

**MONITORING HEART RATE DERIVED INDICES AND DC-
POTENTIAL OF THE BRAIN OVER A 12-WEEK ENDURANCE
TRAINING PERIOD IN RECREATIONAL RUNNERS**

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ABSTRACT

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Purpose: The present study examined the agreement between HR-derived indices obtained over different recording situations (morning upon waking, in the lab, and during nocturnal measurements). Changes in DC-potential were examined following a high-intensity training (HIT) week by comparing them to a control week. Moreover, the relationship between adaptation to endurance training and the changes of morning HR-derived indices were examined. **Methods:** Eighteen subjects performed all testing and measurements (10 men, age 36.4 ± 6.1 yr; height 178.3 ± 5.2 cm; weight 77.8 ± 6.2 kg; VO_{2max} 55.7 ± 6.0 ml/kg/min; 8 women, age 34.1 ± 7.5 yr; height 166.8 ± 6.7 cm; weight 61.7 ± 7.5 kg; VO_{2max} 47.1 ± 4.8 ml/kg/min). During the 12-week period, daily DC-potential and HR-derived indices were obtained upon waking, together with nocturnal HR recordings. Endurance performance was assessed at three time points (PRE, MID, POST) via a 3km Time Trial (TT) and a VO_{2max} test until exhaustion, with the analysis of maximal velocity (V_{max}). **Results:** There was an acceptable agreement between the home and lab measurement for HR (ICC = .754), questionable agreement for Ln RMSSD (ICC = .647) and Ln HF (ICC = .575), and poor agreement for Ln LF (ICC = .427), Ln TP (ICC = .492), and DC-potential (ICC = .291). Agreement between nocturnal and morning measurements ranged from poor to questionable due to low ICC (range from .368 to .683), despite a small typical error. DC-potential did not change following a HIT training period ($p > 0.05$). Weekly morning HR changes significantly correlated with changes in endurance performance over the 8-week intervention period. **Conclusion:** The lab measurements seem to moderately agree with home measurements for time-domain indices. The nocturnal and morning measurements agreement ranged from poor to moderate, thus being not recommended to compare results between these different methods. DC-potential was not affected by changes in training load as observed in HIT periods. Weekly morning HR seems to be the best index to use when evaluating adaptations to endurance training.

Keywords: methodology, endurance, heart rate variability, monitoring, DC-potential.

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“In science there are no experts, only expertly argued points based on evidence”

ABBREVIATIONS

ANS	Autonomic Nervous System
a-vO ₂	Arterio-venous oxygen difference
CNS	Central Nervous System
DC	Direct Current
ECG	Electrocardiogram
EEG	Electroencephalography
HF	High Frequency
HR	Heart Rate
LF	Low Frequency
LT	Lactate Threshold
NN	Normal to Normal
OP	Omega Potential
PNS	Parasympathetic Nervous System
RMSSD	Square root of the mean squared differences of successive NN intervals
RR	R-R Interval
SDNN	Standard Deviation of the NN interval
SNS	Sympathetic Nervous System
SV	Stroke Volume
SWS	Slow Wave Sleep
TP	Total Power
TT	Time Trial
TTE	Time to Exhaustion
VO ₂ max	Maximal Oxygen Consumption
VO ₂ peak	Peak Oxygen Consumption
VLF	Very Low Frequency
VT1	First Ventilatory Threshold
VT2	Second Ventilatory Threshold

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ABSTRACT

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1. Introduction

Endurance physical activity is inherent to humans, even since the Palaeolithic era, where the impact of natural selection was largely influenced by the role of oxidative metabolism for survival reasons (Koch et al., 2008). This hunter-gatherer lifestyle adopted by our human ancestors required a large aerobic activity and may have influenced the current human neurobiology (Boullosa et al., 2013). Over history, different examples of exceptional endurance achievements are documented, as it is the case of Pheidippides, a Greek messenger that covered 40.8 km without stopping from the battlefield of Marathon to Athens to announce the Greek victory over the Persians in 490 BC.

Endurance events have gained importance throughout history. Probably the first reported endurance competition was the running race *dolichos*, which consisted in a 5 km distance competition that was first introduced in the Ancient Olympic games in 720BC. Later on, in 1896, with the celebration of the first Modern Olympic games, numerous endurance events, such as marathon running or cycling, started to raise the importance of endurance sports.

With the passage of time, endurance sports became more and more professionalised, being one of the first training programs documented in the 1920's. In efforts to understand how exercise affected the human physiology, different research centres, such as the Harvard Fatigue Lab or Cambridge lab led by A.V. Hill, started challenging, in the 1920's, the human body, and were able to anticipate our modern view of exercise physiology. Based on some of the empirical and practical data obtained from coaches and scientists after half a century of the Olympic games, the basis of training was then established, as an attempt of improving the performance of the elite athletes. The main purpose of this approach was to provide training loads that were effective in improving the athlete's final performance.

In the latter decades, endurance events have benefited from a further increase in sport professionalism, which helped athletes from all around the world to take part in international competitions. This increase in endurance events importance has led to a

greater interest in the exercise physiology research, aiming at using exercise to better understand the human physiology, and also using the human physiology to better understand how to train athletes of different levels.

Nowadays, every nation aims to improve the physical performance of their athletes to its upper limit, which requires a large training load, including high training volumes and intensities. In turn, the optimal athletic training requires sufficient recovery to restore homeostasis, but enough training load to induce the desired training adaptations. Unluckily, a usual mismatch in training that involves too large training load with insufficient recovery is frequently seen, and results in the state of non-functional overreaching, and when continued for several months, in overtraining syndrome (Meeusen et al., 2013).

In the latter years, the importance of the recovery process has been recognized as a potential area, and a more scientific approach to the recovery process has been investigated in efforts to optimize the balance between load and recovery. When approaching this balance, different systems play an important role, but the importance of the nervous system and its different components, as the autonomic nervous system, has been highlighted as one of the physiological variables that may have a huge impact on optimising training adaptations.

For some of the reasons mentioned above, the topic of this Master thesis may be of interest for sport scientists, coaches or athletes aiming to understand the importance of including autonomic nervous system information in efforts to balance the load and recovery to optimise performance. It will help to understand the methodological issues related to its monitoring and how to obtain a clearer picture of the training adaptation from the correct analysis of the data obtained from the athletes.

2. Endurance training

Endurance training usually involves both high volumes and frequency of training, with modifications of intensity, in efforts to improve the event performance. This chapter will look firstly at the endurance performance determinants, and once the physiological basis is explained, the adaptations that occur following endurance training will be presented. To conclude the chapter, as every athlete is different, the factors that affect the different individual adaptations to a same training stimulus are reviewed.

2.1 Endurance performance determinants

Endurance can be defined as the capacity to sustain the highest possible velocity or power output needed for a certain time or distance. Performance in endurance events has been traditionally explained by three physiological factors: VO_2max , exercise economy and lactate threshold (Di Prampero et al., 1986).

The first one, VO_2max , reflects an individual's maximal rate of aerobic energy expenditure, it is known to represent the upper limit of endurance performance and has been long associated with success in endurance sports (Jones & Carter, 2000). As VO_2max is calculated as the product of maximal cardiac output (Q_{max}) and the maximal arterio-venous oxygen difference (a- $v\text{O}_2$ difference), any improvement of VO_2max would come from an increase in either stroke volume (SV) (maximal HR remains the same) or in a- $v\text{O}_2$ difference (Midgley et al., 2006). The mechanism responsible for increasing SV is an increase in myocardial stress (mechanical overload), which has been suggested to occur at an intensity $>75\%$ of VO_2max (MacDougall & Sale, 1981). In efforts to improve the a- $v\text{O}_2$ difference, an increase in skeletal muscle capillarisation is needed, and this happens following increases in capillary pressure resulting from increases in blood flow (Hudlicka et al., 1992), which can be attained by both low and high training intensities.

The second one, exercise economy, has been defined as the oxygen uptake required at a given absolute exercise intensity. Different studies have reported the close relationship between exercise economy and endurance performance (Conley & Krahenbuhl, 1980; Saunders et al., 2004). It has been suggested that good exercise economy is partly

related to the total volume of endurance training performed, together with the neuromuscular characteristics, since the best economy values are often found in older or more experienced athletes, or those who complete a large weekly training mileage (Jones, 1998). With the increased success of East African runners, the role of economy has been suggested as one of the distinct factor of their performances over their European fellows, as their maximal oxygen uptake is similar across the high-level runners irrespective of their nationality (Wilber and Pitsiladis, 2012). Furthermore, an exceptional running economy developed over the years, together with maintaining the VO_2 max values, has been attributed to be the key in the success of Paula Radcliffe when breaking the marathon world record (Jones, 1998).

The third one, lactate threshold or metabolic threshold, can be defined as the exercise intensity corresponding to the increase in blood lactate above resting levels (Lactate Threshold; LT) and the associated changes in gas exchange (Ventilatory Threshold; VT). This threshold has been shown to be a powerful predictor of endurance performance, and exercise above this threshold is associated with a nonlinear increase in metabolic, respiratory and perceptual stress, which may cause a rapid decrease in performance (Farrell et al., 1979). Following successful endurance training programmes, this threshold can be positively shifted, and this shift allows the athlete to be able to sustain a higher power output or a higher running velocity without experiencing the associated increase in fatigue (Jones & Carter, 2000).

Growing evidence showing the importance of neuromuscular contribution to endurance performance has been reported in the last decade. Although endurance sport events require high aerobic power, athletes must also be able to maintain a relatively high velocity over the course of a race that requires muscle power and neuromuscular skills. Furthermore, in usual race situations like closing a gap during the race, or even for sprinting before the finish line for the race victory, are situations where they may benefit from a better neuromuscular condition (Jones & Carter, 2000). Paavolainen et al. (1999) showed that in addition to aerobic power and economy, the neuromuscular characteristics were also related to the 5-km running performance. They emphasized that in homogeneous athletes, the combination of VO_2 max, running economy, and neuromuscular characteristics could explain the differences in the final 5-km

performance. These findings emphasized the role of neuromuscular and anaerobic characteristics in elite endurance athlete.

In the latter years, especial attention has been directed to understand the factors that make East African runners so successful over long distance running. The research group led by Sano (2015) has focused on studying the muscle-tendon interaction and has found that their unique anthropometric characteristics (i.e., Achilles tendon length) result in a reduction in gastrocnemius activation during submaximal running and a lower tendinous stretch-shortening loading. These findings support the idea that an efficient storage and recoil of elastic energy, may be a key factor in running economy, thus, being the neuromuscular system of great importance for a better performance across athletes with similar maximal oxygen uptake. This is supported by the extensive research done on the lower limb stiffness and running performance (Kubo et al., 2015; Saunders et al., 2004).

2.2 Physiological adaptations to endurance training

The magnitude of the training response depends on the duration of the exercise bouts, their intensity and the frequency with which they are performed, along with the individual factors (Jones & Carter, 2000). As it can be expected, endurance training causes adaptations at different levels.

At the pulmonary level, no structural adaptations are reported in the literature, and adaptations come from functional improvements in respiratory muscle strength and endurance, together with minute ventilation (McKenzie, 2011). At the cardiovascular level, there is an improved delivery of oxygen from the atmospheric air to the mitochondria due to adaptations at different levels. An increase in $a-vO_2$ is typically seen in previously untrained individuals, however, when chronic endurance training is applied, there is a greater increase in SV (Blomqvist & Saltin, 1983). The increased SV is a result from an improved cardiac function, specifically due to increases in left ventricular size, myocardial contractility and end-diastolic volume (Jones & Carter, 2000). Oxygen carrying capacity is also increased due to changes at the blood level, with an increase in red blood cell production and haemoglobin (Hb), together with an

increase in plasma volume (Warburton et al., 2004). Likewise, the HR of individuals after endurance training is reduced during resting and at submaximal intensities (Blomqvist & Saltin, 1983).

At the hormonal level, endurance trained individuals generally become more sensitive toward hormones (i.e., epinephrine, insulin) in the peripheral tissues such as muscle, liver and fat (Kjaer et al., 2008). Endurance training will result in a basal decreased level of circulating reproductive hormones (i.e., testosterone, estradiol), and an increase in circulating plasma cortisol levels (Tremblay et al., 2005; Consitt et al., 2002). Furthermore, high intensity exercise will result in an acute increase in growth hormone (GH) secretion (Weltman et al., 1992), together with a downregulation of the pro-inflammatory cytokine production (Steinacker et al., 2004).

Once the common adaptations following endurance training have been explained, it is necessary to expand on the training design factors that will influence these adaptations. It is known that the total length of the training program influences the magnitude of the adaptations, as an example, improvements in VO_2max for untrained people are usually around 5 to 15% with short-term endurance training programs (Jones & Carter, 2000). However, Hickson et al. (1981) reported that VO_2max increased by 23% over 9 weeks of endurance training, but the majority of this increase (14%) occurred after only 3 weeks. There is some evidence that during longer term training programmes, VO_2max will eventually stabilise, with subsequent improvements in performance resulting from continued improvements in submaximal factors such as exercise economy and lactate threshold (Jones, 1998; Rusko, 1992)

Together with the length of the training intervention, the importance of the training intensity should be kept in mind. It is known that endurance training results in a marked increase in the oxidative capacity of skeletal muscle (Jones & Carter, 2000). However, a recent review done by Bishop et al. (2014) summarizes that training intensity appears to be an important determinant of improvements in mitochondrial function (measured as the rate of ATP production in isolated mitochondria, MAPR), suggesting that repeated fluctuations of O_2 consumption seem necessary to largely increase it. However, training volume seems to be an important determinant of training-induced improvements in

mitochondrial content (measured as citrate synthase, CS), as found in strong correlations between training volumes and changes in skeletal muscle CS.

In highly trained athletes who have been training for years, their adaptations do not seem to be related to the length of the training, but rather to the intensity of their training, thus, the target is to optimise their training distribution (as understood by volume and intensity interaction). Different approaches to endurance training have been investigated during the last decades searching for the optimal balance between intensity and volume. Since the paper of Fiskerstrand & Seiler (2004) describing the training intensity distribution in Norwegian elite rowers, and later on suggesting that there could be some evidence for an optimal distribution (Seiler & Kjerland, 2006), an increased number of research studies looking at the training intensity distributions and how this could be linked to endurance performance. In order to standardize these studies, training intensity was generally divided into three zones defined by the assessment of the different thresholds: Zone 1 ($<VT_1$), Zone 2 ($VT_1 - VT_2$), and Zone 3 ($>VT_2$). Stöggl and Sperlich (2014) concluded that a training intervention where the zone 2 was avoided would have a greater improvement in key endurance parameters like VO_2 peak or Time to Exhaustion (TTE), and this has been supported by many other studies (Muñoz et al., 2014; Plews et al., 2014). However, Jones (1995) reported that a large part of the training towards the marathon world record of Paula Radcliffe was performed at zone 2.

2.3 Factors affecting individual adaptation to endurance training

The previous chapter highlighted the different adaptations that could be expected to take place following endurance training, unfortunately, individual training adaptations do not always follow the same time course of adaptation, but may differ between subjects. Research studies usually report training adaptations as group mean and standard deviation, and this does not reflect the inter-individual variation in the response to the same training stimulus (Mann et al., 2014). From a practical point of view, it is of great importance to understand the factors that may affect the individual differences when adapting to a similar training program, because this will provide us with a tool to individualise the training program for specific subjects or athletes.

It is known that individuals that show a low training response to one variable (i.e; 3km Time Trial) do not necessarily show a low training response in other parameters (i.e; $VO_2\text{max}$) (Scharhag-Rosenberger et al., 2012), making the concept often used in the literature of high-responders or low-responders even more complex. From a physiological point of view, many factors have been considered to play an important role on these adaptations. A recent review by Mann et al. (2014) suggests that different factors like genetic, psychological stressors, gender, age, training status, and sleep, may influence on the individual adaptation to a fixed training intervention.

The influence that genotype, heredity and phenotype might have in the training adaptation to a fixed stimulus was first investigated in 1980s by comparing the within-pair and between-pair training adaptation of monozygotic twins, finding that there was less variation of training responses within pairs, suggesting that certain adaptations were genotype-dependent (Hamel et al., 1986). In the need for a larger study looking at the influences of genotype and heredity, the HERITAGE Family Study (Bouchard et al., 1995) recruited more than 130 families and studied their responses to a 20-week training program. They found that baseline $VO_2\text{max}$, together with age and sex, had little effect on $VO_2\text{max}$ increase after the intervention, thus, not being an important factor for $VO_2\text{max}$ adaptation. However, Bouchard et al. (2011) later found that the responses of $VO_2\text{max}$ could be explained by variance in 21 single nucleotide polymorphisms (SNPs).

Individual variation in the metabolic stress imposed on each training session has been suggested to be a significant factor to individual variation in training responses (Mann et al., 2014), and this is likely to be caused by the prescription of the training intensity with methods that show large inter-individual variation in blood lactate and time to exhaustion to a fixed % $VO_2\text{max}$ or % HR_{max} (Scharhag-Rosenberger et al., 2010). The psychological stress that life events has on subsequent training adaptations has not been completely investigated, but it has been suggested to affect the recovery phase, as it can be seen with a slower recovery of muscle function in the 60 min following a strenuous exercise bout in subjects with higher life associated stress (Stults-Kolehmainen & Bartholomew, 2012).

Latter research suggests that variable training intervention based on individual changes (i.e; morning HRV) would improve the individual adaptations compared to a fixed training intervention. Previous studies (Kiviniemi et al., 2007; 2010) have shown that programming training based on changes of morning HRV (i.e., RMSSD) is a potential tool to maximise individual adaptations. The multiple factors that could affect adaptation should be, if possible, standardised in efforts to better understand the training adaptations process. It should be kept in mind that if individuals do not respond to a certain parameter, as subjects have individual patterns of response, this does not mean that they wont improve in other parameters, thus, being possible to term the same subject as ‘responder’ and ‘non-responder’ in different variables (Mann et al., 2014).

2.4 Training monitoring

Every athlete trains in efforts to improve their performance, especially high-level athletes who try to perform their best at major events, such as the Olympic games. To achieve that goal they usually increase their training volume, intensity or frequency. These modifications are continuously adjusted, to either increase or decrease the level of fatigue, depending on the goal of the phase of training they are at, and these modifications are done based on different objective or subjective markers.

Fatigue should be ideally assessed during all the different phases (figure 1) to ensure that they reach the desired level of fatigue, and later on, to ensure that adaptation to training is occurring and is not hampering the athlete’s adaptation to training. However, because of the multi-factorial nature of fatigue and the inherent complexities of trying to monitor it in the athlete (Halsen, 2014), it is usually done over the various training phases, so that the training program can be adjusted and individualized between each training cycle (Buchheit, 2014).

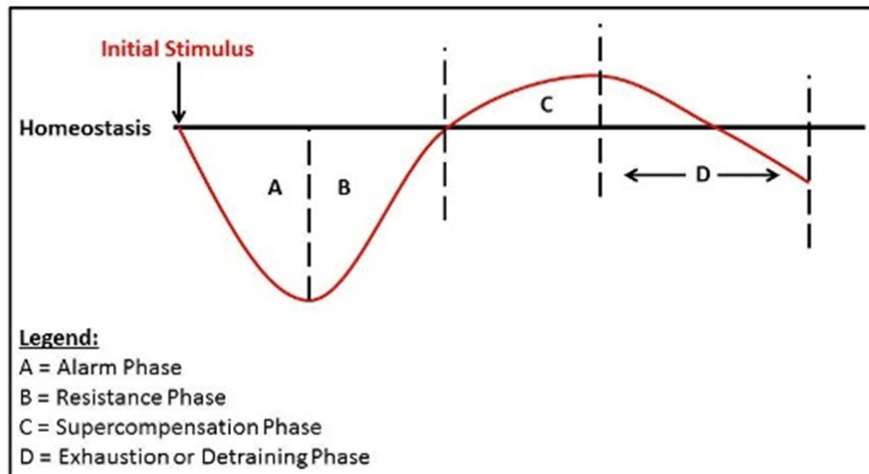


Figure 1. The stress-response model based on Selye's general adaptation syndrome theory. Modified from Selye, 1956.

Due to the complex nature of fatigue, different approaches have been proposed in the literature, and monitoring tools have been mainly divided in two components: external load (i.e; power output in cycling) and internal load (i.e; perceived effort).

External load is defined as a measure of work rate, and is independent of his internal characteristics. In sports like cycling where it can be measured through a power meter, it is easier to monitor it, as you would be able to get objective data of the work rate done after every single training session. On the contrary, in individual or team sports involving running, they have to rely on time-motion analysis, where the tracking of the athlete through GPS will give valuable information about the time spent at different speeds, despite being limited in outdoors situations where wind speed and other factors can influence the measurement (Dellaserra et al., 2014)

Internal load can be defined as the relative physiological and psychological stress imposed in the athlete from different sources (i.e., training loads, family issues). The most commonly used are:

- Rate of Perceived Exertion (RPE) is one of the most commonly quantitative tools, and it is used to rate the perception of effort after each training session or after a competition. It is often combined with other internal (i.e., HR) and external load measures (i.e., Watts).

- Training impulse (TRIMP) is used as a training load tool, and is calculated based on the duration and intensity (measured as mean HR) of the training session (Bannister & Calvert, 1980). There have been different derivations from the original TRIMP, involving RPE or individualised HR-zones, attempting to obtain more accurate individual training load data.
- Lactate concentrations have shown to vary according to the exercise intensity and duration, thus, being a good tool to monitor the session metabolic load. However, different environmental and methodological may limit its daily use (i.e., ambient temperature, hydration status, or sampling procedure).
- Heart Rate (HR) is the most common marker used to assess internal load in athletes due to the strong relationship that it has with submaximal exercise oxygen consumption. Some limitations as environmental and day-to-day variation must be taken into account when interpreting this data.
- Questionnaires and diaries have been an easy and inexpensive way of determining the responses to training sessions and competitions, as they provide subjective information of important issues like perceived fatigue or quality of sleep. However, these questionnaires and diaries must be validated with physiological data, as some subjects might manipulate the data reported.

A combination of both internal and external load provides more valuable information about the status of the athlete, as it is a ratio between what the external work that the athlete actually does, and how his body is reacting to this load. Vesterinen et al. (2014b) recently showed that a ratio between average HR of the session and mean running speed is an effective tool to monitor endurance adaptations. From the different internal load tools mentioned above, indices obtained from HR data have received increasing interest in the latter years (Buchheit, 2014), with growing evidence suggesting that it can accurately inform about positive or negative adaptations to training (Plews et al., 2013a).

3 Autonomic nervous system

The autonomic nervous system (ANS) can be defined as the system of nerves that regulates the function of all innervated tissues and organs throughout the vertebrate body except striated muscle fibres. The ANS is, together with the endocrine system, responsible for maintaining the internal milieu. This control is made from efferent signals that go to the periphery of the body. The essential role of the ANS in these integrative homeostatic and allostatic programs, is to distribute specific signals generated in the central nervous system to the various target organs in order to keep the component cells, tissues and organs in an optimal environment for their function (Jänig, 2006; p. 2). The integration of the different systems that are responsible for maintaining homeostasis can be seen in figure 2.

Autonomic modulation is normally fast and occurs within seconds, contrary to the hormonal system. Most target tissues regulated by the ANS react under physiological conditions to only one of the autonomic systems, but a few of them react to both (Jänig, 2006; p. 24). The heart is one of the organs modulated by the ANS. The sympathetic branch (SNS) is modulated by preganglionic neurons at T1-T5 and postganglionic neurons at superior cervical ganglion, stellate ganglion and upper thoracic ganglia (superior and middle cervical ganglion).

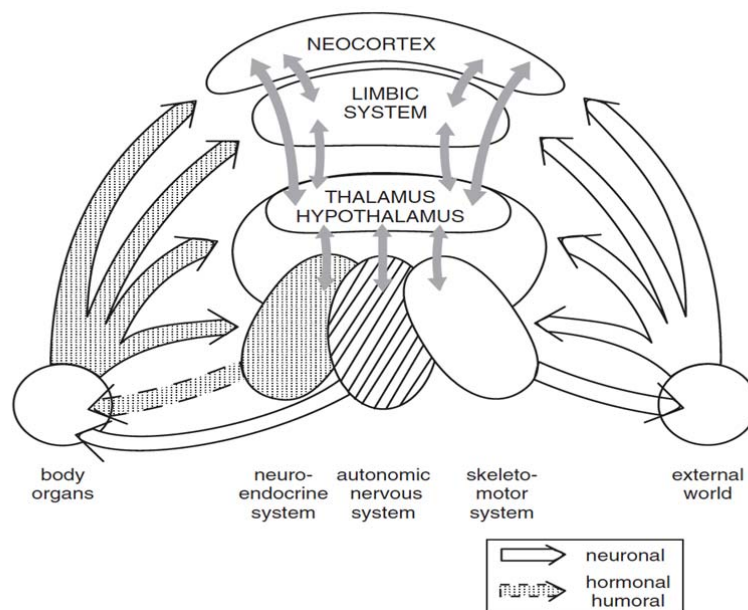


Figure 2. Model representing the integration of the different systems that take part in the maintenance of a stable internal milieu in humans. Modified from Jänig and Häbler (1999)

The parasympathetic branch (PNS) is modulated by the nucleus ambiguus (preganglionic neurons) and the cardiac plexus (postganglionic neurons) (Jänig, 2006; pp. 16-24). The SNS stimulation is done by the nerve endings that are distributed over the heart ventricles, which when stimulated, result in an increase in heart rate and in contractility. However, the PNS stimulation is done at the sinus node, which connects with the atrial fibers, and produces the opposite reaction, which is a decrease in heart rate, and a decrease in contractility strength in a lower magnitude (Guyton & Hall, 2003; pp. 112-113).

3.1 HRV as an index of ANS status

In sports, every time a training stimulus is applied to the body, the aim is to break the homeostasis, with a final goal of pushing the body to restore this homeostasis and adapt to that stimulus, also called supercompensation, becoming more prepared when facing a similar stimulus (Selye, 1956). This homeostasis, as stated above, is partly regulated by the ANS, so the addition of information of the ANS may be important in the training process as it could potentially be monitored how it changes after applying different loads, optimising the training load and the recovery needed (Buchheit, 2014).

The relevance of heart rate variability dates to a century ago, when a Dutch cardiologist called Karel Frederik Wenckebach reported that a variable pulse rate was a sign of a healthy heart (Wenckebach, 1914). The clinical importance of HRV became more popular when it became an independent predictor of mortality following cardiovascular infarction in the late 1980s (TaskForce, 1996). With later availability and development of digital ECG recorders, HRV had the advantage of providing additional valuable time-efficient physiological information at a low cost (Buccheit, 2014), and this interest has been reflected in the amount of increasing annual publications (Heathers, 2014).

To further understand HRV, it might seem to be the oscillation between consecutive instantaneous heart rates, but what it is really measuring is the variation of the time between consecutive beats, as it is usually derived from the RR-interval (RRI) recordings due to the facility of recognising them, as shown in figure 3 (a-b) (Aubert et al., 2003). In order to interpret the results from these recordings, different methods have

been proposed to evaluate the variability of RR intervals, the most popular and widely used are time-domain and frequency-domain methods (TaskForce, 1996).

Time-domain methods are the simplest to perform, as they are easily to obtain with simple statistical methods that could be done on a simple excel spreadsheet (Buchheit, 2014). However, it was said that their main limitation was the discrimination of the activity of the different autonomic branches (Aubert et al., 2003). The most frequently used parameters are:

- Standard deviation of the normal-to-normal interval (SDNN) over the recorded time interval. These parameters represents the total variance of HR, but is largely influenced on the length of the recording period, thus, when compared, it should be done from recording with the same durations (TaskForce, 1996)
- Square root of the mean squared differences of successive normal-to-normal intervals (RMSSD). This parameter is the most commonly used, especially in sport science research. It is known that RMSSD is an index of the parasympathetic modulation, and is not very affected by breathing pattern, thus, being highly recommended for field settings (Buchheit, 2014).

Frequency-domain methods were first introduced by Akselrod et al. (1981) to quantitatively evaluate the beat-to-beat cardiovascular control. Power spectral density (PSD) analyses give information of how power distributes as a function of frequency, and the computation of power in defined frequency regions could give information about the contribution of the different ANS branches (Aubert et al., 2003).

The most commonly used spectral analysis is Fast Fourier Transform (FFT) due to the simplicity of the algorithms used, and the spectral components calculated from this method are (figure 3c):

- Very low frequency (VLF) represents the frequency band < 0.04 Hz, and is though to relate, among other factors, to thermoregulation and kidney functioning.
- Low frequency (LF) represents the frequency range of $0.04 - 0.15$ Hz, which represents oscillations linked to regulation of blood pressure and vasomotor tone, and is considered to be regulated by both parasympathetic and sympathetic outflows.

- High frequency (HF) represents the frequency range of 0.15 – 0.4 Hz, which reflects the effects of respiration on HR (also referred to as respiratory sinus arrhythmia), is considered to represent the parasympathetic efferent activity.
- Total Power (TP), which represents all the frequency bands below 0.4 Hz, and it is considered as an index of total variability.

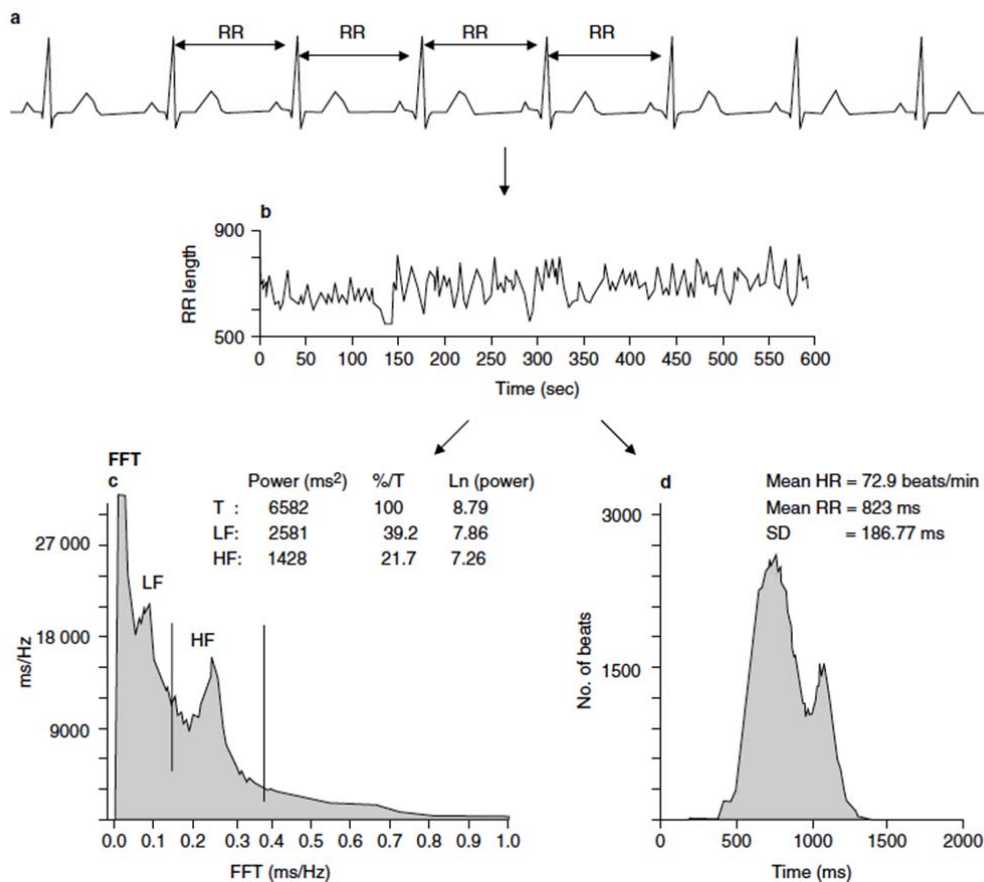


Figure 3. Analysis of the heart rate variability obtained from a resting recording. (a) graphical representation of consecutive RR intervals; (b) frequency domain analysis graph; (c) time domain analysis results; (d) results from a 24h recording. Obtained from Aubert et al., (2003).

It is generally accepted that time-domain indices mainly reflect respiratory sinus arrhythmia, which is mediated by parasympathetic vagal outflow, thus, being considered as vagal tone indices (TaskForce, 1996; Goldstein et al., 2011). Regarding the frequency-domain indices, HF band has been shown to decrease, together with LF and TP, after a complete vagal blockade (Martinmäki et al., 2006), being considered as a marker of vagal tone (TaskForce, 1996). On the other hand, the interpretation of LF

band remains controversial (Reyes del Paso et al., 2013), as it has been traditionally proposed to reflect sympathetic tone, or to be affected by both parasympathetic and sympathetic branches. However, growing evidence show that LF is not correlated with sympathetic outflow (Baumert et al., 2009), and that it does not increase, but rather decreases, when cardiac beta-adrenergic stimulation (which increases HR and plasma norepinephrine levels) is applied (Ahmed et al., 1994). Finally, it is currently suggested that LF band reflects the modulation of cardiac autonomic outflows by baroreflexes (Goldstein et al., 2011).

3.2 HRV as a tool to monitor endurance training adaptations

Due to the increasing interest in monitoring parameters linked to performance and fatigue, in efforts to improve the load-recovery balance, HRV has been proposed as a promising tool for adjusting training loads and preventing the athlete to fall into an overreaching state (Meeusen et al., 2013).

The 1990's decade represents the start point of HRV use in sports, and from there on, there has been a big increase in HRV studies reporting cross-sectional and longitudinal data from athletes of different levels. Over these years, different and contradictory findings have been reported, showing some studies that these measures are sensitive to fitness improvements, fatigue, overload and detraining, while others have not (Plews et al., 2013a).

Measures of autonomic activity, measured at baseline, have been suggested to predict endurance performance (Flatt & Esco, 2014; Vesterinen et al., 2014a), and training responses in untrained or relatively trained subjects (Hautala et al., 2003; Buchheit et al., 2010). Studies looking at the time-course of changes in the ANS status and endurance performance are various (Pichot 2002; Plews et al., 2013b) suggesting that positive and negative changes in endurance performance are linked to changes in HRV. However, due to high day-to-day variation in HR-derived indices, it is suggested to assess training adaptations over changes on weekly averages, and not from single data points (Plews et al., 2012; Buchheit, 2014). Furthermore, resting HRV (measured as

nocturnal TP) has been recently proposed as a marker of trainability of high-intensity training for endurance runners (Vesterinen et al., 2014).

As HRV represents the vagal tone, it is normally higher during resting states. Night recordings have been suggested as more reliable measurements due to the independence of the measurement on environmental factors, and may better discriminate from real changes in the autonomic nervous system (Pichot et al., 2000). Despite being night recordings a more controlled situation, Hynynen et al. (2006) found that HRV after awakening is more sensitive to chronic athletic stress suggesting that these measurements could provide important additional information of the ANS status. Moreover, the measure that could be collected more frequently (morning measure) is likely to be the most powerful one, as it can decrease the noise of measurement when performed daily (Buchheit, 2014).

3.3 Methodological factors affecting HRV

Billman (2011) suggested recently on his work on the HRV history that: *“The internal and external consistency of the methods used have received comparatively less research interest than the understanding of the autonomic, cardiac and circulatory which creates those methods”*. Similar to this, it has been argued that the contradictory findings published to date in the sports science field may be related to methodological inconsistencies and/or partial misinterpretation of the data rather than to limitations of HRV (Buchheit, 2014).

Studies looking at HRV reliability are usually done by means of the test-retest method, with the retest usually done after several days or weeks (Cipryan et al., 2013). It is known that the activity of the ANS system is very sensitive to different external and internal factors, thus, the inter-day variability can consequently be significant. These factors may contribute to the inconsistent results found in HRV studies (Cipryan et al., 2013; Buchheit, 2014).

Reliability studies have also shown a higher CV and associated random error, especially for the frequency-domain parameters (Pinna et al., 2007; Al Haddad et al., 2011),

suggesting that the time-domain methods may be more suitable for reducing the noise of successive measurements. Plews et al. (2012) suggested that single-day data was not enough to conclude whether an athlete is suffering from overreaching or overtraining, due to the high day-to-day variability of the ANS and HRV, so he suggested to use a 7-day rolling average in order to reduce the error and be able to obtain more accurate information about the ANS status of an athlete. A time-domain parameter was used in this study (RMSSD values), due to the reasons above mentioned. This new perspective of the HRV monitoring (averaging consecutive days) aims to assess more accurately the changes in the ANS status.

Additionally, the different methods of assessing the cardiac ANS adaptation to training found in the literature are uneven: repeated measures of resting diurnal HRV (Buchheit et al., 2010; Vesterinen et al., 2013), resting nocturnal HRV (Nummela et al., 2009), exercise HR (Scharhag-Rosenberger et al., 2009), post-exercise HRR (Lamberts et al., 2009), and post-exercise HRV (Buchheit et al., 2008). These variables are thought to have a different time course of adaptation over a training intervention, and it remains to be investigated whether they would provide an equivalent level of information on the training adaptations (Buchheit et al., 2010).

In the following figure (4), an example of the different measurements that can be carried out throughout the day are shown. HRV can be measured in many different situations, however, not only the different measurements possible, but also the duration (1 min, 5 min, 4 h) and the position of the measurement (supine, standing, or seated), are part of the different methodological variations that may affect to the controversial findings in the literature (Esco & Flatt, 2014; Plews et al., 2012; Hynynen et al., 2006).

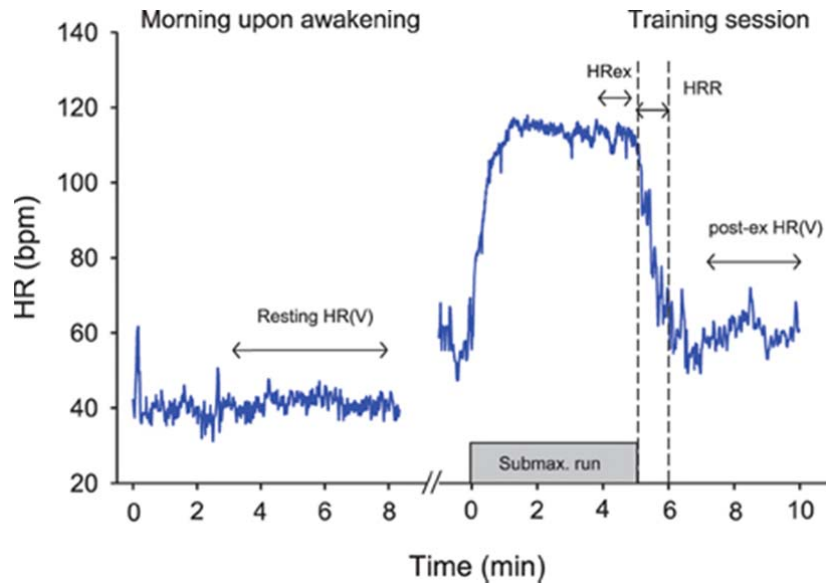


Figure 4. Example of different HR-derived measurements obtained during the day time: resting heart rate variability (HRV), exercise heart rate (HR), heart rate recovery (HRR), and post-exercise HRV (Buchheit, 2014).

Furthermore, another of the methodological variations when comparing between different studies is the methods used to calculate HRV, using either time-domain or frequency-domain parameters. In the latter years, the use of time-domain parameters, such as RMSSD, has increased due to its simplicity, and due to its lower sensitivity to free paced breathing, and makes it an ideal parameter to measure in day-to-day monitoring under spontaneous breathing in athletes (Buchheit, 2014).

4 Central nervous system

The central nervous system (CNS) is a complex system containing more than 100 billion neurons that constantly receive information. One of the most important functions of the CNS is to process incoming information in a way that appropriate mental and motor responses will occur (more than 99% of all the sensory information is considered by the brain as irrelevant). When important sensory information arrives, it is immediately channelled into proper integrative and motor regions of the brain to cause desired responses, this is the integrative function of the CNS (Guyton & Hall, 2006; p. 555).

Traditionally, the role of the CNS on endurance performance – understood as fatigue appearance - has been controversial. Early works from Mosso (1915) proposed that fatigue was from both central origin (nervous) and from peripheral origin (muscle). Later on, the group led by Hill (1923) argued that endurance performance was limited peripherally by an accumulation of metabolites at muscle level because of the limiting capacity of the heart to supply oxygen to muscles, thus causing muscle anaerobiosis in the skeletal muscle. In the latter years, a growing body of evidence has supported the important role that the CNS plays in fatigue appearance, and thus, endurance performance. One of the theories, the so called “central governor” model, was first proposed by Noakes (2011), and suggested that the CNS regulates exercise specifically to insure that each exercise bout ends whilst homeostasis is retained in all bodily systems, and that the limiting factor of endurance performance resides in the brain.

The most important role of the CNS is to control the various bodily functions by controlling the contraction of appropriate muscles throughout the body, the contraction of the smooth muscle in the internal organs, and the secretion of active chemical substances by exocrine and endocrine glands in the body (Guyton & Hall, 2006; p. 556). Operating in parallel to this axis is the ANS (as shown before with the figure 5), which has been explained in the previous chapter.

Three major levels of the central nervous system have specific functional characteristics: the spinal cord level, the subcortical level (lower brain area), and the

cortical level (higher brain area). For instance, the spinal cord level can cause walking movements, and different reflexes (i.e., control of blood vessels). The lower brain area controls most of the subconscious activities of the body (i.e., equilibrium, arterial pressure, anger, etc). For the higher brain area, it is considered a large memory storehouse which functions always in association with the lower brain centres to precisely determine the operations to be done (Guyton & Hall, 2006; p. 558).

This review will focus on the role of the cerebral cortex (the outer cortical layer of the brain), which is made up of neuron's bodies, each of them receiving thousands of synaptic inputs, giving rise to a complex processing capability (Guyton & Hall, 2006; p. 685). The cerebral cortex consists of two hemispheres, such as right and left hemispheres. Each hemisphere can be divided into the following most prominent lobes: frontal, parietal, occipital and temporal. These lobes are responsible for a variety of bodily functions (as seen in figure 5):

- Frontal lobe is involved with personality, emotions, problem solving, motor development, reasoning, planning, parts of speech and movement.
- Parietal lobe is responsible for sensation (e.g. pain, touch), sensory comprehension, recognition, perception of stimuli, orientation and movement.
- Occipital lobe is responsible for visual processing.
- Temporal lobe is involved in dealing with the recognition of auditory stimuli, speech, perception and memory.

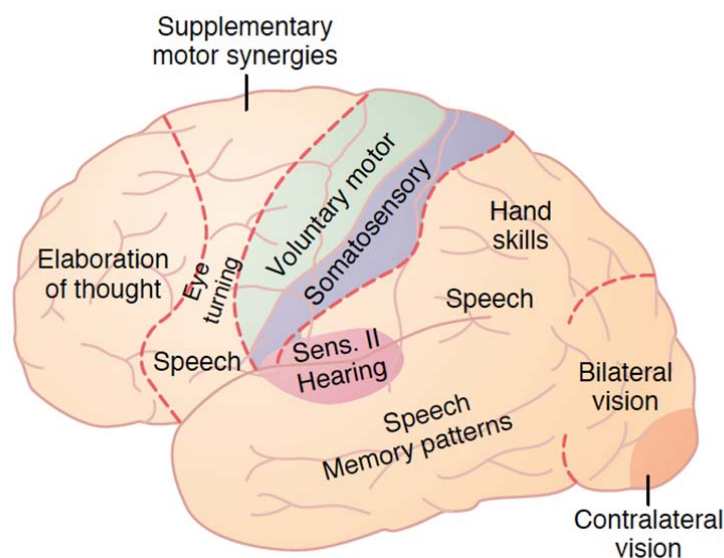


Figure 5. Functional areas of the human cerebral cortex (Guyton & Hall, 2006; p.715)

The CNS is known to be an extremely complex system that is far away from being completely understood. Recent research in the sports field has shown that a decline on motor cortex activity plays a role in endurance exercise termination (Robertson & Marino, 2015), supporting the previously mentioned line that suggests that the brain regulates exercise performance. In addition, the increasing interest of identifying the readiness of an athlete to train or to perform optimally, makes attractive to every sport scientist the integration of a parameter that would provide us with information about the status of the CNS. Despite the increasing amount of research trying to understand the influence of the CNS on exercise performance, empirical data available for evaluating the readiness of the CNS to perform optimally is lacking.

4.1 Neurophysiological basis of EEG and DC recordings

The electroencephalography (EEG) method was first developed by the German psychiatrist Hans Berger, and was considered as an historical breakthrough as it provided a new neurologic and psychiatric tool at that time (Tudor et al., 2004). The EEG records electrical activity from the cerebral cortex, this electrical activity is measured in microvolts (μV) and it must be amplified by a factor of 10^6 in order to be displayed. Most of what it is recorded is known to be originated from neurons, but there are other possible sources including action potentials, post-synaptic potentials, and chronic neuronal depolarization (Rowan & Tolunsky, 2003; p. 1).

Complex neuronal electrical activity generates EEG signals that translate into random and changing EEG waves. The physiological understanding of the underlying mechanisms of EEG rhythmicity, although not completely understood, is mediated through two main processes. Firstly, the interaction between cortex and thalamus, due to the activity of the thalamic pacemaker cells, lead to rhythmic cortical activation. Secondly, is based on the functional properties of large neuronal networks in the cortex that have an intrinsic capacity for rhythmicity. The combination of both results in the creation of recognizable EEG patterns, varying in different areas of cortex, that allows to make sense of the complexity of brain waves (Rowan & Tolunsky, 2003; p. 2).

Traditionally, frequencies higher than 4 Hz were considered as valuable, and a major obstacle was encountered in attempts to faithfully record slow events (also called DC-potentials). Electrode drifts that produced artefactual slow signals tended to saturate the amplifier's dynamic range, and they also pushed the polygraph recorder pens out of scale. To discard these problems, the EEG amplifiers became furnished with an in-built high-pass filter, thus, all kinds of slow signals, whether physiological or artefactual, were eliminated (Vanhatalo et al., 2004).

While it is evident that fast EEG activity has a neuronal origin, slow EEG signals may arise from a variety of sources, including both neuronal and non-neuronal generators. The work of Birbaumer et al. (1990) suggests that the slow potentials represent a measure of excitability of cortical neuronal networks. Based on his findings, the surface potential is affected by the level of polarisation or depolarisation of the dendritic tree in vast networks of pyramidal cells, thus, indicating the level of excitability of the underlying cortical tissue.

The introduction of the DC-potential in the sport field can be attributed to the work of Ilyukhina (1982), where the findings are summarized upon the results of the investigation done on 2900 healthy athletes. The purpose of this work was to recognise the mechanisms of regulation of functional states of the organism as a whole, searching for an integral parameter that would accurately reflect the adaptability of the organism during changing external environmental conditions. From these experimental studies, it was suggested that the time course of the very slow processes of secretory organs and muscles, depended on changes in the state of the CNS, and that was accompanied by reorganisation of very slow activity of the brain and spinal cord. From these findings, he concluded that it was demonstrated the universality of very slow processes as a physiological parameter of the state of the organs and tissues.

From a practical point of view, different patterns of decreasing or increasing the potential following a loading test would demonstrate an optimal activation of the nervous regulation (i.e., 25% increase of the initial values 3 min after the loading). Furthermore, a decrease of more than 50% would mean an unfavourable sign of exhaustion or disturbance of the nervous system. To conclude, Ilyukhina et al. (1982) suggested that the time-course of the DC-potential can be used to assess changes in the

functional state of the athletes, and that this would enable the training process and recovery to be optimised depending on the daily state of this parameter. It remains to be elucidated whether these findings can be practically applied and be meaningful in athletes.

To the best of our knowledge, there are not many studies linking EEG patterns and performance. Del Percio et al. (2009), together with Babiloni et al. (2010), have attempted to test the hypothesis that ‘neural efficiency’ (measured as power decrease in the alpha rhythms, 8-12 Hz) is optimised in elite athletes. Their studies were limited to cross-sectional data and to explosive sports like karate, fencing or rifle shooting, where decisions are made in a relatively short period of time. Their findings showed that elite athletes exhibited lower readiness potential (RP) and mean potential (MP) amplitude, meaning a reduced cortical information processing. They suggested that these rhythms reflected the functional mode of the thalamo-cortical and cortico-cortical loop that facilitates/inhibits the integration of both sensorimotor and cognitive information into the brain, and that these low- and high-frequency alpha rhythms were associated with global brain arousal and the subject’s attentive readiness. Whether these alpha rhythms may change longitudinally with training, and if these changes would provide a window of trainability/readiness remains to be explored.

4.2 Methodological factors affecting EEG and DC-potential measurements

Different factors may influence the recordings of the DC-potential, similarly to those found in EEG recordings, thus, the methodological factors that affect both the EEG and the DC-potential recordings will be presented. To start, the measurements of voltage, obtained from the scalp, always refers to a relative difference in potential energy between two points being monitored. The potential measured is dependent upon the activity present at the ground measure, and the location of a reference electrode should be ideally silent in electrical activity. This relativity of bioelectric recording must be taken into consideration when interpreting EEG data and when forming subsequent conclusions. Ideally, a ground or distant reference point would be a point in space which is electrically stable and which would contribute no fluctuations of electrical

- Delta activity (< 4Hz band) is not present in the normal adult, and when present, it implies some kind of cerebral dysfunction (i.e., vascular disease).
- Theta activity (4-7 Hz band) is usually present in the waking adult, and it should be symmetrically distributed to represent normal function, otherwise it would represent an underlying structural injury, but usually less malignant than what is found in the Delta band.
- Alpha activity (8-13 Hz band) is the principal activity of the normal adult, being at its best when the subject is in a relaxed state with closed eyes, and a certain degree of symmetry between hemispheres is needed for a normal functioning of the brain.
- Beta activity (> 14 Hz band) is always present, and an absence of it may represent an abnormality, and a certain degree of symmetry is expected if there is a correct functioning of the brain.
- The frequency band used in this study is further reduced to a 0-0.5 Hz band, and it is called DC-potential or ultra/intra slow activity (Vanhatalo et al., 2004).

To conclude this chapter, it is necessary to highlight the need of standardizing the protocol when using EEG data in research, especially because the abovementioned factors will influence the results obtained. From a practical point of view, to be able to use the information obtained from the scalp, in the field, it is first needed to be easy to use, and to be consistent with the results obtained in similar controlled conditions. These requirements are necessary to be able to differ between a real change in the state of the CNS with confounding factors (i.e., noise of the measurement).

5. Aim of the study

The discrepancies found in the literature regarding HRV have been mainly attributed to methodological differences between experiments (Buccheit et al., 2014). To the best of our knowledge, there is limited research regarding these methodological differences, and one of the important issues is the reliability of HR-derived indices comparing home-based measurements versus lab-based measurements done on the same day (\pm 2h). Furthermore, the different methods used in the literature (i.e., nocturnal measurements) to assess HR-derived indices have not been compared to see whether they differ or not when monitored on the same day.

On the other hand, new methods that could potentially monitor fatigue are needed in order to make better decisions upon modifying an athlete's training. To the best of our knowledge, there is no empirical data looking into DC-Potential changes from home to lab measurements done on the same morning. Furthermore, changes in DC-potential following an increase in training intensity have not been reported. To conclude, as both HR and HRV (i.e., RMSSD) have been suggested to be related with changes in endurance performance, it is important to determine which index would better predict the changes in performance seen over the 8week intervention period.

The aims of the study were:

1. To compare the HR-derived indices and DC-potential obtained in the morning at home with those obtained in the lab in the same morning.
2. To compare HR-derived indices from morning and nocturnal recordings obtained on the same day.
3. To determine if DC-potential is changed following a high-intensity training period when compared to changes over a low-intensity training period.
4. To determine if HR or RMSSD could predict the changes in endurance performance occurred over the 8week training intervention.

6. Methods

6.1 Study design

The study was designed to compare different monitoring variables throughout the 15 weeks period (Figure 7). Subjects performed endurance tests and basal testing at three time points (PRE, MID and POST). Every subject was assigned with a device (Omegawave Oy; Espoo, Finland) that was automatically synchronised with the lab computer and daily checked by a researcher. Subjects were instructed after the PRE measurements how to self-assess HR-derived indices and DC-Potential at home, and were requested to do it daily throughout the intervention period. Moreover, a Garmin Forerunner 610 was assigned and used by every subject during all their training sessions, and to were requested to wear it during at least 4 nights a week. Training and nocturnal data was sent to a project researcher once a week, and later analysed.

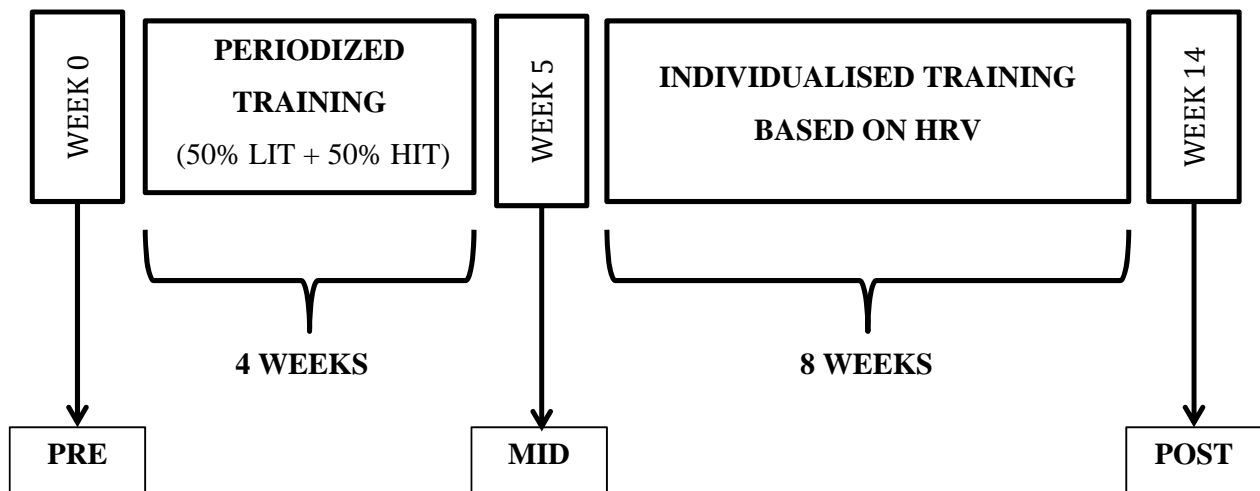


Figure 7. Experimental design of the study. Low-intensity training (LIT); High-intensity training (HIT).

6.2 Subjects

Twenty recreational runners were recruited, but only eighteen subjects performed all testing and measurements (10 men, age 36.4 ± 6.1 yr; height 178.3 ± 5.2 cm; weight 77.8 ± 6.2 kg; $VO_2\text{max}$ 55.7 ± 6.0 ml/kg/min; 8 women, age 34.1 ± 7.5 yr; height 166.8 ± 6.7 cm; weight 61.7 ± 7.5 kg; $VO_2\text{max}$ 47.1 ± 4.8 ml/kg/min). The inclusion criteria on entering the study were: have a background of at least two years of endurance training, age between 18-45 for men and 18-50 for women, body mass index (BMI) less than 30, non-smoker, and a health status without any chronic diseases or prescribed medications. Before the start of the project, all subjects underwent medical screening to ensure that they presented a normal ECG pattern. After comprehensive oral and written explanations of the study, all subjects gave their written informed consent. The Ethics Committee of the University of Jyväskylä, in accordance with the Declaration of Helsinki, approved this study.

6.3 Training intervention

The training intervention had a total duration of 12 weeks that was divided into two parts. During both parts of the study, the subjects were allowed to perform once a week a circuit training strength session and one training session of a different mode than running (i.e., cycling).

i) The first part of the study consisted of a 4-week preparation period where subjects followed a periodized training program with half of their weekly sessions done at low-intensity and the other half was done at moderate- or high-intensity. In this first part of the study, training was adjusted individually to maintain their usual frequency and volume of training, however training intensity was changed. This period followed a classical structure where three weeks of progressive training was followed by one week of low-intensity training with reduced volume.

ii) The second part of the study consisted of an 8-week training period guided by morning RMSSD values. Individual optimal area was established as the smallest worthwhile change (SWC) of RMSSD, based on the recordings obtained from the first part of the study. If the subject 7-day rolling RMSSD went below or above this SWC

line, subjects were asked to rest or perform low-intensity training. On the other hand, if the 7-day rolling RMSSD stayed within the SWC area, high-intensity training sessions were scheduled.

6.4 Data collection and analysis

Maximal Incremental Treadmill Test. The incremental running test was done on a motorized treadmill (Rodby RL3500E, Vänge, Sweden) with the inclination set at 0.5°. Before the test, subjects were asked not to do any vigorous physical activity two days before, and were allowed to warm up for 5 min at the corresponding first stage speed of the running test. The test was started at a speed of 7 km/h for women, and 8 km/h for men, and was further increased every 3 min by 1 km/h until volitional exhaustion. The test was considered as maximal when: RER was higher than 1.05, reported maximal HR was achieved, and/or VO₂ consumption showed a plateau. VO₂max value was taken from the highest VO₂ value over a 60 s period. Before each test session, the gas analyser (Oxycon Mobile, Viasys Healthcare GmbH, Hoechberg, Germany) was calibrated, and breath-by-breath data of ventilation and respiratory gases, as well as HR (Suunto T6, Suunto Oy, Vantaa, Finland), were continuously monitored throughout the test.

3km Time Trial. Every subject ran a maximal 3000m time trial (TT) in a 200m indoor running track (Jyväskylä, Finland). Timing was done by an experienced researcher, and feedback was individually given after every lap. HR (Garmin Forerunner 610, Garmin Ltd., UK) was recorded throughout the test and was used for analysis together with the ending time.

Diurnal OMEGAWAVE measurements. Upon waking, subjects were asked to place two electrodes (one in the hand palm and another one in the mid of the forehead, as indicated by the manufacturer, as seen in the figure 8) in order to quantify the direct current potential of the brain (Omegawave Oy; Espoo, Finland) from the scalp (at Fpz area following international placement 10-20). The subjects were asked to stay in a supine position, with eyes closed and in a quiet environment. The measurement length was until the DC-potential stabilisation, and usually lasted 4 minutes. Together with this, the subjects were asked to measure their RR intervals (Omegawave Oy; Espoo,

Finland) placing a heart rate belt over their chest. The RR data was recorded together throughout 4 minutes and analysed with the Omegawave software. Because of software limitations, artefact correction was not possible, thus, measurements with no more than 2 artefacts were taken into account. Breathing was not controlled and was free paced, as research has shown that RMSSD remains consistent across different breathing patterns (Esco & Flatt, 2014). The individual mobile device of every subject was synchronized with a computer placed in the Physiology Lab (KIHU) and daily updates of their HRV profile was done every morning. Furthermore, during the measurement weeks (PRE, MID and POST), all lab tests were performed between 07.00 and 08.00 hours, with the subjects lying down on a stretcher in a supine position. The room was kept at a comfortable temperature, and before every measurement started, lights were turned off after verifying that there was a complete silent situation.



Figure 8. Example of the placement of both electrodes: a) on the palm of the dominant hand, and b) on the Fpz 10-20 international placement on the forehead.

Nocturnal HR-derived indices. The subjects were asked to measure nocturnal RR intervals during at least four nights per week throughout the training study. A HR monitor (Garmin Forerunner 610, Garmin Ltd., UK) was used to record RR intervals with a sampling frequency of 1000 Hz. The recording started before going to bed and was stopped upon waking in the morning. The first 30 min of recording was excluded and the following continuous 4-hour period was accepted for the analysis if the imposed

cut-off of the erroneous RR intervals was lower than 33%. The acceptable RR interval data was processed and analysed using the Firstbeat Sports heartbeat analysis software (version 2.0.0.9, Firstbeat Technologies Ltd., Jyväskylä, Finland). RR interval recordings were first scanned through an artefact detection filter of the Firstbeat PRO software to exclude all falsely detected, missed and premature heart beats (Saalasti, 2003). Time-domain and Frequency-domain data was obtained from these recordings.

HIT week or LIT week. HIT week was considered when there was no more than one low-intensity session and the rest of the sessions performed were high-intensity training. A lower training load and lower time spent above VT1 was expected for the control week (LIT) versus the HIT week. Seven day rolling DC-potential, HR and RMSSD, both in absolute and relative changes, were analysed.

6.5 Statistical analysis

The data was analysed using SPSS 19 (SPSS Inc, Chicago, USA). All data are presented as mean (SD). The distribution of each variable, as well as the difference between tests, was visually inspected for normality distribution by Q-Q plots, and further explored with the Shapiro-Wilk normality test.

To assess the reliability of measures of HR-derived indices different methods have been proposed in the literature (Atkinson & Nevill, 1998). In this study, a specifically designed spreadsheet was used (Hopkins, 2000), which provides reliability statistics for consecutive pairs of trials for each individual when there are at least two trials. HRV data was log transformed in order to assure a better distribution of the data. The following indices were chosen to assess reliability: i) Pearson's product-moment correlation analysis; ii) typical error of measurement with 95% confident intervals; iii) Intraclass correlation coefficient (ICC) with 95% confidence intervals; iv) Coefficient of Variation (CV); and v) Limits of Agreement (LoA) with the bias between pair of measurements ± 1.96 SD.

To assess the relationship between morning measures (upon awaking) and lab measurements assessed in the same morning, a total of 33 cases from 18 subjects were

obtained. To establish the agreement between nocturnal and morning measurements, data from 18 subjects all over the 12 week period was obtained, and a total of 494 cases were accepted for analysis.

To assess the sensitivity of DC-Potential to high-intensity training (HIT) periods, all training sessions during the last 8 week period were visually inspected for each subject, to obtain a control week where training load is low and done only in a low-intensity (LIT) manner, while a HIT period was established as a week where all except one of the trainings were done with high-intensity. Training load data, calculated from a modified TRIMP version (Lucia et al., 1999) was calculated for each session and week. Furthermore, the time above the VT2 was calculated to better show the difference between training periods. Due to the large CV of the daily DC-potential, a 7-day rolling average was used as it has been proposed for HRV measurements (Plews et al., 2012), and the difference between the first (1st) and last (7th) day of each period was compared in absolute and relative values to the control week using a paired student's t-test, with significance established at $p < 0.05$. From the 18 subjects, we were only able to establish a control week and a HIT week for 13 subjects. From these subjects, a total of 20 cases were taken into analysis.

To evaluate the training adaptation, HR and RMSSD, both at a single point and as a weekly average, were analysed. Prior the calculations, uniformity of the variables were examined. A total of 14 subjects were taken into analysis. Pearson's product-moment correlation analysis was used to determine the association between relative changes in endurance tests and the HR-derived indices. The following criteria were adopted to interpret the magnitude of the correlation (r) between test measures: < 0.1 , trivial; 0.1-0.3, small; 0.3-0.5, moderate; 0.5-0.7, large; 0.7-0.9, very large; and 0.9-1.0, almost perfect.

7. Results

Agreement between Home and Lab HR-indices

A summary of the different reliability indices used can be found in table 1. There was a significant difference between home and lab for all the indices except for Ln LF and Ln HF ($P > 0.05$). The moment Pearson correlation showed that HR had a large correlation ($r = .809$), while Ln HF ($r = .575$) and Ln RMSSD ($r = .647$) had a moderate agreement, with the rest of the measurements having a low agreement. When examining closely the data, large individual variations were found. In HR, 17 cases fall within ± 5 bpm, while 8 cases had differences larger than 10 bpm between the home and lab measurements. Figure 9 shows the difference within each pair of measurements for the Ln RMSSD.

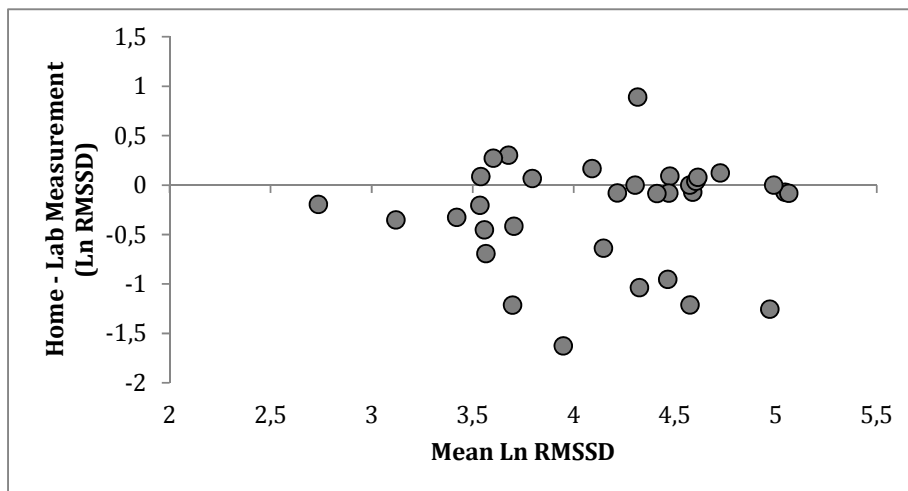


Figure 9. Bland Altman plot with the within-subject differences between the measurement done at home and in the lab in Ln RMSSD.

Table 1. Agreement of HRV parameters between two tests done the same morning at home and in the lab.

	Cases	Test		Reliability				
		Home (i)	Lab (ii)	Pearson Correlation	Typical Error [95% CI]	ICC [95% CI]	CV (%)	LoA Bias [\pm 1.96 SD]
HR	33	52.6 (7.9)	56.5 (11.6) *	.809 *	4.93 [4.0; 6.5]	.754 [.555; .872]	8.9	3.9 [\pm 13.7]
Ln RMSSD	33	4.28 (0.63)	4.01 (0.65) *	.647 *	0.38 [0.31; 0.51]	.647 [.390; .810]	9.8	- .27 [\pm 1.06]
Ln LF	32	6.81 (1.05)	6.54 (1.03)	.427 *	0.79 [0.63; 1.05]	.427 [.092; .675]	12.7	- .27 [\pm 2.18]
Ln HF	32	6.67 (1.35)	6.18 (1.34)	.575 *	0.88 [0.70; 1.17]	.575 [.283; .769]	15.1	- .49 [\pm 2.43]
Ln TP	32	7.69 (0.99)	7.31 (0.97) *	.492 *	0.70 [0.56; 0.93]	.492 [.172; .717]	9.8	- .38 [\pm 1.94]
DC-Potential	30	15.94 (12.6)	18.15 (11.1) *	.293	9.98 [7.95; 13.42]	.291 [-.077; .589]	81.6	4.03 [\pm 19.88]

ICC = Intraclass Correlation Coefficient; CV = Coefficient of Variation; LoA = Limits of Agreement; Bias = mean difference between criterion and repeated measure; [95% CI] = 95% confidence intervals for typical error and ICC. Significance (*) is set at $P < 0.05$. HR = Heart Rate; Ln RMSSD = Natural logarithm of the square root of the mean squared differences of successive normal-to-normal intervals; Ln LF = Natural logarithm of Low Frequency power; Ln HF = Natural logarithm of High Frequency power; Ln TP = Natural logarithm of Total Power; DC-Potential = Direct current potential of the brain.

Agreement between Nocturnal and morning HR-indices

A summary of the different reliability indices used can be found in table 2. There was a significant difference between nocturnal and morning measurements for every variable ($P < 0.05$). A large correlation was found in the variables HR ($r = 0.692$), Ln RMSSD ($r = 0.641$) and Ln TP ($r = 0.532$), while a moderate correlation was found in Ln HF ($r = 0.460$). Despite the acceptable agreement in Ln RMSSD and HR, the limits of agreement plot showed large within-subject differences between measurements (figure 10).

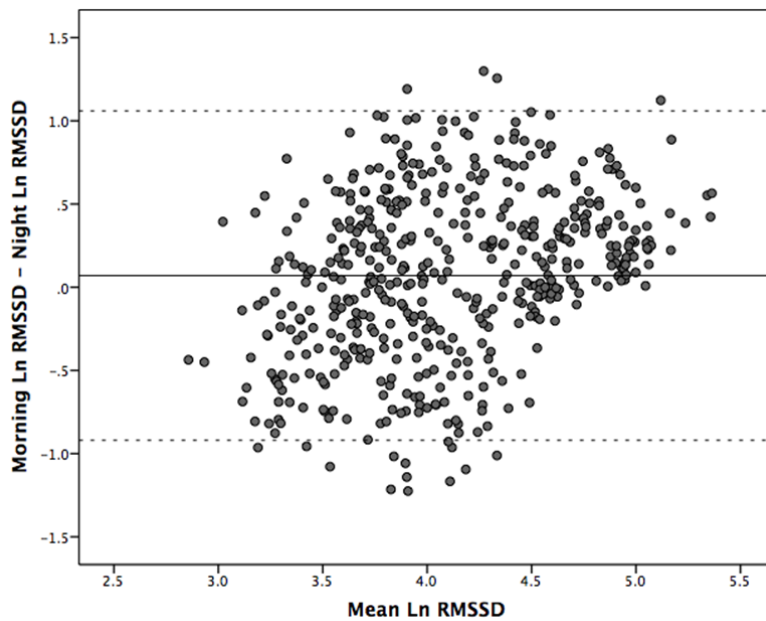


Figure 10. Bland-Altman plot of Morning and Nocturnal Ln RMSSD.

Table 2. Agreement of HRV parameters during two tests done at night and in the following morning.

	Test			Reliability				
	Cases	Nocturnal	Morning	Pearson Correlation	Typical error [95% CI]	ICC [95% CI]	CV (%)	LoA Bias [\pm 1.96 SD]
HR (bpm)	494	53.8 (6.6)	54.2 (7.8) *	.692 *	4.1 [3.85; 4.36]	.683 [.633; .728]	7.4	0.4 [\pm 11.3]
Ln RMSSD (ln ms)	494	4.06 (0.50)	4.13 (0.65) *	.641 *	0.36 [.34; .38]	.619 [.562; .671]	9.1	.07 [\pm 0.99]
Ln HF (ln ms²)	494	7.94 (0.67)	6.33 (1.34) *	.460 *	0.84 [.79; .90]	.368 [.289; .442]	22.2	-1.61 [\pm 2.33]
Ln TP (ln ms²)	494	8.56 (0.75)	7.42 (0.95) *	.532 *	0.59 [.56-.63]	.518 [.450; .580]	13	-1.14 [\pm 1.64]

ICC = Intraclass Correlation Coefficient; CV = Coefficient of Variation; LoA = Limits of Agreement; Bias = mean difference between criterion and repeated measure; [95% CI] = 95% confidence intervals for typical error and ICC. Significance (*) is set at $P < 0.05$. HR = Heart Rate; Ln RMSSD = Natural logarithm of the square root of the mean squared differences of successive normal-to-normal intervals; Ln HF = Natural logarithm of High Frequency power; Ln TP = Natural logarithm of Total Power;

Sensitivity of DC-Potential to High-Intensity training periods

There was a significant difference ($P < 0.05$) between training load in the control week (225 ± 100 AU) and the HIT week (333 ± 108 AU). In addition, there was significant difference between the time spent above the VT2 in the control week (6 ± 8 min) and the HIT week (135 ± 56 min). There were no significant difference of the relative changes from day 1 to day 7, in the 7day rolling RMSSD, the 7day rolling HR, and the 7day rolling DC-potential indices, between the control and the HIT week ($P > 0.05$). Furthermore, there were no significant differences between the absolute changes from the day 1 to the day 7 in the abovementioned variables, between the control week and the HIT week ($P > 0.05$). In the figure 11, an example can be seen of the differences between a control week and a HIT week in one of the cases.

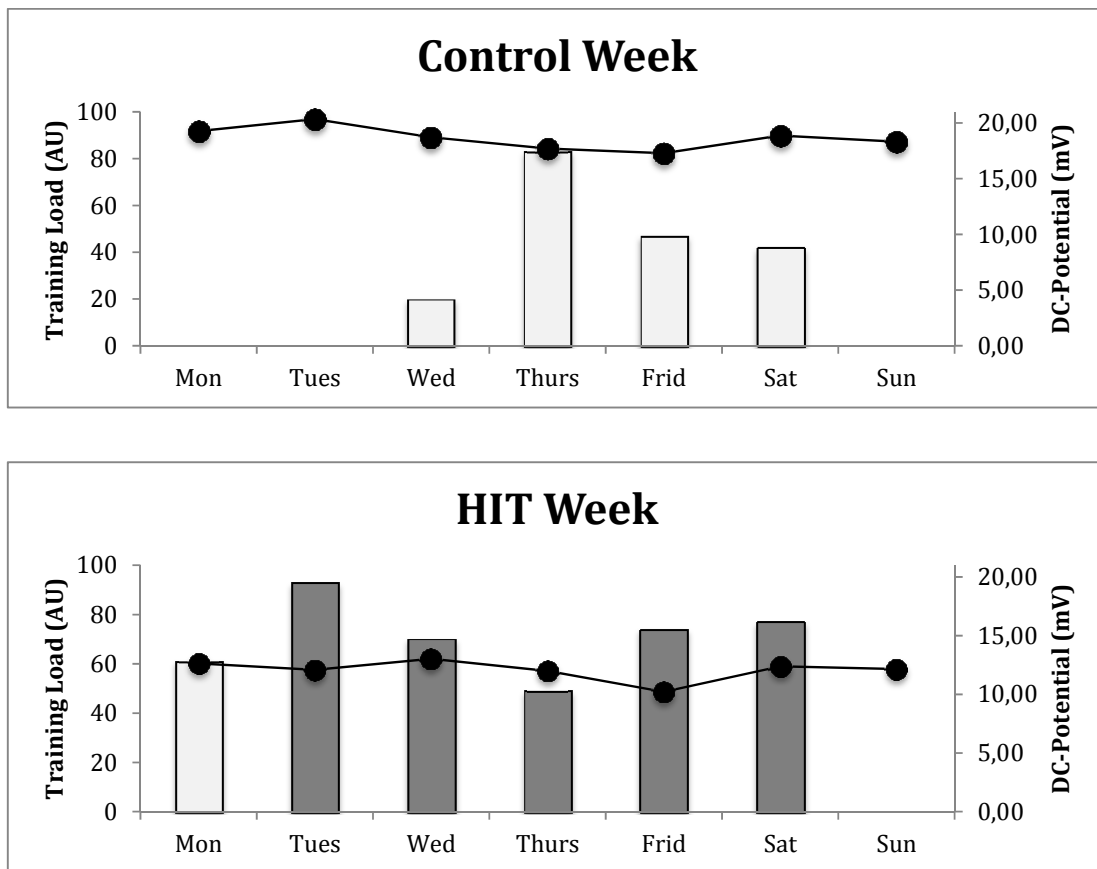


Figure 11. A graphical example comparing a control week and a high-intensity week of one subject during the 8-week period. Dark grey bars represent HIT sessions, while light grey bars represent LIT sessions.

Prediction of endurance performance changes with HR-derived indices

No relationships were found between changes in morning HR (single data point) from MID to POST and relative changes in Vmax speed ($r = 0.094$), $VO_2\text{max}$ ($r = -0.167$), and speed in 3km TT ($r = 0.072$). Positive correlations were found between weekly HR average relative changes from MID to POST and relative changes in Vmax speed ($r = -0.732$; $P < 0.01$) and 3km TT ($r = -0.636$; $P < 0.05$) (Figure 12), and a tendency was found with $VO_2\text{max}$ ($r = -0.53$; $P = 0.051$). Positive correlation was found between changes in RMSSD (single data point) from MID to POST and relative changes in $VO_2\text{max}$ ($r = 0.650$; $P < 0.05$), and no correlations were found with Vmax ($r = -0.132$), and with 3km TT ($r = 0.055$). No correlations were found between relative changes in weekly RMSSD average from MID to POST and relative changes in Vmax speed ($r = 0.328$), $VO_2\text{max}$ ($r = 0.187$), and speed in 3km TT ($r = 0.444$).

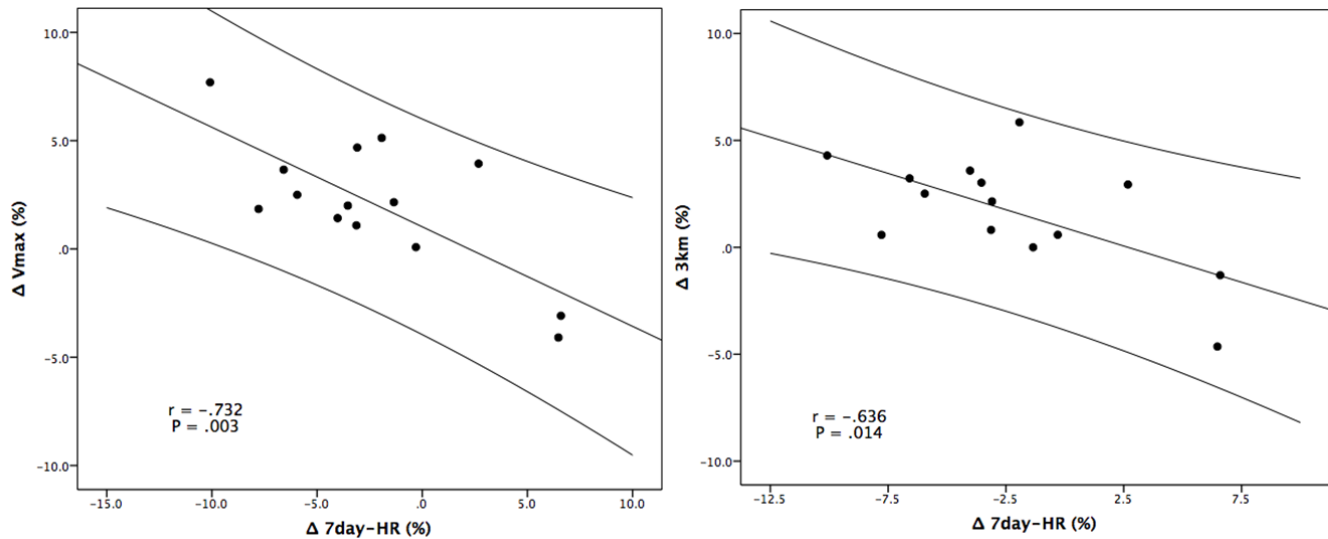


Figure 12. Relative changes from MID to POST measurements (8 weeks) in weekly HR and relative changes in: i) speed at Vmax; ii) speed at 3km TT.

8. Discussion

To the best of our knowledge this is the first study to examine: 1) the agreement between HR-derived indices obtained at home and in the lab in the same morning; 2) the agreement between HR-derived indices obtained during nocturnal and morning recording; 3) to examine the effects of high intensity endurance training on DC-potential; 4) and to determine the relationship between changes in HR-derived indices and changes in endurance performance.

The main findings of the study were: i) the agreement between HRV measures in the same morning (home vs lab) showed acceptable agreement for HR, and questionable for RMSSD, and Ln HF values; ii) the nocturnal and morning measurements showed poor and questionable agreement for most of the indices, but HR and RMSSD seemed to be the ones that have larger agreement; iii) DC-potential did not seem to be changed following increases in training load, as seen during a HIT week period; iv) endurance performance changes were correlated with weekly morning HR average changes, but not with weekly RMSSD changes.

Agreement between Home and Lab HR-indices

The results of our study showed that there was an acceptable agreement between home and lab measurements for HR (seen as ICC > .70), a questionable agreement for Ln RMSSD and Ln HF (ICC .50 - .70), and variables such as Ln LF, Ln TP, and DC-potential had a very poor agreement (ICC < .50). Conclusions from our findings cannot be compared directly to HRV reliability studies (Al Haddad et al., 2011; Cipryan et al., 2013) due to the inherent difference in the study design. These results show slightly lower CV for time-domain variables (i.e., RMSSD), but higher difference with frequency-domain parameters such as Ln HF and Ln LF when compared to the study of Al Haddad et al. (2011). Moreover, for frequency-domain methods, results show larger typical error and ICC when compared to the inter-day reliability (Cipryan et al., 2013).

This is the first study to address the agreement between successive measures of DC-potential of the brain, done in different environments (home and lab) on the same day (within 2h). Our results showed that the agreement is very poor (CV = 81.6%; ICC =

0.291). The origin of this low agreement between measurements may come from different sources. First of all, the associated noise of the measurement is a factor that has to be taken into account when using successive measurement. On the other hand, this low agreement may reflect a different state of the CNS, as the second measurement did not represent a completed rested state, as it was the first measurement. A different state of the CNS may be expected after 1-2h after awakening, and this might be reflected in the large differences between both measurements. However, these changes were in some cases larger than what Ilyukhina et al. (1982) reported to be a change from an optimal state to an exhaustion state (change in 50% of the initial value), making difficult to conclude that this change was due to a change in the state of the CNS.

One of the limitations of the study is the standardization of the transport used (i.e., cycling) when subjects moved towards the lab. The influence that cycling has on a subject's subsequent measurement might have had an effect in some of the subjects, as some subjects had very large differences between measurements (up to 18 bpm). Furthermore, the influence of being in a laboratory should not be undervalued, as for some subjects this first measurement done at the lab might have caused some excitation or stress. In our study breathing pace was not controlled, and it is known that breathing has an influence in the results, especially in frequency-domain methods (Buchheit, 2014). It is also possible that a difference in the breathing, together with possible voluntary or involuntary actions (like moving, swallowing, sneezing, coughing, or yawning) done during the home measurement, would have influenced the lower agreement found in HR-derived and DC-potential indices.

Agreement between Nocturnal and morning HR-indices

Our findings show that the agreement between morning and nocturnal HR-derived indices range from poor (for the frequency-domain indices, Ln HF and Ln TP) to questionable (for the time-domain variables, HR and Ln RMSSD). The nocturnal recordings have been extensively used in HRV research studies (Nummela et al., 2009; Hynynen et al., 2006; 2010; Pichot et al., 2000; 2002), due to the suggested less sensitivity of the measurement to environmental factors, and because it may represent a condition free of external disruptive events (Pichot et al., 2000). However, it has been criticised because the 4h recording period analysed does not distinguish between sleep stages, and thus, does not represent a standardised period, but rather a continuous

variation between sleep stages where different vagal or sympathetic dominance can be found (Buchheit et al., 2004). Another method to measure nocturnal HRV has been proposed by Buchheit et al. (2004). This method limits the measurement to a selected slow wave sleep (SWS) period, which is the deepest stage of sleep, and is characterized by a large vagal tone, and regular respiratory patterns. However, this method has not been compared directly with consecutive morning measurement as done in this study, neither it has been compared with the 4h method used in the present study. Thus, whether the analysis of the SWS period is in agreement with the morning measurement remains to be explored.

Recently, Buchheit (2014) suggested that the possible effects that different patterns of sleep (time spent at different sleep stages) may have on these recordings have been overlooked, and these different patterns may change over time independent from training-related changes in ANS status. In our study, our results show that parameters measured during night recording and morning recording might differ up to around 1 Ln ms^2 (figure 9), which may not be highly meaningful at low values (2-3 Ln ms^2), but it can be much more than meaningful at high values (4-5 Ln ms^2).

The differences in the agreement between both methods might be partly explained by the different timing of the measurement. The first one, the morning measurement, aims to measure the HRV in a rested situation after awakening, where the levels of circulating hormone and body temperature are low. On the other hand, the nocturnal measurement, aims to measure the HRV of a subject over the night, and this period includes different levels of hormonal secretion (i.e., growth hormone), and brain activity throughout the different sleeping stages. Thus, it cannot be expected to obtain the same result, but to give a partial agreement, and possibly to change similarly over time. Future studies should look at the relationship between nocturnal HRV (with both methods: SWS and 4h recording) and quality of sleep (measured as efficiency of sleep and time at different stages), as it could be reflecting a different adaptation than the morning measurement (i.e., efficiency of sleeping).

Sensitivity of DC-Potential to High-Intensity training periods

The results of our study show that neither of the measured variables (7day rolling RMSSD, 7day rolling HR and 7day rolling DC-potential) change following high

intensity periods, when compared to low-intensity periods. HR-derived indices are known to be affected by different factors. Vagal tone can be decreased the following day after a training session with a large part of the training conducted above the second ventilatory threshold (VT2), and can be increased following a low-intensity session below the first ventilatory threshold (VT1) (Seiler et al., 2007). However, following a longer high-intensity training period, the athlete can fall in an overreached state, where evidence of up-regulation of vagal tone has been also reported (Le Meur et al., 2013). Other factors different than training load (i.e., life stresses) have a large influence on HR and HRV (Buchheit, 2014). These different findings make the interpretation of the changes in HR and HRV even more difficult. Based on our findings, it can be suggested that, due to the high day-to-day variation of HR and HRV, together with the different changes expected following high-intensity or low-intensity periods, the interpretation of training solely based on HR and HRV is not recommended. It can be argued that HR-derived indices are more sensitive to chronic changes, as they have been extensively proposed as a parameter to chronic fatigue (Buchheit, 2014).

Furthermore, for DC-potential measurement, the 7-day rolling average was chosen due to its high CV even when done within 2h (CV = 81%). Whether high-intensity sessions have an impact on the state of the CNS (measured as DC-potential) has not been studied before. It is suggested that some of the factors that might have influenced the absence of changes in 7-day DC-potential following HIT periods are the standardised conditions that may be needed to get a reliable measure (Appendix I), and probably makes this tool difficult to use in field settings. Acute changes were found (in single-point DC-potential) following high-intensity training sessions, but of different direction and magnitude. Thus, it remains to be elucidated whether individual patterns may help optimize the usage of DC-potential as a monitoring tool.

Furthermore, future research should look at the influence of the placement currently used for DC-potential measurement in the field, and to compare it to reference placement (Cz position, Vertex) used in research (Ilyukhina et al., 1982), as it may not reflect what the current research suggests. Based on our results, none of the abovementioned parameters should be used independently, and decisions upon training session changes should be done based on the individual variation of the ANS system,

and together with other parameters (i.e., subjective perception of effort) (Buchheit, 2014)

Prediction of endurance performance changes with HR-derived indices

The prediction of endurance performance based on HR-derived indices has been extensively explored in the literature (Buchheit, 2014). However some of the studies have been limited only to positive responders (Buchheit et al., 2010), or have not compared it to VO₂max changes (Plews et al., 2013b). Our findings suggest that weekly morning HR average is the most sensitive parameter to evaluate changes in endurance performance over time, and that it may be more sensitive than RMSSD in single data-point (except for VO₂max) and weekly average.

Single data-points of HR-derived indices for evaluating adaptations to endurance training have received large critics in the last years (Plews et al., 2013b; Buchheit, 2014), arguing that it might hinder the bigger picture that could provide weekly average. Our results show that weekly morning HR was more sensitive to changes in performance than weekly RMSSD changes, as suggested in previous research (Plews et al., 2013b). Resting HR might have changed due to an increase in heart volume and contractility, which would lead to a higher resting stroke volume, and this can be also induced by an increase in blood volume (Aubert et al., 2003), which are common adaptations following endurance training. Furthermore, the lack of relationship found with resting RMSSD might be due to the lower influence of resting vagal tone changes on endurance performance.

The associated day-to-day variation, the influence of environmental factors, and the small error associated with the measurement device, might have had an effect on the low relationship found with RMSSD in this study. Research in this field is limited, and future studies should be looking at the relationship between changes in HR-derived indices and changes in endurance performance in larger samples and in different trained populations (from sedentary to well-trained athletes), in order to be able to make better decision-based changes in training programs.

9. Conclusions

Based on our findings, it is recommended to use the different HRV measurements independently from each other, as the agreement between them is most of the time questionable or even poor. These indices might mirror different changes in the ANS system at distinct time courses.

Furthermore, it is suggested that when introducing new monitoring tools in the training programs, methodological consistency, together with standardization of the measurement, should be regarded as the first step done before making training adjustment based upon the measurement changes. Otherwise, it would not be possible to differentiate between real changes and changes caused by confounding factors. To conclude, it is highly recommended the use of a classical parameter, as it is morning HR, especially when averaged weekly, in order to evaluate the adaptation course following endurance training programs.

Practical Applications

From the practical point of view, morning measures upon waking at home represent the most easy-to-use method to measure HRV (as it takes only 5 min), and it has been suggested to be more sensitive to chronic fatigue changes (i.e., overtraining), when compared to other methods (i.e., nocturnal recordings). Additionally, tracking weekly changes in morning HR can be an effective, easy to implement, and a free way of evaluating adaptations to endurance training over time.

10. References

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11. APPENDIX

OMEGAWAVE INSTRUCTIONS

For the correct collection of data of the O-potential, the following conditions should be met according to the manufacturer:

- Calm and quiet environment, with no excessive light.
- Loud electronic devices should be switched off, with no working magnetic field generators near the measurement place (i.e., TV or phones).
- The room should have a comfortable air temperature (i.e., 19-24°C).

Furthermore, the measurement should be conducted around the same time each day, to avoid the effect of biological rhythms, and before the first meal of the day.

While conducting the measurements, the following steps should be followed:

1. The subject should remain in a relaxed vertical state for at least one minute prior to a measurement.
2. After the first step, the subject should then lie down and relax for around 2 min, allowing the body to enter a stable condition, and after this period, the measurements begins and would last about 2 or 3 min.

Other requirements during the measurement process are:

1. Lying down on the back without a pillow support.
2. Arms alongside the body.
3. Eyes closed.
4. Legs straightened out without crossing them.
5. Breathing should be natural, without excessive depth or increased speed.
6. It is advised to refrain from the following during a measurement: talking, moving, swallowing, sneezing, coughing, or yawning.