	153.7	156
Mean HR*	(1/min)	41.085	43.45	36.685	32.595	36.55	40.06
STD HR	(1/min)	3.095	11.24	5.185	3.11	3.555	4.58
RMSSD	(ms)	62.5	73.3	55.6	55.85	93.35	103.9
NN50	(count)	31.5	55	35.5	57	75.5	107.5
pNN50	(%)	15.45	26.65	19.75	35.55	41.25	54.4
RR triangular index		15.333	22.889	15.973	20.452	22.0855	20.95
TINN	(ms)	0	115	0	0	0	0
VLF (0−0.04 Hz)	ms2)	6542.5	13215	13279.5	16417	11284.5	15736.5
LF (0.04−0.15 Hz)	(n.u.)	64.7	59.1	78.5	72.15	54	51.2
HF (0.15−0.4 Hz)	(n.u.)	35.3	40.9	21.5	27.85	46	48.8
Total	(ms2)	10410.5	15588.5	14310	17417.5	16165	18671.5
LF/HF	(ms2)	1.943	1.602	3.767	2.677	1.187	1.114
SD1	(ms)	44.25	51.95	39.45	41.6	66.2	73.7
SD2	(ms)	142.7	345.25	252	223.5	205.75	207.95
nervous score		Calm	nervous	calm	calm	calm	calm
sgm5mins		AVER-AGE	AVER-AGE	AVER-AGE	AVER-AGE	AVER-AGE	AVER-AGE

Table 4: results of 6 race horses

After examining our results individually, I then performed a statistical test in Microsoft Excel, the T-Test. You can see the results in the following table:

Mean RR*	0.043747778*
STD RR (SDNN)	0.03016*
Mean HR*	0.079222*
STD HR	0.305324
RMSSD	0.499862
NN50	0.788472
pNN50	0.896102
RR triangular index*	0.054968*
TINN	0.667556
VLF (0–0.04 Hz)*	0.195631
LF (0.04–0.15 Hz)	0.90402
HF (0.15–0.4 Hz)	0.90402
Total	0.14307
LF/HF	0.318887
SD1	0.25404
SD2*	0.032278*

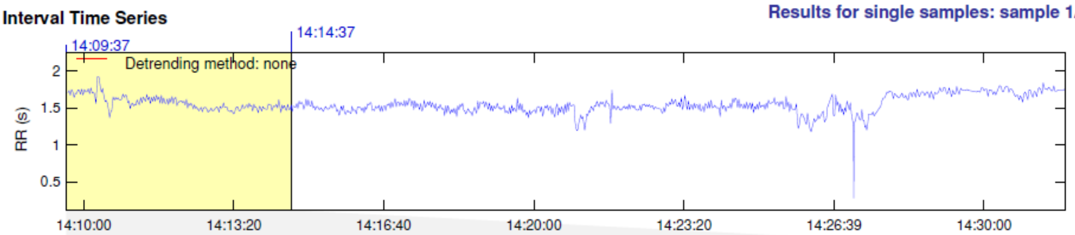
Table 5: results of the Statistical T-Test. The ones with a value of p<0.1 are significant. These are marked with an * in the table.

It is the ones with values of p<0.1 that are significant here. As you can see these are the Mean RR, Mean Heart Rate, SDNN, RR triangular index, SD2. The following documents are examples of our results. They are results for two of the horses in the study. The first 5 pages (20-24) are results for pleasure horse 4 and the last two pages (25-26) are results for race horse 2.

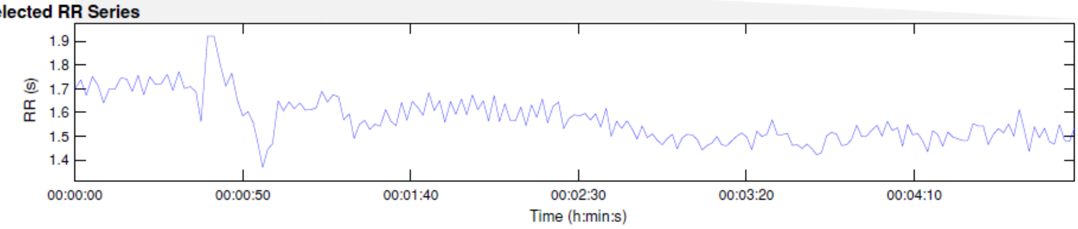
HRV Analysis Results

Results for single samples: sample 1/5

RR Interval Time Series



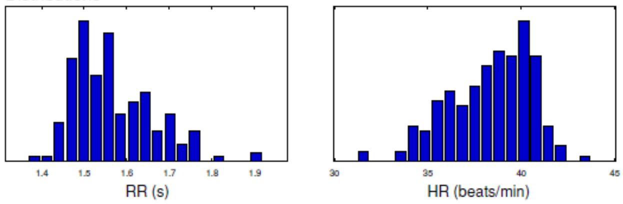
Selected RR Series



Time–Domain Results

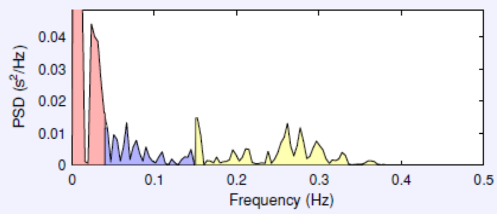
Variable	Units	Value
Mean RR*	(ms)	1568.1
STD RR (SDNN)	(ms)	94.3
Mean HR*	(1/min)	38.39
STD HR	(1/min)	2.21
RMSSD	(ms)	62.5
NN50	(count)	81
pNN50	(%)	42.6
RR triangular index		15.917
TINN	(ms)	0.0

Distributions*



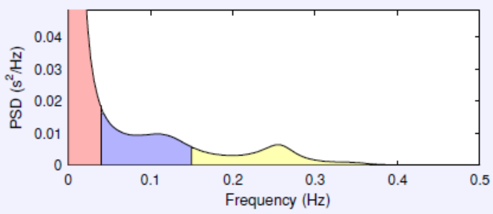
Frequency–Domain Results

FFT spectrum (Welch's periodogram: 256 s window with 50% overlap)



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	2565	69.7	
LF (0.04–0.15 Hz)	0.0664	417	11.3	37.4
HF (0.15–0.4 Hz)	0.1523	696	18.9	62.6
Total		3678		
LF/HF		0.599		

AR Spectrum (AR model order = 16, not factorized)

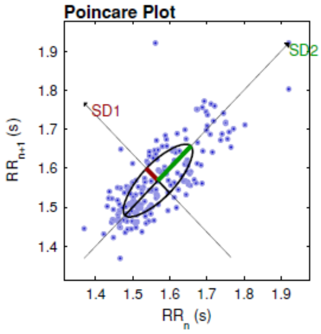


Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	5773	76.9	
LF (0.04–0.15 Hz)	0.0430	1077	14.3	62.0
HF (0.15–0.4 Hz)	0.2539	660	8.8	38.0
Total		7511		
LF/HF		1.632		

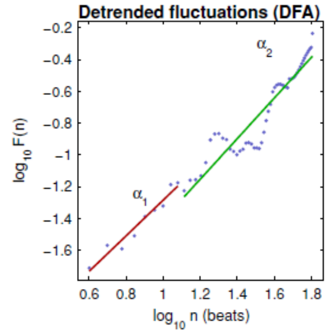
Nonlinear Results*

Variable	Units	Value
Poincare plot		
SD1	(ms)	44.3
SD2	(ms)	125.8
Recurrence plot		
Mean line length (Lmean)	(beats)	26.07
Max line length (Lmax)	(beats)	158
Recurrence rate (REC)	(%)	42.60
Determinism (DET)	(%)	99.04
Shannon Entropy (ShanEn)		3.727
Other		
Approximate entropy (ApEn)		0.913
Sample entropy (SampEn)		1.443
Detrended fluctuations (DFA): $\alpha 1$		1.121
Detrended fluctuations (DFA): $\alpha 2$		1.279
Correlation dimension (D2)		3.755

Poincare Plot



Detrended fluctuations (DFA)



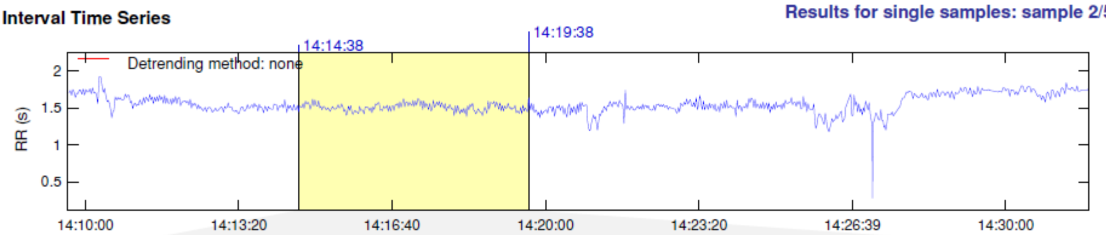
*Results are calculated from the non–detrended selected RR series.

Kubios HRV, version 2.0
Department of Physics
University of Kuopio, Finland

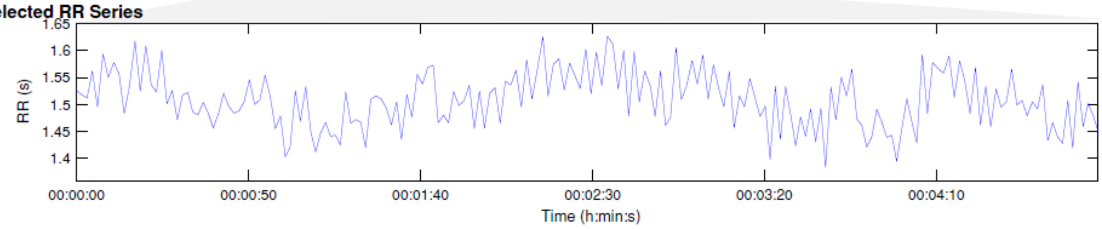
HRV Analysis Results

RR Interval Time Series

Results for single samples: sample 2/5



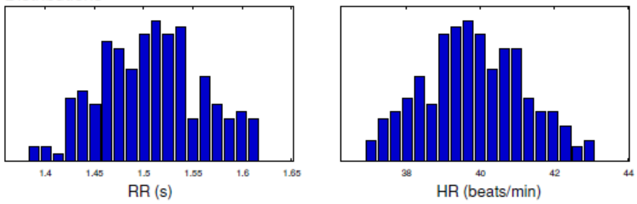
Selected RR Series



Time–Domain Results

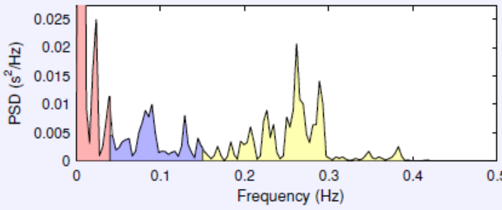
Variable	Units	Value
Mean RR*	(ms)	1508.0
STD RR (SDNN)	(ms)	51.3
Mean HR*	(1/min)	39.83
STD HR	(1/min)	1.36
RMSSD	(ms)	62.1
NN50	(count)	101
pNN50	(%)	51.3
RR triangular index		13.200
TINN	(ms)	0.0

Distributions*



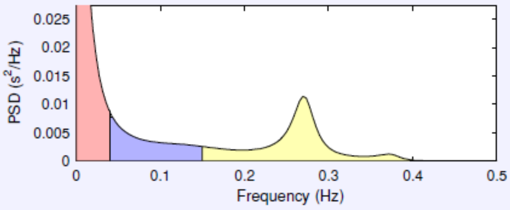
Frequency–Domain Results

FFT spectrum (Welch's periodogram: 256 s window with 50% overlap)



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0078	1280	53.2	
LF (0.04–0.15 Hz)	0.0898	392	16.3	34.9
HF (0.15–0.4 Hz)	0.2617	732	30.4	65.1
Total		2404		
LF/HF		0.536		

AR Spectrum (AR model order = 16, not factorized)

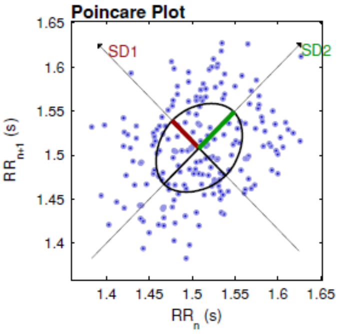


Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	1105	49.4	
LF (0.04–0.15 Hz)	0.0430	427	19.1	37.7
HF (0.15–0.4 Hz)	0.2695	705	31.5	62.3
Total		2238		
LF/HF		0.606		

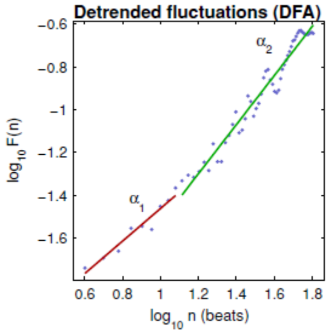
Nonlinear Results*

Variable	Units	Value
Poincare plot		
SD1	(ms)	44.0
SD2	(ms)	57.7
Recurrence plot		
Mean line length (Lmean)	(beats)	6.24
Max line length (Lmax)	(beats)	25
Recurrence rate (REC)	(%)	15.92
Determinism (DET)	(%)	93.56
Shannon Entropy (ShanEn)		2.487
Other		
Approximate entropy (ApEn)		0.860
Sample entropy (SampEn)		2.048
Detrended fluctuations (DFA): $\alpha 1$		0.761
Detrended fluctuations (DFA): $\alpha 2$		1.147
Correlation dimension (D2)		4.487

Poincare Plot



Detrended fluctuations (DFA)



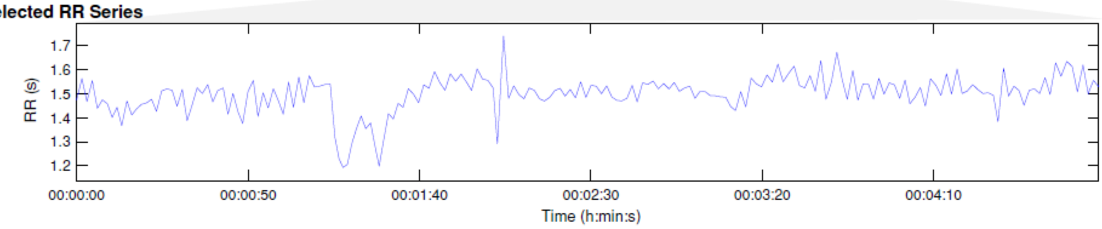
*Results are calculated from the non–detrended selected RR series.

HRV Analysis Results

RR Interval Time Series

Results for single samples: sample 3/5

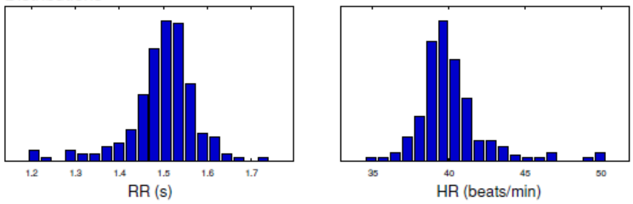
Selected RR Series



Time–Domain Results

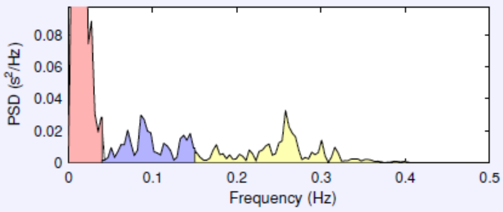
Variable	Units	Value
Mean RR*	(ms)	1497.9
STD RR (SDNN)	(ms)	78.6
Mean HR*	(1/min)	40.17
STD HR	(1/min)	2.29
RMSSD	(ms)	79.4
NN50	(count)	100
pNN50	(%)	50.3
RR triangular index		14.286
TINN	(ms)	0.0

Distributions*



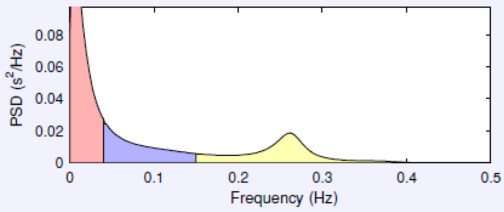
Frequency–Domain Results

FFT spectrum (Welch's periodogram: 256 s window with 50% overlap)



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0195	3897	60.0	
LF (0.04–0.15 Hz)	0.0859	1254	19.3	48.3
HF (0.15–0.4 Hz)	0.2578	1344	20.7	51.7
Total		6495		
LF/HF		0.934		

AR Spectrum (AR model order = 16, not factorized)

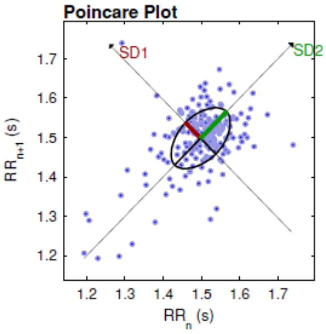


Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	3033	53.7	
LF (0.04–0.15 Hz)	0.0430	1244	22.0	47.6
HF (0.15–0.4 Hz)	0.2617	1368	24.2	52.4
Total		5645		
LF/HF		0.910		

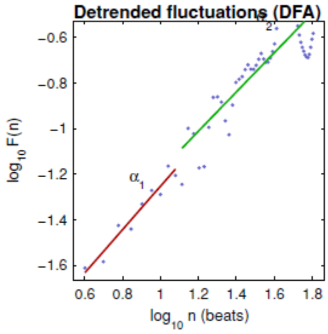
Nonlinear Results*

Variable	Units	Value
Poincare plot		
SD1	(ms)	56.3
SD2	(ms)	96.1
Recurrence plot		
Mean line length (Lmean)	(beats)	12.67
Max line length (Lmax)	(beats)	91
Recurrence rate (REC)	(%)	44.77
Determinism (DET)	(%)	98.04
Shannon Entropy (ShanEn)		3.283
Other		
Approximate entropy (ApEn)		0.951
Sample entropy (SampEn)		1.759
Detrended fluctuations (DFA): α1		0.952
Detrended fluctuations (DFA): α2		0.861
Correlation dimension (D2)		3.675

Poincare Plot



Detrended fluctuations (DFA)

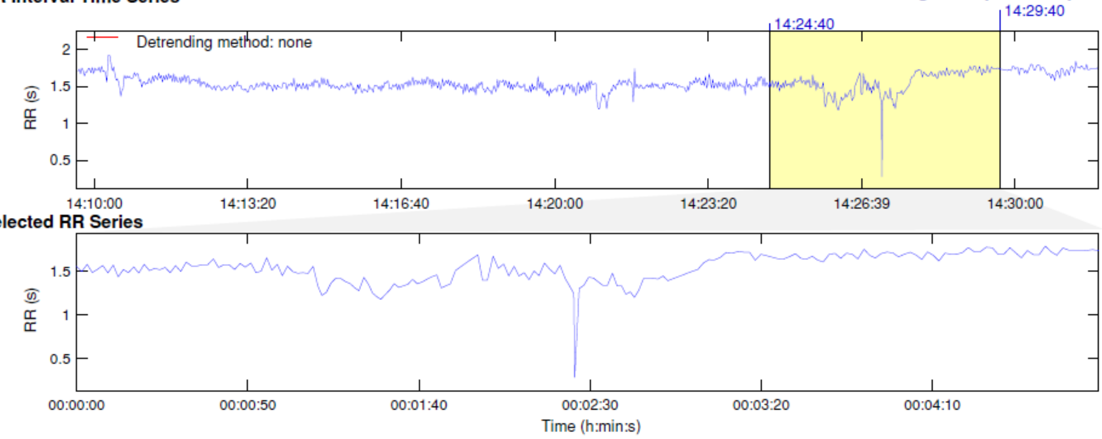


*Results are calculated from the non–detrended selected RR series.

HRV Analysis Results

RR Interval Time Series

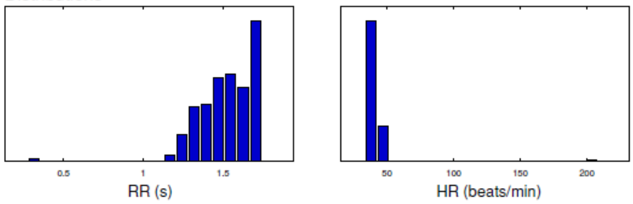
Results for single samples: sample 4/5



Time–Domain Results

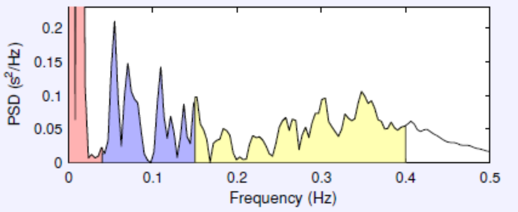
Variable	Units	Value
Mean RR*	(ms)	1531.6
STD RR (SDNN)	(ms)	175.6
Mean HR*	(1/min)	40.30
STD HR	(1/min)	13.13
RMSSD	(ms)	124.4
NN50	(count)	94
pNN50	(%)	48.2
RR triangular index		24.500
TINN	(ms)	305.0

Distributions*



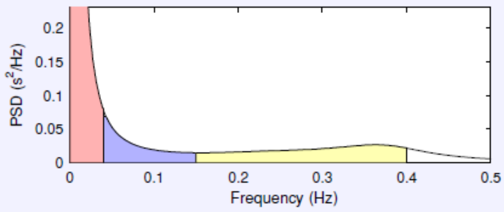
Frequency–Domain Results

FFT spectrum (Welch's periodogram: 256 s window with 50% overlap)



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	21103	51.5	
LF (0.04–0.15 Hz)	0.0547	7293	17.8	36.7
HF (0.15–0.4 Hz)	0.3477	12591	30.7	63.3
Total		40987		
LF/HF		0.579		

AR Spectrum (AR model order = 16, not factorized)

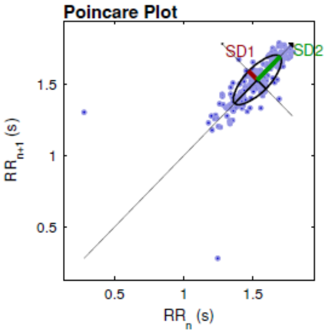


Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	17924	69.2	
LF (0.04–0.15 Hz)	0.0430	2972	11.5	37.2
HF (0.15–0.4 Hz)	0.3633	5020	19.4	62.8
Total		25915		
LF/HF		0.592		

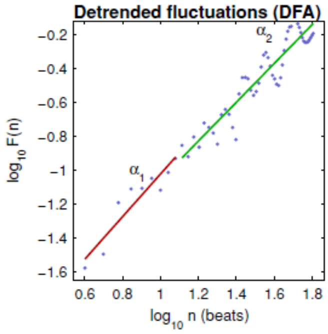
Nonlinear Results*

Variable	Units	Value
Poincare plot		
SD1	(ms)	88.2
SD2	(ms)	232.3
Recurrence plot		
Mean line length (Lmean)	(beats)	17.10
Max line length (Lmax)	(beats)	90
Recurrence rate (REC)	(%)	45.45
Determinism (DET)	(%)	99.01
Shannon Entropy (ShanEn)		3.631
Other		
Approximate entropy (ApEn)		0.843
Sample entropy (SampEn)		0.989
Detrended fluctuations (DFA): $\alpha1$		1.258
Detrended fluctuations (DFA): $\alpha2$		1.144
Correlation dimension (D2)		3.182

Poincare Plot



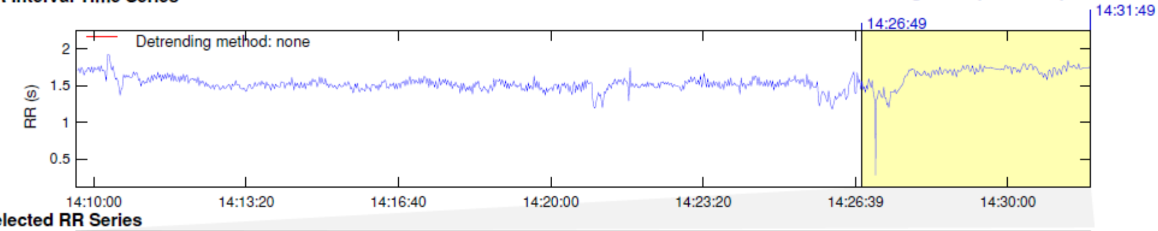
Detrended fluctuations (DFA)



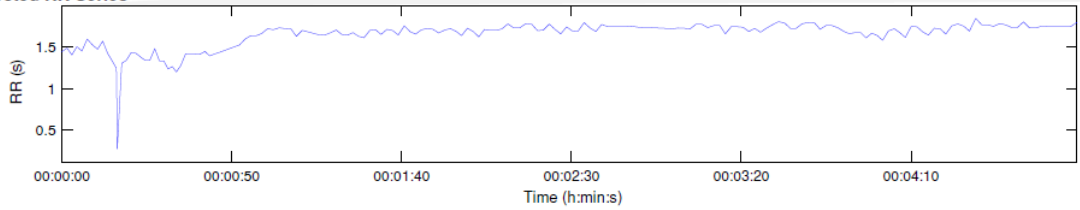
*Results are calculated from the non–detrended selected RR series.

RR Interval Time Series

Results for single samples: sample 5/5



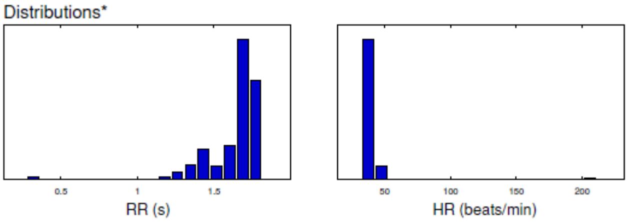
Selected RR Series



Time–Domain Results

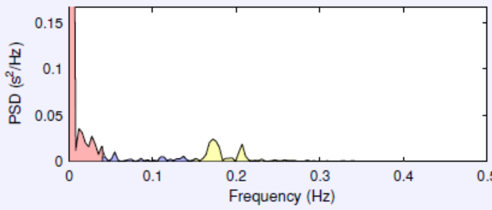
Variable	Units	Value
Mean RR*	(ms)	1640.9
STD RR (SDNN)	(ms)	171.0
Mean HR*	(1/min)	37.66
STD HR	(1/min)	13.59
RMSSD	(ms)	117.5
NN50	(count)	68
pNN50	(%)	37.4
RR triangular index		11.438
TINN	(ms)	255.0

Distributions*



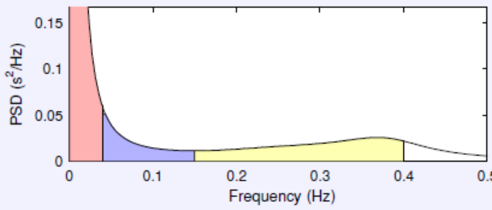
Frequency–Domain Results

FFT spectrum (Welch's periodogram: 256 s window with 50% overlap)



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	3799	81.2	
LF (0.04–0.15 Hz)	0.0547	250	5.3	28.4
HF (0.15–0.4 Hz)	0.1719	632	13.5	71.6
Total		4681		
LF/HF		0.396		

AR Spectrum (AR model order = 16, not factorized)

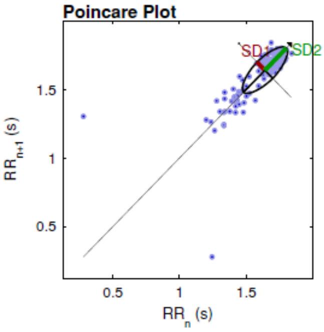


Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	14890	68.8	
LF (0.04–0.15 Hz)	0.0430	2222	10.3	32.9
HF (0.15–0.4 Hz)	0.3672	4528	20.9	67.1
Total		21640		
LF/HF		0.491		

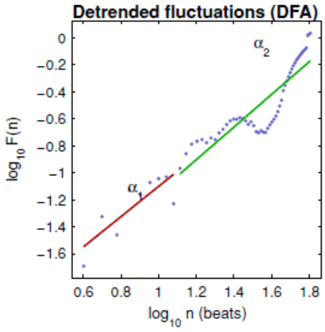
Nonlinear Results*

Variable	Units	Value
Poincare plot		
SD1	(ms)	83.3
SD2	(ms)	227.0
Recurrence plot		
Mean line length (Lmean)	(beats)	60.21
Max line length (Lmax)	(beats)	160
Recurrence rate (REC)	(%)	65.28
Determinism (DET)	(%)	99.92
Shannon Entropy (ShanEn)		4.576
Other		
Approximate entropy (ApEn)		0.687
Sample entropy (SampEn)		0.749
Detrended fluctuations (DFA): $\alpha1$		1.123
Detrended fluctuations (DFA): $\alpha2$		1.208
Correlation dimension (D2)		3.378

Poincare Plot



Detrended fluctuations (DFA)

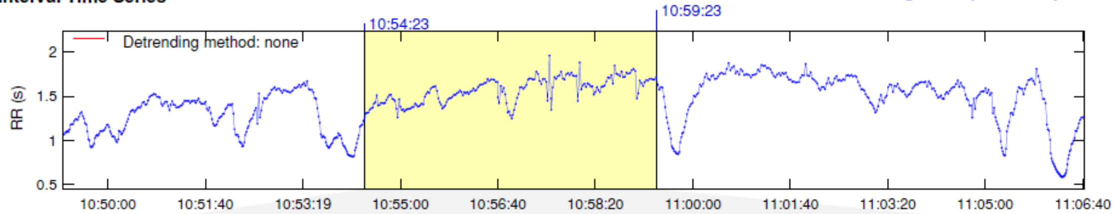


*Results are calculated from the non–detrended selected RR series.

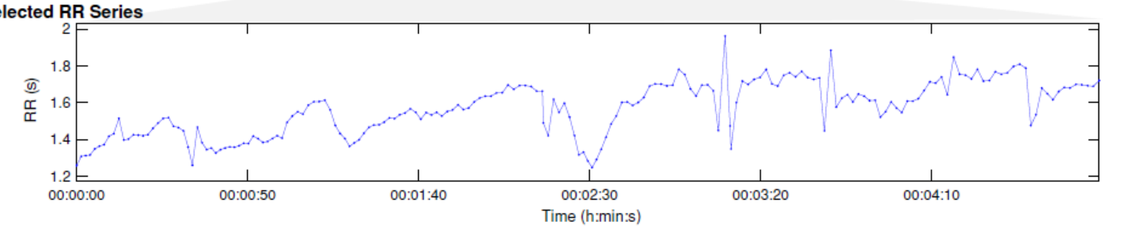
HRV Analysis Results

RR Interval Time Series

Results for single samples: sample 1/2



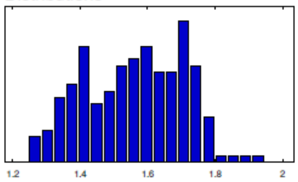
Selected RR Series

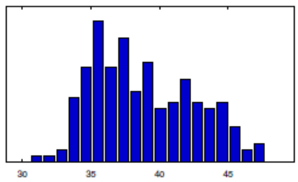


Time–Domain Results

Variable	Units	Value
Mean RR*	(ms)	1559.9
STD RR (SDNN)	(ms)	146.1
Mean HR*	(1/min)	38.81
STD HR	(1/min)	3.72
RMSSD	(ms)	89.9
NN50	(count)	46
pNN50	(%)	23.8
RR triangular index		21.556
TINN	(ms)	0.0

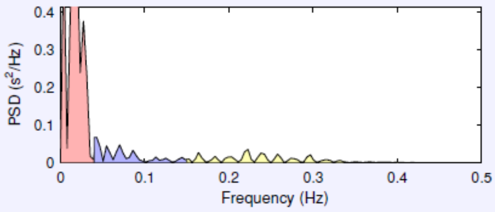
Distributions*





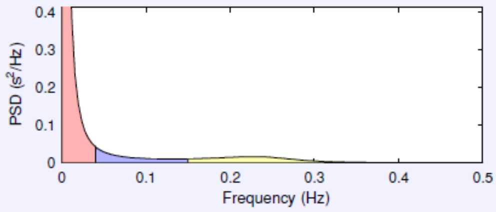
Frequency–Domain Results

FFT spectrum (Welch's periodogram: 256 s window with 50% overlap)



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0195	11507	74.9	
LF (0.04–0.15 Hz)	0.0430	1896	12.3	49.1
HF (0.15–0.4 Hz)	0.2227	1964	12.8	50.9
Total		15366		
LF/HF		0.966		

AR Spectrum (AR model order = 16, not factorized)

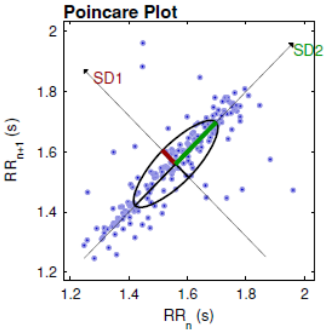


Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	15009	80.1	
LF (0.04–0.15 Hz)	0.0430	1714	9.1	45.8
HF (0.15–0.4 Hz)	0.2266	2025	10.8	54.2
Total		18748		
LF/HF		0.847		

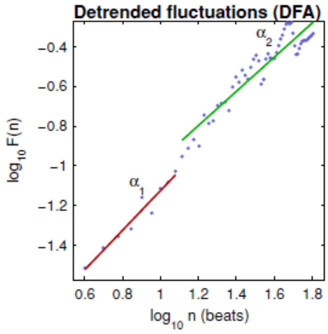
Nonlinear Results*

Variable	Units	Value
Poincare plot		
SD1	(ms)	63.7
SD2	(ms)	195.5
Recurrence plot		
Mean line length (Lmean)	(beats)	12.38
Max line length (Lmax)	(beats)	118
Recurrence rate (REC)	(%)	37.84
Determinism (DET)	(%)	99.24
Shannon Entropy (ShanEn)		3.249
Other		
Approximate entropy (ApEn)		0.778
Sample entropy (SampEn)		0.857
Detrended fluctuations (DFA): $\alpha 1$		0.996
Detrended fluctuations (DFA): $\alpha 2$		0.860
Correlation dimension (D2)		2.313

Poincare Plot



Detrended fluctuations (DFA)



*Results are calculated from the non–detrended selected RR series.

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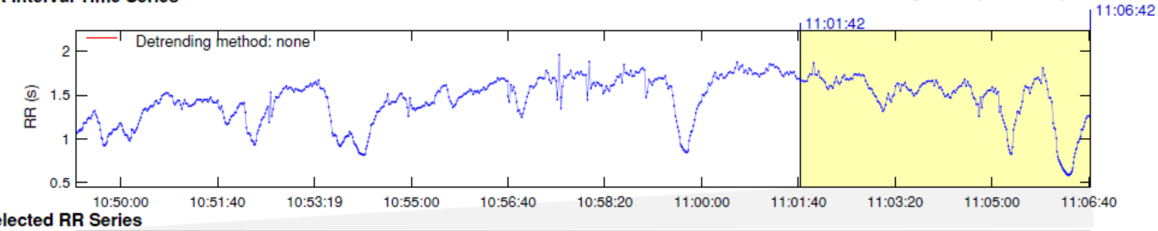
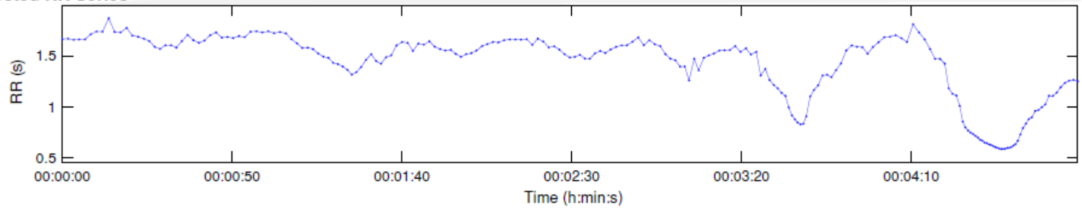
HRV Analysis Results

RR Interval Time Series

Results for single samples: sample 2/2



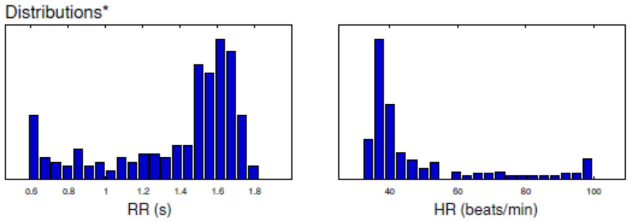
Selected RR Series



Time–Domain Results

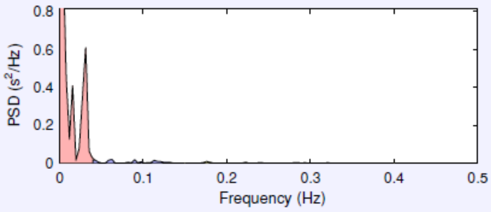
Variable	Units	Value
Mean RR*	(ms)	1379.3
STD RR (SDNN)	(ms)	350.7
Mean HR*	(1/min)	48.09
STD HR	(1/min)	18.76
RMSSD	(ms)	56.7
NN50	(count)	64
pNN50	(%)	29.5
RR triangular index		24.222
TINN	(ms)	230.0

Distributions*



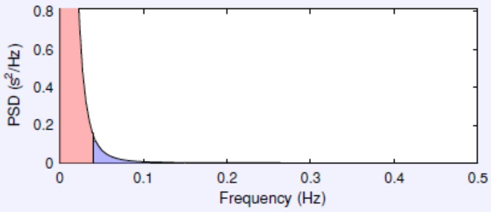
Frequency–Domain Results

FFT spectrum (Welch's periodogram: 256 s window with 50% overlap)



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	14923	94.4	
LF (0.04–0.15 Hz)	0.0625	614	3.9	69.1
HF (0.15–0.4 Hz)	0.1758	274	1.7	30.9
Total		15811		
LF/HF		2.238		

AR Spectrum (AR model order = 16, not factorized)

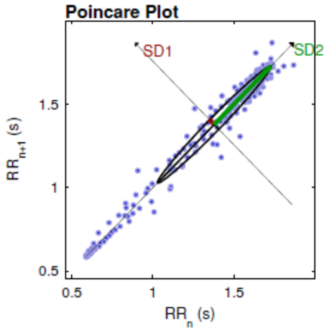


Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	71820	96.0	
LF (0.04–0.15 Hz)	0.0430	2516	3.4	85.2
HF (0.15–0.4 Hz)	0.1523	438	0.6	14.8
Total		74774		
LF/HF		5.742		

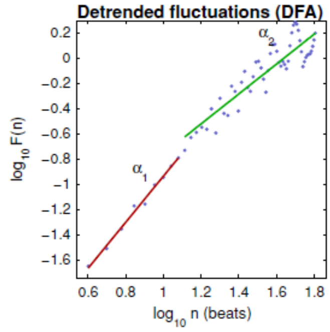
Nonlinear Results*

Variable	Units	Value
Poincare plot		
SD1	(ms)	40.2
SD2	(ms)	495.0
Recurrence plot		
Mean line length (Lmean)	(beats)	33.69
Max line length (Lmax)	(beats)	208
Recurrence rate (REC)	(%)	56.21
Determinism (DET)	(%)	99.88
Shannon Entropy (ShanEn)		4.066
Other		
Approximate entropy (ApEn)		0.489
Sample entropy (SampEn)		0.405
Detrended fluctuations (DFA): $\alpha 1$		1.826
Detrended fluctuations (DFA): $\alpha 2$		1.186
Correlation dimension (D2)		2.516

Poincare Plot



Detrended fluctuations (DFA)



*Results are calculated from the non–detrended selected RR series.

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Discussions

The autonomic nervous system is a system that works unconciously to controll bodily functions, cardiac regulation, vasomotor activity and certain reflex actions. The cardic centre of the medulla oblongata is controlled via the hypothalamus. These send sympathetic and parasympathetic stimuli to the cardiac plexus at the base of the heart. These send additional fibres forming the cardiac nerves via sympathetic ganglia to the SA and AV nodes. As the sinus node is rich in acetylcholinesterase, the effect of any vagal impulse is brief because the acetylcholine is rapidly hydrolyzed. Parasympathetic influences exceed sympathetic effects probably via two independent mechanisms: (1) a cholinergically induced reduction of norepinephrine released in response to sympathetic activity; and (2) a cholinergic attenuation of the response to an adrenergic stimulus. An understanding of the modulatory effects of neural mechanisms on the sinus node has been enhanced by spectral analysis of HRV. The efferent vagal activity is a major contributor to the HF component (Von Borell et al, 2007).

The health of the heart muscle and heart rate varability should improve with exercise. The higher the heart rate variability the healthier the heart muscle. Too much variability though can also have a detrimental effect on the health of the animal, for example it could create an arrhythmia. Respiratory sinus arrhythmia (RSA) is heart rate variability in synchrony with respiration, by which the R-R interval on an ECG is shortened during inspiration and prolonged during expiration. It is a physiologic phenomenon that reflects the relationship between the respiratory and cardiac systems. This is less significant in horses than humans due to their low heart rate but could still be a factor to consider when analysing the heart rate variability. The autonomic nervous system response to athletic training and rehabilitative exercise programs after various disease states is thought to be a conditioning phenomenon. HRV data should be useful in understanding the chronological aspects of training and the time to optimal conditioning as it relates to the autonomic influences on the heart (Task ,1996).

HRV analysis is an important tool nowadays for several reasons and is used by many people throughout the world today simply to monitor their training progress in various sports. HRV became popular to assess autonomic stress and pain reactions especially in nonverbally communicating animals using HRV analysis based on easy-to-use automatic RR-detectors. (Stucke et al, 2015).

(Von Borell et al, 2007) stated that free movement of horses during IBI measurement should be avoided (Parker et al, 2010) to minimise arte-facts, but also prolonged restraint could cause stress resulting in an increased sympathetic activity (Vitale et al, 2013). Thus, for HRV analysis, horses should be in resting conditions in a restricted area, but which is sufficiently wide to allow free movement. (Von Borell et al, 2007). This is what we did during our study as it was performed on the horses when they were in complete rest, while freely standing in their box but not restrained. For reasons of standardisation across different studies incorporating HRV, 5-min recordings have been suggested as a recommended recording length unless the nature of the study dictates another design (Sloan et al, 1994). When analysing longer IBI sequences, averaging the results obtained from 5-min overlapping periods can sometimes minimise some of the difficulties (e.g. non-stationary data) encountered with longer segments of data. Various studies have demonstrated that analysing 5-min segments of IBI data in the time-, frequency- and non-linear domains deliver results comparable to, or even better than, analysing 24 h of data (Von Borell et al, 2007). Therefore in our own study we analyse the average of two different 5 minute segments after they became stationary.

Automatic "filters" which exclude some intervals from the original RR sequence (e.g., those differing by more than 20% from the previous interval) should not replace manual editing as they are known to behave unsatisfactorily and to have undesirable effects leading potentiaily to errors (Task Force, 1996). In short term HRV measurements, AV-blocks occur in a third (Rietmannet al., 2004a) of horses that are resting. Arrhythmias may complicate HRV analysis by distorting HRV parameters and only data segments without arrhythmia should be used for frequency-domain analysis (Physick-Sheard et al, 2000; Rietmann et al., 2004b). Therefore, under resting conditions longer equine ECG sequences are needed to ensure a 5 min ECG sequence without arrhythmia(Von Borell et al, 2007). With the polar system that we used, no manual editings are possible so horses with AV blocks are included in our study. We only did very basic artefact corrections but according to (vendig et al, 2016) this is acceptable. Vendig et al. (2016), have found significant correlations between HRV values evaluated by polar and telemetric ECG in rest and in walk. Others have found that such RR-detectors are sensitive to technical errors (Marchant-Forde et al., 2004; Parker et al, 2010; Jonckheer-Sheehy et al., 2012) and (Marchant-Forde et al, 2004) have defined five error types in IBI identification using an auto-matic RR-detector (Polar®Electro Oy, Kempele, Finland) in comparison with a simultaneously recorded ECG (Stucke et al, 2015)(Von Borell, 2007). While total power and HF power components were over-estimated, LF power components were underestimated

(Marchant-Forde et al., 2004). In recent studies, the IBI data of humans and dogs derived from anautomatic RR-detector (Polar®810s and Polar®RS800CX) were not different from those calculated with a recordedECG. (Parker et al, 2010) compared an automatic RR-detector (Polar®S810i) with a gold standard ECG in horses tied in a resting position, free in the box and free on pasture, and found the same five error types as described by (Marchant-Forde et al, 2004). Error type 4 is the most frequently observed artefact in horses (Parker et al, 2010) which is characterised by failure to correctly identify one or more R-peaks resulting in a long and incorrect IBI. Horses often show a prominent T-wave (Parker et al, 2010) which can be incorrectly detected as R-peak resulting in two short IBIs in the place of one (error type 5) (Stucke et al, 2015). The methods expressing overall HRV and its long- and short-term components cannot replace each other. The selection of method used should correspond to the aim of each particular study (Task, 1996). Based on our results it seems to be that long term parameters reflect training adaptation and short term parameters reflect quick adaptation to recent physiologic stress and rather corresponds to the type of behavior (nervous/calm) of horses. Multipie studies have demonstrated that short-term measures of HRV rapidly return to baseline after transient perturbations induced by such manipulations as mild exercise, administration of short acting vasodilators, transient coronary occlusion, etc. More powerful stimuli, such as maximum exercise or administration of long acting drugs may result in a much more prolonged interval before return to control values (Task, 1996). This is why we recorded the data in the mornings before the animals had exercised. Frequency domain methods should be preferred to the time-domain methods when investigating short-term recordings. (Task, 1996),

Temperament has been commonly investigated alongside heart rate variability. Although most papers in the published literature on emotional states and HRV relate to humans, there are strong arguments that the same principles can be applied to non-human mammals since (Task, 1996) stated that the phylogenetic ‘old’ limbic system is considered as the neural substrate for emotions and is similarly present in both humans and other mammals, (Janig et al, 1977); (Kautzner et al, 1995), electrical stimulation of the hypothalamus and the limbic system in animal models lead to similar emotional responses to those seen in humans (Chapleau et abboud, 2001); (Arora et al, 2004) the endogenous impact of emotions is transmitted via the vegetative nervous system in both humans and other mammals, and; (Carney et al, 2005) the functional control of vagal tone is similar in all mammals (Porges, 1995) (Von Borell et al, 2007). In our study, because of the low number of horses there was no statistical analysis performed on values reflecting behaviour, calm or nervous, only observations were made. Calm horses in the pleasure horse group had higher pNN50, higher HF and lower LF/HF ratio representing higher actual vagal tone. (Von Borell et al, 2007) stated that when using geometrical analysis, recordings of at least 20 minutes (but preferably 24 hours) should be used to ensure the correct performance of these methods. According to this, our results based on the geometrical evaluations should be looked at carefully. It seems to be that nervous or calm behaviour is rather reflected in the frequency domain analysis values. In our group of race horses there is only one nervous horse, so no real observations could be done. Looking at the frequency domain values of this single nervous horse no special observations could be done but looking at the geometrical evaluation, higher SDNN, TINN and SD2 values were detected which rather reflect higher HRV values. The behavioural effect might have been overcome by the training and age in the case of these race horses.

RMSSD (root mean square of successive differences) is used to estimate the high frequency beat-to-beat variations that represent vagal regulatory activity. It represents short term components of HRV. Three of the horses had higher RMSSD (PH 1, PH3 and PH4). These horses are all lame horses and according to (Vendrig et al, 2016) lame horses had lower HRV which is in contradiction to our study. Vendrig only had 2 horses so this is too small a sample number to prove anything. We have 3 horses but we still do not have a high enough number of horses in our study to prove anything by this. However, this indicates that these horses have higher vagal activity and therefore higher parasympathetic influence which is similar to the findings of Kovacs et al. (2015) in lame cows. According to Kovacs et al. the increased parasympathetic influence can be explained by the increased endogenous opioid levels. The current geometrical methods are possibly inappropriate to assess short-term changes in heart rate variability therefore their significance is questionable here.

PH5 has a mean heart rate of 49.51 beats per minute which is a very high heart rate which is possibly abnormal and could be the sign of some type of disease. She appeared clinically healthy but no cardiological examinations have been performed on her. Therefore we thought to exclude her from some of our analysis.

5.1) Stress

Additionally, HRV analysis has also been used to assess stress and susceptibility to stress in horses. Basal autonomic states could be considered as an index for an individual's susceptibility to stress, with individuals with low vagal tone potentially being more vulnerable to stress. In horses, baseline resting levels of LF, HF and their ratio has been found to differ between habitual crib-biting and normal control horses (Bachmann et al, 2003). Other research reports on a relationship between indices of HRV (increased mean HR, LF and HF/LF and decreased HF) and stress related behaviour exhibited as a result of enforced backward movement in horses (Von Borell et al, 2007). An increase in the LF/HF ratio indicates a shift towards sympathetic dominance. In our results PH4 and PH1 have the lowest values for LF/HF ratio. These are the calmest of the pleasure horses. This can indicate an increase in physical and psychological stress. LF/HF ratio is a measure of sympathovagal balance but one has to appreciate that this measure may also be influenced by other physiological functions like thermoregulation or myogenic activity of vessels. In the study done by (Erber et al, 2012) they stated that, neither branding nor microchip implantation was associated with a decrease in HRV, but the increase in HR alone provided evidence of increased sympathetic activity. They recently found a decrease in HRV in foals at weaning (Erber et al., 2011). This all proves that stress decreases heart rate variability. It also shows that stress could be directly linked with whether the horse is a nervous type horse or calm type horse. A nervous horse is a lot more likely to become stressed in everyday life. Positive emotions may significantly increase the HF component of a power spectrum (Tiller et al, 1996), (McCraty et al, 1995) whereas the opposite occurs with negative emotions. However in our study race horse 3 and 4 of the race horses have the lowest HF values and these are 2 calm horses. This again may be that the stress has been overcome by training and age. (Von Borell et al, 2007) stated that in another study done by Friedman and Thayer (Friedman and Thayer, 1996), they examined HRV in people who suffered from panic attacks or blood phobia and found that those who experienced panic attacks had lower vagal tone than those with a blood phobia, while normal controls exhibited the highest tone. Children that exhibited greater flexibility in vagal tone in response to social and attention tasks demonstrated fewer behavioural problems at a later age (Porges et al, 1996). It seems apparent, therefore, that vagal tone is a useful indicator for determining the physiological and psychological ability of someone to respond to stress (Von Borell et al, 2007). This all shows that heart rate variability is an important indicator of stress in horses and also gives us valuable information in how they cope with such stress.

5.2) Training

Training is a commonly investigated method of heart rate variability analysis in horses too. Horses nowadays are mostly used for sport so it is very important in the race horse industry and other sporting industries to be able to monitor a horse's progress in training and other such things. (Von Borell et al, 2007) stated that several horse studies have shown an effect of physical effort and training on cardiac function and sympathovagal balance (Thayer et al, 1997), (Kuwahara et al, 1997), (Physick-Sheard et al, 2000), (Visser et al, 2002), (Kato et al, 2003). In general, resting HR in horses is significantly decreased by training but one study has failed to find any training related changes in the vagal tone when HRV was recorded at rest (Kuwahara et al, 1999). Other work found that under challenging conditions (behaviour tests); untrained horses showed more pronounced elevations in HR (though not significant) and associated decreases in HRV parameters (Viser et al, 2002). (Von Borell et al, 2007). It was found that resting HR decreased, and HRV did not change with training by (KuwaharaM et al, 1999). This is in contrast to our study and to (Vendigs et al, 2016). Intensive physical exertion led to decreased LF power and increased HF power was found by (Physick-Sheard et al., 2000; Cottin et al, 2006). On the long term, training increased LF power and LF/HF ratio (Kuwahara et al., 1999; Hada et al., 2006), meaning there was relative sympathetic predominance under resting conditions. These are very interesting observations as they are opposite to what we found. In our study, comparing horses in training and non-trained, significant differences were visible in SDNN, mean HR, triangular index and SD2, so all reflecting total power and long term variability. Based on this it seems that training adaptations are more reflected by the geometrical values than by the frequency domain analysis. An explanation for the high mean RR and low SDNN and STD HR is that they possibly had extreme vagal influence. (Von Borell et al, 2007) stated that in humans cardiovascular and cardiorespiratory functions are higher in elderly athletes than in age-comparable sedentary groups. Basically age-associated decline in HRV parameters is due to lifestyle too and not solely to aging. We cannot state for sure but we believe that the differences between the pleasure and race horses are possibly due to training rather than the aging but aging is still a viable possibility. PH4 and PH2 were highly trained during their sporting career and have an AV block on auscultation with PH4 possibly having an advanced AV block. We could not evaluate this as we did not do an ECG during this study. PH2 and PH4 have just ended their career (show jumping and dressage throughout their whole lives), 3 horses have never been under high level training and PH6 ended his career as a dressage horse more than 10 years ago. PH 2 and PH4 still have some influence from the training they used to do on their heart rate variability parameters but PH6's career ended too long ago for it to have any influence on his heart variability parameters. Aging may also be a factor here and not just PH6's previous career. The high SDNN observed in PH3 is comparable to the race horses. However this is not associated with training or behaviour and suggests that there are further influences which should be taken into account. PH3 has the lowest SDV1 and the highest value for SDV2 out of the leisure horses, which correlates with the SDNN.

5.3) Behaviour

A behavioural test was done on non-trained horses by (Visser et al, 2002).They found that the HR increased and HRV decreased but this is not significant. HRV analysis in horses appears to be a sensitive measure of both physical and emotional stress responses. When measuring and analysing HRV in horses, it is preferable to use a system that stores ECG due to the characteristics of equine t-waves. It is recommended that you pay particular attention to the occurrence of ectopic beats and edit data accordingly, and while it is not always necessary to shave the electrode site, gel should be used liberally to enhance the signal transmission. We did not have the ability to edit our data correctly in our own study with the use of Polar S810 so performing this kind of behavioural test was not an option for us unfortunately.

4) Conclusion

In conclusion to our study, we discovered that training has a significant effect on heart rate variability parameters. We believe the differences between the race horses and the pleasure horses is mainly due to training because of the significant differences in the SDNN, mean HR, TINN, and SDV2. It was because of the clear influence of training on the heart rate variability parameters of PH2 and PH4 compared with the other pleasure horses that can be seen. This is what makes us believe that the differences between these two groups is more due to training than aging but at the same time further studies should be done to prove this, as we have not ruled out for sure that aging does not have an effect. Behaviour may have an effect on heart rate variability parameters also but our study did not have a large enough sample group to prove anything on this subject. Also in our study, huge individual differences and other factors may also influence heart rate variability, therefore further studies are necessary.

5) Summary

We performed this study for several reasons. We wanted to look at the resting heart rate variability in horses as this had never been done properly before. We investigated the influence of the autonomic nervous system on the Heart Rate Variability and how this influenced calm or nervous type horses differently. We looked at how training induces changes in the autonomic nervous system and compared our results between race horses and pleasure horses. We also wanted to investigate how stress and behaviour relate to heart rate variability and whether heart rate variability was a good measure of stress. Our hypothesis was that calm horses with higher parasympathetic influence of the autonomic nervous system are less stressed healthier horses and that training has an influence on heart rate variability parameters. We had 2 different groups of horses in our study. The first group were 6 race horses all in the height of their training careers, the second a group of pleasure horses and older retired sport horses. We used The Polar S810 to measure Heart Rate Variability. This is a non-invasive method and we performed this on our horses first thing in the morning while at rest in their own boxes. We measured 5 minute intervals and took an average of two of these to get our results. We analysed these with Kubios Heart rate variability analysis system. We discovered that training had a significant effect on heart rate variability parameters. Behaviour may also have an effect but a larger study needs to be done as we did not have a big enough sample number during our own study. Further studies also need to be done because of huge individual variation.

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7) References

Andre E. Aubert, Bert Seps and Frank Beckers (2003): Heart Rate Variability in Athletes; Laboratory of Experimental Cardiology, School of Medicine, K.U. Leuven, Leuven, Belgium

Arora R, Krummerman A, Vijayaraman P, Rosengarten M, Suryadevara V, Lejemtel T, et al. (2004): Heart rate variability and diastolic heart failure; Pace 2004; 27: 299–303.

Bachmann I, Bernasconi P, Hermann R, Weishaupt MA, Stauffacher M (2003); Behavioural and physiological responses to an acute stressor in crib-biting and control horses; Appl Anim Behav Sci 2003; 82: 297–311.

Becker-Birck, M., Schmidt, A., Lasarzik, J., Aurich, J., Möstl, E., Aurich, C; in press-a (2013): Cortisol release and heart rate variability in sport horses participating in equestrian competitions: Journal of Veterinary Behaviour – Clinical Applications and Research: http://dx.doi.org/10.1016/j.jveb.2012.05.002.

Carney R M, Freedland KE, Veith RC (2005); Depression, the autonomic nervous system, coronary heart disease. Psychosom Med 2005; 67: S29–33.

CCC. Vendrig, BSc, Inge D Wijnberg DVM, PhD, Dipl ECEIM, Meg M. Sleeper VMD, Dipl DACVIM (2016): Heart rate variability in endurance horses, how is the HRV affected by rest and different training settings?: Research project, Faculty of veterinary medicine, Utrecht University, Department of equine sciences

Chapleau MW, Abboud FM (2001). Introduction. In: Chapleau MW, Abboud FM, editors; Neuro-cardiovascular regulation: from molecules to man, vol. 940. Ann. of the New York academy of sciences; 2001. p. XIII-XXII

Cottin F , Leprêtre P.M, Lopes P, Papelier Y, Médigue C, Billat V (2006); Assessment of Ventilatory Thresholds from Heart Rate Variability in Well-Trained Subjects during Cycling

De Vito G, Galloway S D, Nimmo M A, Maas P, McMurray JJ (2002); Effects of central sympathetic inhibition on heart rate variability during steady state exercise in healthy humans. Clin Physiol Funct Imaging (2002); 22: 32–38.

Eberhard von Borell a, Jan Langbein b, Gérard Després c, i, Sven Hansen a, Christine Leterrier d, Jeremy Marchant-Forde e, Ruth Marchant-Forde e, Michela Minero f, Elmar Mohr g, Armelle Prunier h, Dorothée Valance d, Isabelle Veissier i, (2007); Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals — A

Erber R, Wulf M, Rose-Meierhöfer S, Becker-Birck M, Möstl E, Aurich J, Hoffmann G, Aurich C (2011). Behavioural and physiological responses of young horses to different weaning protocols. Stress. doi:10.3109/10253890.2011.606855.

Friedman BH, Thayer JF (1998). Anxiety and autonomic flexibility: a cardiovascular approach. Biol Psychol 1998; 47: 243–63.

Hada T, Ohmura , Mukai K, Eto D, Takahashi T, Hiraga A (2006); Utilisation of the time constant calculated from heart rate recovery after exercise for evaluation of autonomic activity in horses

Heart Rate Variability: Standards of Measurement, Physiological Interpretation, and Clinical Use. Task Force of the European Society of Cardiology and the North: American Society for Pacing and Electrophysiology

Jänig W, Das vegetative Nervensystem. In: Schmidt RF, Thews G, editors (1977): Physiologie des Menschen. Berlin, Heidelberg: Springer-Verilog; 1977. p. 114–45.

Jonchleer-Sheehy Valerie SM, Vinke Claudia M, Ortolani Allessia (2012); Validation of a polar human heart rate monitor for measuring heart rate variability in adult dogs under stationary conditions.

J. Sztajzel ′, M. Jung 2, K. Sievert I, A. Bayes de Luna J (2008): Cardiac autonomic profile in different sports disciplines during all-day activity: Introduction: Cardiac vagal control, emotion, psychopathology, and health (Science direct) Biological Psychology 74 (2007) 113–115

Junichiro Hayano, Fumihiko Yasuma, Akiyoshi Okada, Seiji Mukai and Takao Fujinami (1996): Respiratory Sinus Arrhythmia: A Phenomenon Improving Pulmonary Gas Exchange and Circulatory Efficiency: http://dx.doi.org/10.1161/01.CIR.94.4.842: Circulation. 1996; 94: 842-847. Original published August 15, 1996 http://circ.ahajournals.org/content/94/4/842

Kamath MV, Fallen EL(1995); Correction of the heart rate variability signal for ectopic and missing beats. In M Malik, AJ Camm (eds.): Heart Rate Variability. Armonk, NY, Futura Publishing Co., Inc., 1995, pp. 75-85.)

Kato T, Ohmura H, Hiraga A, Wada S, Kuwahara M, Tsubone H(2003). Changes in heart rate variability in horses during immersion in warm spring water. Am J Vet Res 2003; 64:1482–5.

Kautzner J (1995); Reproducibility of heart rate variability measurements. In: Malik M, Camm AJ, editors. Heart rate variability. Armonk, N.Y.: Futura Publ. Comp., Inc.; 1995. p. 165–71.

Kovács Levente, Fruzsina Luca Kézér , Viktor Jurkovich , Margit Kulcsár-Huszenicza, János Tőzsér (2015); Heart Rate Variability as an Indicator of Chronic Stress Caused by Lameness in Dairy Cows

KuwaharaM, Hiraga A, KaiM, Tsubone H, Sugano S (1999). Influence of training on autonomic nervous function in horses: evaluation by power spectral analysis of heart rate variability. Equine Vet J 1999;Suppl. 30:178–80.

Kuwahara M, Suzuki A, Tsutsumi H, Tanigawa M, Tsubone H, Sugano S(1999):Power spectral analysis of heart rate variability for assessment of diurnal variation of autonomic nervous activity in miniature swine. Lab Anim Sci 1999;49:202–8.

Marchant-Forde RM, Marchant-Forde JN (2004): Pregnancy-related changes in behaviour and cardiac activity in primiparous pigs. Physiol Behav 2004; 82:815–25.

Marc M, Parvizi N, Ellendorff F, Kallweit E, Elsaesser F (2000): Plasma cortisol and ACTH concentrations in the warmblood horse in response to a standardized treadmill exercise test as physiological markers for evaluation of training status: J Anim Sci
2000; 78: 1936-46.

Mareike Becker-Bircka, Alice Schmidta, Juliane Lasarzika, Jo¨rg Aurichb, Erich Mo¨ stlc, Christine Auricha(2013): Cortisol release and heart rate variability in sport horses participating in equestrian competitions: aGraf Lehndorff Institute for Equine Science, Vienna University of Veterinary Sciences, Neustadt (Dosse), Germany; bDivision of Reproduction, University of Veterinary Sciences, Vienna, Austria; and cDivision of Biochemistry, University of Veterinary Sciences, Vienna, Austria

Mareike von Lewinski a, Sophie Biau b, Regina Erber c, Natascha Ille c, Jörg Aurich d, Jean-Michel Faure b, Erich Möstl e, Christine Aurich(2013): Cortisol release, heart rate and heart rate variability in the horse and its rider: Different responses to training and performance: The Veterinary Journal 197 (2013) 229–232

Marie Peeters MSc, Coline Closson MSc, Jean-François Beckers PhD, Marc Vandenheede PhD(2013): Rider and Horse Salivary Cortisol Levels During Competition and Impact on Performance: Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.

McCraty R, Atkinson M, Tiller WA, Rein G, Watkins AD(1995). The effects of emotions on short-term power spectrum analysis of heart rate variability.
Am J Cardiol 1995;76:1089–93.

M. E. Covalesky, C. R. Russoniello and K. Malinowski, PhD 1(1992): Effects of show jumping performance stress on plasma cortisol and lactate concentrations and heart rate and behaviour in horses; Mircean M, Giurgiu G, Mircean V, Zinveliu E (2007): Serum cortisol variation of sport horses in relation with the level of training and effort intensity; Bull Univ Agric Sci Vet Med Cluj Napoca 2007; 64:488-92.

Parker Matthew O, Goodwin Deborah, Eager Rachel A, Marlin David (2010); Comparison of Polar W heart rate interval data with simultaneously recorded ECG signals in horses.

Physick-Sheard PW, Marlin DJ, Thornhill R, Schroter RC (2000). Frequency domain analysis of heart rate variability in horses at rest and during exercise. Equine Vet J 2000; 32:253–62. .

R. Erber, M. Wulf, M. Becker-Birck, S. Kaps, J.E. Aurich, E. Möstl, C. Aurich (2012); Physiological and behavioural responses of young horses to hot iron branding and microchip implantation; The Veterinary Journal 191 (2012) 171–175

Porges SW, Doussard-Roosevelt JA, Portales AL, Greenspan SI(1996). Infant regulation of the vagal “Brake” predicts child behaviour problems: a psychobiological model of social behavior; Dev Psychobiol 1996;29:697–712

Porges SW (1995). Orienting in a defensive world: mammalian modifications of our evolutionary heritage. A Polyvagal Theory. Psychophysiology 1995;32:301–18.

Rietmann TR, Stuart AEA, Bernasconi P, Stauffacher M, Auer JA, Weishaupt MA (2004): Assessment of mental stress in warmblood horses: heart rate variability in comparison to heart rate and selected behavioural parameters. Appl Anim Behav Sci 2004; 88:121–36.

Schmidt, A., Aurich, J., Möstl, E., Müller, J., Aurich, C., (2010a); Changes in cortisol release and heart rate variability during the initial training of 3-year-old sport horses; Hormones and Behavior 58, 628–636.

Schmidt A, Biau S, Mo¨stl E, Becker-Birck M., Morillon B, Aurich J, Faure, JM, Aurich C, (2010b). Changes in cortisol release and heart rate variability in sport horses during long-distance road transport: Domest. Anim. Endocrinol. 38, 179-189.

Schmidt, A., Mo¨stl, E., Wehnert, C., Aurich, J., Mu¨ ller, J., Aurich, C., (2010d); Cortisol release and heart rate variability in horses during road transport. Horm. Behav. 57, 209-215.

S. Kinnunent, R. Laukkanen, J. Haldis, O Hanninent and M Atalayt (2006): Heart rate variability in trotters during different training periods
EQUINE EXERCISE PHYSIOLOGY 7: Equine vet. J., Suppl. 36 (2006) 214-217

Sloan RP, Shapiro PA, Bagiella E, Myers MM, Bigger JT, Steinman RC (1994): Brief interval heart period variability by different methods of analysis correlates highly with 24-h analyses in normals. Biol Psychol 1994; 38: 133–42.

Stucke Diana, GroBe Ruse Mareile, Lebelt Dirk (2015); measuring heart rate variability in horses to investigate the autonomic nervous system activity- pros and cons of different methods.

Task Force of the European Society of Cardiology, North American Society of Pacing and Electrophysiology (1996): Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Circulation 1996; 93: 1043–65.

Thayer JF, Hahn AW, Pearson MA, Sollers III JJ, Johnson PJ, Loch WE(1997). Heart rate variability during exercise in the horse. Biomed Sci Instrum 1997;34:246–51.

Tiller WA, McCraty R, Atkinson M. Cardiac coherence(1996): a new, non-invasive measure of autonomic nervous system order. Altern Ther Health Med 1996;2:52–65

Tulppo MP, Makikallio TH, Takala TE, Seppanen T, Huikuri HV (1996); Quantitative beat-to-beat analysis of heart rate dynamics during exercise; Am J Physiol (1996); 271: H244–H252.

Tyler, C.M., Golland, L.C., Evans D.L., Hodgson, D.R. and Rose, R.J. (1998): Skeletal muscle adaptations to prolonged training, overtraining and detraining in horses; *P'iigers Arch. Eur: J. Physiol.* 436, 391-397.

Visser EK, van Reenen CG, van der Werf JTN, Schilder MBH, Knaap JH, Barneveld A, et al. (2002); Heart rate and heart rate variability during a novel object test and a handling test in young horses. Physiol Behav 2002; 76:289–96.

Von Borell, E., Langbein, J., Despres, G., Hansen, S., Leterrier, C., Marchand-Forde, J., Marchand-Forde, R., Minero, M., Mohr, E., Prunier, A., Valance, D., Veissier, I., (2007): Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – A review. Physiology and Behavior 92, 293–316

Voss B, Mohr E, Krzywanek H(2002). Effects of aqua-treadmill exercise on selected blood parameters and on heart-rate variability of horses. J Vet Med A Physiol Pathol Clin Med 2002;49:137–43.

Witold Kedzierski PhD a, Anna Cywinska PhD(2014): The Effect of Different Physical Exercise on Plasma Leptin, Cortisol, and Some Energetic Parameters Concentrations in Purebred Arabian Horses: Journal of Equine Veterinary Science 34 (2014) 1059–1063

Witold Kedzierski PhD a, Katarzyna Strzelec PhD b, Anna Cywi_nska PhD c, Sylwester Kowalik PhD a (2013):Salivary Cortisol Concentration in Exercised Thoroughbred Horses: Journal of Equine Veterinary Science 33 (2013) 1106-1109

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