RELATIONSHIPS BETWEEN TRAINING, NOCTURNAL HEART RATE VARIABILITY AND MORNING CORTISOL SALIVA LEVELS IN YOUNG ENDURANCE ATHLETES

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Spring 2019
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ABSTRACT

Kuorelahti Titta. 2019. Relationships between training, nocturnal heart rate variability and morning cortisol saliva levels in young endurance athletes. Biology of physical activity, University of Jyväskylä, Bachelor's Thesis in Exercise Physiology, 45 pp.

Introduction. In order to reach the peak levels of performance, athletes must find a way to combine intensive training with sufficient amount of recovery. This is especially important in endurance sports where both training volume and intensity are relatively high. It is not always easy for the athletes to tell the difference between normal training-induced fatigue and non-functional overreaching which is why researchers have pursued to develop methods to assess the recovery from training. There are several ways to evaluate physiological stress and recovery of which HRV and cortisol secretion are very different, but both commonly used. The aim of this study was to search what changes and trends occur in stress and recovery state markers HRV and salivary cortisol in young endurance athletes and what are the relationships between these two stress markers.

Methods. Seven well-trained junior athletes (age 16-18), who trained for cross-country skiing year-round, participated in the study. The study protocol included a seven weeks long measurement period where the HRV and morning salivary cortisol measurements were made every other week to allow for four testing weeks. The nocturnal HRV was measured with a contact-free sleep tracking Emfit QS-device that reported the magnitude of HRV with a time-domain variable rMSSD. The free cortisol levels were measured from the saliva that was collected by participants themselves immediately after waking up using the Passive Drool-method. The study protocol did not alter the athletes' training programmes but the training characteristics (volume, intensity, load) from each athlete were followed during the measurements, using electronic training diaries.

Results. The only significant changes in the training characteristics occurred in the weekly training volume and weekly training load ($p<0.05$). The nocturnal HRV values decreased during the three testing weeks and then increased to the last one, while the morning salivary cortisol level showed a reverse effect by decreasing during the first three test weeks and then increased to the last one. However, the changes within the stress markers were also not considered significant. The average cortisol values varied between 58 and 72 nmol/l. The changes in the morning salivary cortisol level and nocturnal HRV, showed a high negative correlation when the HRV levels were low and the cortisol levels high (-0.893, $p<0.01$; -0.857, $p<0.005$). The significant correlation was found also between the salivary cortisol level and the three-day training intensity (0.811, $p<0.05$). No other correlations between the training characteristics or the stress markers were considered significant, even thought the correlation coefficients were often relatively high.

Conclusions. The results of the present study authenticate the assumption that ANS and circulating hormones work together and are both responsible from the regulation of stress reactions and that the co-operation of these two stress systems is stronger in more stressful conditions. Significant connections between the morning salivary cortisol levels and the training intensity were also found denoting that salivary cortisol could serve important information about the recovery state of body after hard training. The participants' absolute cortisol values were notably higher that they should on average person (reference interval for morning salivary cortisol levels in adults is 4–28 nmol/l (Association for Clinical Biochemistry 2012) which can be considered as a finding that endurance training causes hypercortisolism for young endurance athletes or as a sign of non-functional overreaching or overtraining. HRV did not show any significant associations with any of the training characteristics. In further research, it would be important to find more subjects to do this type of study in order to get more significant and valid findings.

Key words: heart rate variability, cortisol, stress, recovery, endurance training.

ABBREVIATIONS

ACTH	adrenocorticotropic hormone
ANS	autonomic nervous system
HF (ms^2)	high frequency variation of R-R intervals (0.15-0.40 Hz)
HPA-axis	hypothalamic-pituitary-adrenal-axis
HR	heart rate
HRV	heart rate variability
LF (ms^2)	low frequency variation of R-R intervals (0.04-0.15 Hz)
LF/HF	ratio of high to low frequency variation in R-R intervals.
NFO	non-functional overreaching
NN	normal-to-normal interval
NN50	number of interval differences of successive NN intervals greater than 50ms
OT	overtraining
pNN50	proportion derived by dividing NN50 by the total number of NN intervals
PNS	parasympathetic nervous system
PSD	power spectral density
RRI (ms)	time between adjacent heart beats
rMSSD (ms)	the square root of the mean squared differences of successive R-R intervals, estimate of short-term components of HRV.
RSA	respiratory sinus arrythmia
SA node	sinoatrial node
SDNN (ms)	standard deviation of R-R intervals, estimate of overall HRV
SNS	sympathetic nervous system
TP	total power
VLF (ms^2)	very low frequency variation of R-R intervals (<0.04 Hz)
VO_{2max}	maximal oxygen uptake

CONTENTS

1 INTRODUCTION

In order to reach the peak levels of performance, athletes must find a way to combine intensive training with sufficient amount of rest and recovery. This is especially important in endurance sports where both training volume and intensity are relatively high. Endurance training is often based on an overload principle where the intention is to disturb the balanced state of body, the homeostasis, through the training-induced physical stress. After the exercise, an adequate recovery period leads to restoration of homeostasis and former physical capacity. The physical capacity can also be improved during the recovery period because of the adaptation of the physiological systems underlying homeostasis. The efficiency of these systems increases in response to repeated training sessions, leading to smaller disturbance of homeostasis.

The body pursues to restore the disturbed homeostasis with coordinated activation and control of neuroendocrine and autonomic nervous system (ANS). ANS is very sensitive to stress body encounters and responses to it by altering the activating its two subsystems: parasympathetic nervous system (PNS) and sympathetic nervous system (SNS). The activity of PNS has been observed to decrease during stress and return to normal values during the recovery which makes it a promising marker of stress and recovery. Neuroendocrine system, in turn, reacts to stress by activating the hypothalamic-pituitary-adrenal-axis, leading to elevated concentrations of circulating glucocorticoids. By measuring the levels of glucocorticoid stress hormones from plasma, urine or saliva, it is also possible to evaluate the stress and recovery state of body. (Ulrich-Lai & Herman 2009.)

It is not always easy to tell the difference between normal training-induced fatigue and non-functional overreaching. Today, there are numerous applications and devices on the market that athletes can use to measure their recovery state and with that information possibly avoid the unwanted state of overreaching and overtraining. Heart rate variability (HRV) is one of these promising stress markers because of its close relations to the functions of ANS. The use of HRV in monitoring the athlete's state of fatigue and possible signs of overreaching and overtraining has been studied in several researches and as a matter of fact, it has turned out to be a quite

reliable method to determine the recovery from training (Baumert et al. 2006; Hynynen et al. 2007; Kajaia et al. 2017; Pichot et al. 2000; Plews et al. 2012; Plews et al. 2014).

There are also other methods to assess the recovery from training, one of which is measuring the free cortisol levels in body. Cortisol is probably the best known glucocorticoid hormone and it has been generally named to stress hormone because of its relatively quick response to almost any type of stress. Studies have observed the relationships between this stress marker and exercise induced stress and overtraining symptoms and found significant associations between them both (Alghadir et al. 2015; Bandyopadhyay et al. 2012; Hill et al. 2008; Jacks et al. 2002; Roberts et al. 1993). Cortisol levels can be quantified a few different ways (measurements from saliva, blood serum and urine) of which measuring cortisol levels from saliva is the easiest to perform yet still showing good reliability. Reference intervals for morning cortisol levels in adults are 4–28 nmol/l when measured from saliva and 171-536 nmol/l measured from serum (Association for Clinical Biochemistry 2012).

Although there are many researches that have pursued to evaluate the stress and recovery of professional and recreational athletes, it is not easy to find literature focusing on junior athletes. This is understandable considering that the training loads in elite athletes are somewhat higher than they are in juniors, making the line between functional and non-functional overreaching even thinner. However, unlike professionals, junior athletes have to deal with other stress sources in their lives. For example, almost every junior athlete is still at school and they have to find a way to combine the relatively large amount of training with their studies. Junior athletes are also going through great changes in life during and after puberty which increases the emotional stress they have to deal with. It may also be harder for young athletes to recognize the non-functional overreaching symptoms than it is to professionals who know their bodies very well.

Due to these limitations in the literature, the purpose of this study was to examine what changes and trends occur in stress and recovery state markers HRV and salivary cortisol in young endurance athletes. The other purpose was to examine if there is relationships between these two stress markers and what kind of relationships are they.

2 PHYSIOLOGICAL STRESS AND RECOVERY

2.1 Body homeostasis and stress

The term homeostasis is used in physiology to represent the maintenance of a steady and harmonious state of body (Chrousos & Gold 1992). These constant conditions are continuously challenged by intrinsic and extrinsic forces or threats, called stressors. Stress, in turn, refers to a state in which homeostasis is threatened or is perceived to be so. Body reacts to stressors, which can be both physical and emotional, by counteracting forces called adaptational responses. These responses include many physical or mental reactions that aim to restore the disturbed homeostasis. (Chrousos & Gold 1992; Chrousos 2009).

Adaptational responses are known to be regulated by the autonomic nervous system (ANS) and two hormonal axes: hypothalamic-pituitary-adrenal-axis (HPA-axis) and sympathetic-adrenal-medullary axis (Ulrich-Lai & Herman 2009). The most immediate responses are provided by the ANS that includes sympathetic (SNS) and parasympathetic nervous systems (PNS). Both SNS and PNS are involved in regulation of homeostasis and they provide only short-term alterations to physiological states of body by innervating the end organs through neural pathways. The regulation of homeostasis by HPA-axis acts slower and is more sustained than the regulation by ANS. HPA-axis has a major role in controlling the circulation of glucocorticoid hormones, for example cortisol, that are important in maintaining homeostasis. The sympathetic-adrenal-medullary axis in turn, is regulated by the sympathetic branch of the ANS and it can, for example, rapidly affect heart rate and blood pressure. (Ulrich-Lai & Herman 2009.)

2.2 Endurance training as a physiological stressor

Training and exercise can be considered as stress factors that disturb body homeostasis (Borresen & Lambert 2009). After exercise, during the recovery process, the original steady state is restored. Exercise training is based on adaptative mechanisms of the body which means that the body becomes more efficient in a certain type of exercise when that specific exercise is

repeated several times at the same intensity. In addition, better efficiency of the physiological systems underlying the maintenance of homeostasis may decrease the disturbance of body homeostasis. (Borresen & Lambert 2009).

Endurance refers to the ability of the body to resist fatigue during physical activity. Endurance sports involve relatively large muscle groups and training for these sports usually consists high volumes of training at different intensities. Endurance training alters the homeostatic state of body because it requires many acute changes and results in long-term adaptations in metabolism.

During endurance exercise, the need of energy in the working muscles is increased and most of that energy is produced aerobically in oxidative phosphorylation (Rivera-Brown et al. 2012). Oxidative phosphorylation is dependent on the constant supply of oxygen and cardiovascular and respiratory systems are the ones responsible from the maintenance of oxygen delivery to the working muscles (Wilmore et al. 2008, 222-223). In order to guarantee the sufficient supply of oxygen, these two systems increase the oxygen uptake from environmental oxygen through alveolar ventilation, enhance the diffusion of the oxygen into the blood, accelerate the blood flow to tissues by increasing cardiac output and finally, support the oxygen diffusion into the active muscle cells (Rivera-Brown et al. 2012). Endurance exercise also affects to carbohydrate, fat and amino acid metabolism, since all three of those macronutrients can be used as a source of energy in oxidative phosphorylation. However, in higher intensities, some of the energy must be produced anaerobically from the muscle energy stores or via glycolytic pathway. This often results into production and accumulation of lactic acid, forcing the body to also take care of the removal of lactate. In addition, endurance exercise causes several hormonal changes and alters the thermoregulation, thereby disturbing homeostatic state of body even more. (Maughan & Gleeson 2010; Rivera-Brown et al. 2012).

Endurance training leads to several long-term adaptations in body in order to improve the ability to deliver and utilize oxygen for oxidative phosphorylation and enhances the capacity and performance in endurance exercise (Rivera-Brown et al. 2012). The adaptations include changes in the structures of skeletal muscles, metabolism and cardiovascular and respiratory

systems. Some of the changes occur at rest while some of them only appear during submaximal or maximal exercise. (Wilmore et al. 2008, 224-238.) The main cardiovascular, respiratory, muscular and metabolic adaptations are summarized in Table 1.

TABLE 1. Summary of expected adaptations to long-term endurance training. (Wilmore et al. 2008, 224-238.)

	Variable	Effect
Cardiovascular	Heart size	↑
	Stroke volume	↑
	Heart rate	↓
	Cardiac output	↑ at maximal intensity
	Blood flow	↑
	Systolic blood pressure	↓ at submaximal, ↑ at maximal intensities
	Diastolic blood pressure	↓ at maximal intensity
	Blood volume	↑
Respiratory	Pulmonary ventilation	↓ at submaximal, ↑ at maximal intensities
	Pulmonary diffusion	↑ at maximal intensity
	a-vO_2 difference	↑
Muscular	Type I muscle fiber size	↑
	Capillary density	↑
	Myoglobin content	↑
	Mitochondrial number and size	↑
	The number of oxidative enzymes	↑
Metabolic	Lactate threshold	↑
	Respiratory exchange ratio	↓
	Oxygen consumption	↓ at submaximal, ↑ at maximal intensity

↑, increase; ↓, decrease; a-vO_2 difference, arteriovenous oxygen difference.

2.3 Endurance training and recovery

One crucial feature in athletic training is finding the balance between training and recovery. As already discussed, exercise training disturbs the body homeostasis and causes adaptations in body in order to improve the physical performance. Most of these exercise-induced adaptations occur during the recovery between exercises. The recovery process attempts to return the body to former or even higher level of homeostasis after the exercise. If the recovery after training is insufficient, training adaptations and performance gains might be diminished and the athlete can be drifted into fatigued, or in the worst case, overtrained state. (Bishop et al. 2008.)

Endurance training includes high training loads which makes it extremely important to ensure the adequate recovery after training to avoid the unwanted state of fatigue. Endurance exercise causes accumulation of several metabolites like hydrogen ions in skeletal muscle and during the recovery process, these metabolites are removed and recycled. Body also aims to restore the disturbed body temperature and fluid balance after training and activates the neuroendocrine-immune responses to reconstitute the body homeostasis. (Stanley et al. 2013). In addition, during the recovery process, possible exercise-induced muscle damages are repaired (Bishop et al. 2008).

To ensure the recovery from training, it is important for endurance athletes to pay attention to nutrition, quality and amount of sleep, prevention of illnesses and possible psychological stress factors. Also, the individual differences between athletes and the features of the recent exercises, like training load, intensity and duration, should be taken into account when planning the recovery. (Halson 2013.) Since the importance of the recovery for elite athletes has been widely accepted, researchers have aspired to find different methods and applications to quantify training loads and the recovery state of an athlete. These methods include different kinds of questionnaires, diaries, rating of perceived exertion, speed and power output measurements and physiological monitoring that is based on measurements of physiological variables like heart rate and blood lactate concentration. (Mujika 2017.) In the following chapter, two common methods to evaluate training load and recovery, HRV and cortisol secretion measurements, will be further discussed.

3 METHODS TO EVALUATE PHYSIOLOGICAL STRESS AND RECOVERY

There are several ways to evaluate physiological stress and recovery. Two common methods, HRV and cortisol secretion, are very different but both commonly used. As already discussed in the previous chapter, ANS and HPA-axis are known to regulate stress reactions of body. Variation in HR is closely related to functions of ANS and HPA-axis and in turn controls the circulation of cortisol. In order to understand the relationships between HRV and ANS, it is important to understand the general functions of ANS and how it regulates functions of the heart. In this chapter the general functions of ANS will be presented, such as HRV as a method of evaluating physiological stress. Also, to understand the role of cortisol in stress evaluation, the secretion pathways of cortisol in the HPA-axis will be presented. Finally, the relationships between HRV, cortisol and endurance training will be discussed.

3.1 General functions of ANS and HPA-axis

Autonomic nervous system consists of two subsystems: parasympathetic nervous system (PNS) and sympathetic nervous system (SNS) and it is mainly activated by centers located in the brain stem, spinal cord and hypothalamus (Guyton & Hall 2006, 748). These two subsystems take part in regulation of many bodily functions, for example arterial pressure, gastrointestinal motility and secretion, sweating and body temperature. Primarily, PNS supports systems taking part in growth and restorative system, whereas, SNS is more responsible for increases in metabolic output to deal with challenges outside the body (Porges 1992). Parasympathetic and sympathetic neurons have both inhibitory and excitatory effects on end organs and their effects are often reciprocal to each other. The two subsystems of ANS are continually active and their basal rates of activity are known as parasympathetic (vagal) tone and sympathetic tone. By changing the rate of these tones, it possible for a single nervous system to both increase and decrease the activity of a stimulated organ. (Guyton & Hall 2006, 753-756.)

Among many other functions, ANS has an important role in control of cardiovascular functions. Both subsystems of ANS are involved in the control of cardiac pumping by varying the atrial pressure and cardiac output (Guyton & Hall 2006, 112). Vagal nerves are distributed mainly to

sinoatrial and atrioventricular nodes of heart which control the generation and conduction of rhythmical impulses of the heart. Stimulation of vagal nerves causes the release of hormone acetylcholine, which decreases sinus rhythm and thereby decreases heart rate. Sympathetic nerves in turn, innervate all regions of the heart and have opposite effects on cardiac functions than vagal nerves. Sympathetic stimulation increases heart rate and the force of contraction of all the cardiac musculature. Sympathetic neurons release hormone norepinephrine which is believed to affect to the functions of heart by increasing sodium-calcium permeability, thereby making the generation of action potential easier. Sympathetic nerves can even triple heart rate and double the strength of heart contractions while vagal stimulation can decrease the cardiac output half to the normal values. (Guyton & Hall 2006, 112-122.)

Along with ANS, the hypothalamus-pituitary-adrenal-axis (HPA-axis) is a central control and regulatory system of body that connects the central nervous system with the hormonal system (Kudielka & Kirschbaum 2005). In stress exposure, the hypothalamus releases corticotropin-releasing factor (CRF) into the primary capillary plexus of the hypophysial portal system in the median eminence of the hypothalamus (Guyton & Hall 2006, 955). CRF is then carried to the anterior pituitary, where it sustains the secretion of adrenocorticotropic hormone (ACTH). ACTH in turn, acts in the inner adrenal cortex and activates the release of glucocorticoid hormones like cortisol. (Guyton & Hall 2006, 955; Ulrich-Lai & Herman 2009.) Mechanism for regulation of glucocorticoid secretion is illustrated in Figure 1. Glucocorticoids have many functions in body, including mobilization of stored energy, preventing development of inflammation and potentiating numerous sympathetically-mediated effects, such as peripheral vasoconstriction (Guyton & Hall 2006, 950-954; Ulrich-Lai & Herman 2009). The functions of the main glucocorticoid hormone cortisol will be further discussed in the chapter 3.3.

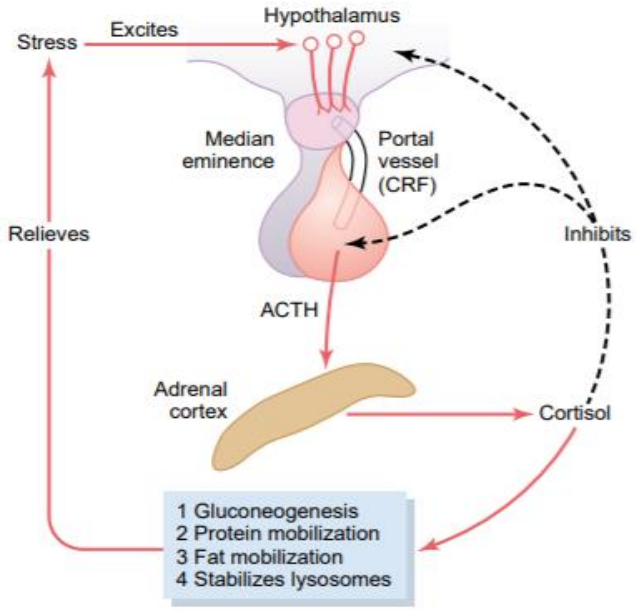


FIGURE 1. Mechanism for regulation of glucocorticoid secretion. ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor. (Guyton & Hall 2006, 955.)

3.2 Heart rate variability

Heart rate variability is a largely studied quality of cardiac rhythm and it refers to variation of instantaneous heart rate and variation of time between two consecutive heart beats. The first documented observations of HRV were already made in 16th century when Hales (1733) observed how fluctuations in HR and blood pressure were related to respiratory pattern with horses (Berntson et al. 1997). After more than two centuries later, the clinical interest of HRV enlarged when two researchers, Wolf (1967) and Hon (1958; Hon & Lee 1963), noticed the relationship between HRV and nervous system status (Berntson et al. 1997). The final clinical significance of HRV was established in late 1980's when it was perceived to be a reliable predictor of mortality after myocardial infraction (Task Force 1996).

Today HRV is used as a marker of sympathetic and vagal activity by researchers from many diverse fields (Task Force 1996). Physiology of HRV is based on the different effects of the two subsystems of ANS innervating SA node because heart rate reflects their modulating effect on the intrinsic firing rate of pacemarker cells (Pumprla et al. 2002). PNS innervates the SA

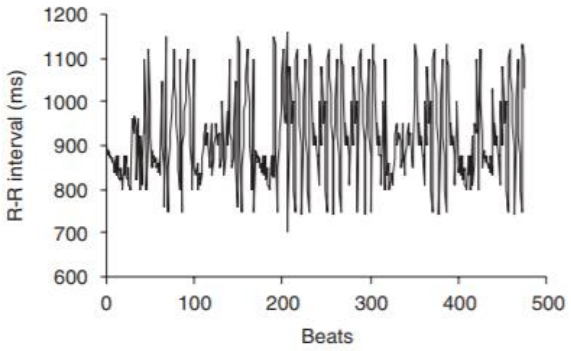
node by a synaptic release of acetycholine which has a short latency function and thereby modifies the cardiac function on beat-to-beat basis. Norepinephrine, released from SNS, in turn is metabolised relatively slow and it alters the cardiac functions with a delay. These different neurotransmitter functions cause PNS and SNS to operate at different frequencies. This feature makes it possible to identify and quantify sympathetic and parasympathetic activity by analysing different frequencies from the recorded fluctuations in heart rate. (Pumprla et al. 2002.)

Some of the beat-to-beat fluctuations in HR are naturally caused by cycles of respiration and the phenomenon is called respiratory sinus arrythmia (RSA). Periodic components of HRV usually aggregate within several frequency bands and in young healthy individuals at rest, respiratory frequency (RSA) is the most remarkable of these bands. (Berntson et al. 1997). RSA is mediated primarily by vagal modulation, since it is noticed to decrease during vagal blockade (Martinmäki et al. 2005; Uusitalo et al. 1996). RSA is usually seen in HRV analysis at high frequencies (HF) which is why it is thought that HF component of HRV is of parasympathetic origin (Berntson et al. 1997).

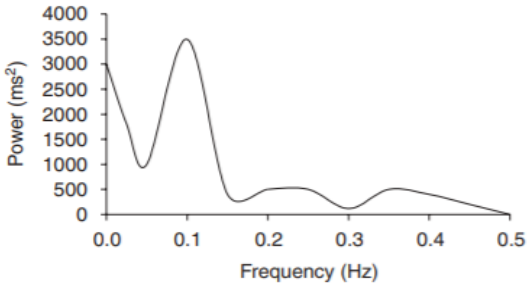
3.2.1 Methods for analysing HRV

When analysing HRV the two most common and valid methods are time-domain and frequency domain analysis (Figure 2). Time-domain analysis starts with an electrocardiographic record, from which each QRS-complex is detected and where normal-to-normal intervals (NN) or instantaneous HR is defined (Task Force 1996). Perhaps the simplest time-domain analysis is standard deviation of NN intervals over selected time (SDNN) which represents the overall variability of RR intervals (RRIs). Another commonly used time-domain measure is the square root of the mean squared differences of successive NN intervals (rMSSD) which is used to measure short term variations in heart rate in high frequencies. Also, the number of interval differences of successive NN intervals greater than 50ms (NN50) and the proportion derived by dividing NN50 by the total number of NN intervals (pNN50) are often presented in time-domain analyses. (Task force 1996.)

Frequency analysis, also known as power spectral density (PSD), describes how power is distributed as function of frequency. Frequency analysis methods can be classified as parametric and non-parametric methods of which the Fast Fourier transform is the most commonly used non-parametric method (Carter et al. 2003). In short term recordings, there are three main spectral components in variation of HR: very low frequency (VLF, <0.04Hz), low frequency (LF, 0.04 – 0.15 Hz) and high frequency (HF, 0.15 – 0.40 Hz). Also, the total power (TP) of HRV is usually calculated, which represents the overall variability of RRIs. (Task Force 1996.)



R-R intervals in time-domain	
AverageNN (ms)	Average of all normal R-R intervals
SDNN (ms)	Standard deviation of all normal R-R intervals
r-MSSD (ms)	Root mean square successive difference
pNN-50 index (%)	Percentage of differences between adjacent normal R-R intervals that are >50ms



R-R intervals in frequency-domain	
Total power (ms^2)	The power in the heart rate power spectrum between 0.00066 and 0.34Hz
VLFP (ms^2)	The power in the heart rate power spectrum between 0.0033 and 0.04Hz
LFP (ms^2)	The power in the heart rate power spectrum between 0.04 and 0.15Hz
HFP (ms^2)	The power in the heart rate power spectrum between 0.15 and 0.36Hz
LFP : HFP ratio	

FIGURE 2. Examples of the time-domain (left) and power spectrum analyses (right). On the left, R-R interval time between each subsequent beat measured over a 7-minute period at rest (~500 beats) and common ways to express heart rate variability in the time-domain are presented. On the right is an example of the power spectrum which shows the magnitude of the variability as a function of frequency. The most commonly found areas in the power spectrum, which represent different influences of sympathetic and parasympathetic nervous system, are displayed in the box below the right figure. (Achten & Jeukendru 2003.)

From these HRV analysing variables mentioned above, rMSSD and HF power have been noticed to have high correlation with each other and they both are thought to be measures of vagal activity. LF power in turn is a more argued component which is considered by some to be a marker of sympathetic modulation and by others to have both sympathetic and vagal influences. A third commonly used component is LF/HF ratio which is thought to describe sympatho/vagal balance or sympathetic modulations. (Task Force 1996.)

3.2.2 Circadian rhythm and other factors affecting HRV

There are numerous factors affecting HRV, including health status, neuropsychological factors like emotions and stress, non-modificable factors like age and gender and lifestyle factors like exercise training (Fatisson et al. 2016). Studies have shown that HRV decreases naturally with aging (Achten & Jeukendru 2003; Carter et al. 2003). Influence of gender to HRV remains more unclear but women seem to have slightly lower HRV values than men (Achten & Jeukendrup 2003; Fatisson et al. 2016; Jensen-Urstad et al. 1997). The psychological side of stress is also an important factor affecting HRV and it has been observed to induce lower HRV values (Fatisson et al. 2016; Michels et al. 2013). Physical activity has been observed to induce higher HRV (Rennie et al. 2003; May et al. 2017) and reduce the decrement of HRV with aging (Achten & Jeukendrup 2003; Carter et al. 2003). Since low HRV is known to be associated with mortality, physical activity could serve as a valuable tool in maintaining cardiac health.

Circadian rhythm in HR and HRV has been observed in numerous studies (Carrington et al. 2003; Furlan et al. 1990). Furlan et al. (1990) committed a 24-hour recording from HRV which indicated daytime being associated with relative sympathetic dominance while vagal activity was more present during the night. Night time decrease in HR, blood pressure and LF/HF component of spectral analysis has been observed, reflecting greater contribution to sympatho-vagal balance (Carrington et al. 2003). HF component of spectral analysis in turn seems to increase during night, also indicating greater vagal dominance. Even though the circadian rhythm of HRV has been widely studied, it is still not completely understood why the sleep and circadian rhythm induce changes in HR, blood pressure and ANS activity around the time of sleep onset. (Carrington et al. 2003).

3.2.3 HRV and endurance training

As already discussed in the earlier chapters, ANS is very sensitive to the stress body experiences and HRV is closely related to the function of the ANS. Parasympathetic activity is generally known to decline during acute physiological stress and return to normal levels during recovery. Since there is close relationship between HRV and ANS function, HRV could serve as a valuable parameter to evaluate ANS recovery from training and adaptations to it.

The effects of endurance exercise and long-term endurance training to HRV differ from each other. Several studies have addressed decreases in HRV variables at the transition from rest to exercise indicating lower parasympathetic control (Carter et al. 2003; Pichon et al. 2004; Saboul et al. 2015; Tulppo et al. 1998). Tulppo et al. (1998) observed the decrease in HF component which decreased as a function of exercise intensity. They also noted that HF power and normalized R-R interval variability almost disappeared when the level of 50-60% of VO_{2max} was reached, indicating disappearance of vagal modulation (Tulppo et al. 1998). Pichon et al. (2004) in turn, noticed significant decrease both in HF and LF component during exercise (components expressed as absolute power (ms^2), $P < 0.05$). In addition to intensity, exercise duration was also established to have a significant effect on HF and LF component by decreasing them with longer training duration (Pichon et al. 2004).

Acutely after exercise and during recovery, HRV tends to return to normal levels, but the effects of endurance exercise may last from a few minutes to hours or even days (Hautala et al. 2001; Martinmäki & Rusko 2007; Mourot et al. 2004; Seiler et al. 2007). Seiler et al. (2007) measured the effects of training intensity and duration to autonomic recovery after exercise in trained athletes and concluded that in low intensities, HRV returns to pretraining values within 5-10 minutes after exercise. Training on higher intensities, over the first ventilatory threshold (the intensity at which an increase in V_E/VO_2 occurred without an increase in V_E/VCO_2) in turn, induced significant delay in HRV recovery ($P < 0.05$) (Seiler et al. 2007). Martinmäki & Rusko (2007) ended up with similar results outlining that LF and HF components of PSD analysis are highly affected by exercise intensity, recovery time and their interaction. Nocturnal HRV values have also been studied on the following night after endurance exercise in order to determine the

long-term effects of endurance exercise. For example, Hynynen et al. (2010) detected the effects of moderate endurance exercise and marathon run on nocturnal HRV and found significant increases in HR ($P < 0.01$) and decreases in rMSSD ($P < 0.01$), HF power ($P < 0.01$) and TP ($P < 0.05$) compared to baseline after both moderate exercise and marathon run. Hautala et al. (2001) found similar decreases in nocturnal HRV on the first day after 75 km cross-country skiing competition ($P < 0.01$). However, HRV values returned to pre-exercise levels already on the second night after the race (Hautala et al. 2001).

Chronic endurance training has been observed to have influences on HRV at rest and during recovery. At rest HRV increases indicating greater parasympathetic activity and lower sympathetic activity, thus contributing to training induced bradycardia (Achten & Jeukendrup 2003; Carter et al. 2003; Stanley et al. 2013; Levy et al. 1998). Endurance trained athletes have also been observed to recover faster from the reduced vagal outflow than untrained individuals (Hautala 2001; Stanley 2013). Tulppo et al. (1998) also stated that better physical fitness leads to smaller decrease in HRV degradation during exercise in submaximal intensities.

Although chronic endurance training seems to increase the proportion of vagal modulation of cardiac events, cumulative effects of heavy endurance training have been established to decrease HRV thus indicating suppressed parasympathetic activity (Baumert et al. 2006; Hynynen et al. 2007; Pichot et al. 2000; Plews et al. 2014). Plews et al. (2014) examined the relationships between HRV and training intensity distribution in international level rowers and observed suppressions in parasympathetic activity after training periods including great amount of high-intensity training. Similar results were found by Hynynen et al. (2007) who reported decrement in nocturnal HRV after an overreaching period in elite cross-country skiers. Pichot et al. (2000) noted that the decrement in HRV occurs progressively during intensive training period and that the HRV rebounds during relative resting week in middle distance runners during their usual training cycle. This rebounding effect of HRV was also observed by Baumert et al. (2006) on rest days following intensified training in track and field and triathlon athletes.

Since HRV has been found to serve information about exercise induced fatigue and recovery from it, numerous studies have investigated if HRV could be used to monitor training and avoid

fatigue and possible non-functional overreaching (NFO) or overtraining (OT). HRV-guided training has been observed to produce positive results on physical performance and training adaptations (Kiviniemi et al. 2007; Vesterinen et al. 2016). Vesterinen et al. (2016) examined how HRV-guided training affected VO_{2max} and 3000 m running performance on recreational endurance runners. Subjects were divided into two training groups with one group following a predefined training program and the other group (HRV-guided group) following moderate and high intensity trainings based on individual HRV measurements. Significant difference was found between groups, since the 3000 m performance was improved for the HRV-guided group (2.1 ± 2.0 %, P = 0.004) but not for the other group (1.1 ± 2.7 %, P = 0.118). However, VO_{2max} was improved by both groups. (Vesterinen et al. 2016.) Kiviniemi et al. (2007) ended up with similar results with moderately fit males, showing significantly greater enhancement in maximal running velocity for HRV-guided training group compared to predefined training group (0.5 ± 0.4 vs. 0.9 ± 0.2 km h^{-1}, P = 0.048, adjusted for baseline values), while no between group differences were found in VO_{2max}.

NFO and OT have usually been observed to be associated with reduced HRV. In recently published study, Kajaia et al. (2017) found significantly lower values in time-domain parameters (mean R-R, SDNN, RMS-SD, pNN50) and in HF component of spectral analysis for the athletes with NFO/OT status compared to athletes without them ($P < 0.05$). These findings reflect lower variation in HR and lower vagal influence on cardiovascular function. They also found significantly higher LF and LF/HF ratio values in NFO/OT athletes, indicating increased sympathetic activity ($P < 0.05$). (Kajaia et al. 2017.) Lower HRV values were also found in studies detecting overtrained cross-country skiers (Hynynen et al. 2007) and triathletes (Plews et al. 2012).

Despite most studies showing strong relationships between reduced HRV and fatigued state of an athlete as well as increased HRV and an enhanced performance, there are also many studies showing diverse results. Studies have shown that increased HRV is not always associated with better aerobic capacity (Achten & Jeukendrup 2003). There have also been studies showing associations between decreased performance and unchanged or even increases in HRV. These divergent findings can be explained due to the methodological approaches adopted, difficulty with defining the state of OT process and the possibility that two types of OT (parasympathetic

and sympathetic) may occur in athletes. (Plews et al. 2013.) However, because some studies have shown these different findings about HRV and its relationship with fatigue, the interpretation of HRV should be always made with caution.

3.3 Cortisol

The steroid hormone cortisol is the most important glucocorticoid hormone in the human body and it is secreted by adrenal cortex. Cortisol has multiple metabolic functions: it stimulates gluconeogenesis and decreases glucose utilization by cells leading to elevated blood glucose concentrations (Guyton & Hall 2006, 951). It also increases the catabolic part of lipid and protein metabolism, stimulates the secretion of insulin and inhibits the inflammatory and allergenic reactions. Such as other steroid hormones, cortisol operates by first activating the intracellural receptors in the cytoplasm of the target cell. The hormone-receptor complex then induces or represses gene transcription by interacting with specific regulatory DNA sequences, called glucocorticoid response elements. The effects of cortisol appear relatively slow because the protein synthetization takes 45 to 60 minutes and the complete development up to several hours or even days. (Guyton & Hall 2006, 951-955.)

Cortisol is generally known as a stress hormone and it has been used in several studies as a marker of stress (Hellhammer et al. 2008; Klaperski et al. 2014; Michels et al. 2013; Putignano et al. 2001). Almost any type of stress, physical or mental, induces an immediate and abundant secretion of ACTH which in turn increases adrenocortical secretion of cortisol within a few minutes (Guyton & Hall 2006, 955). About 30 minutes after the acute stress exposure, plasma cortisol concentration has reached its peak and then starts to decline (Lundberg 2005). Cortisol produces the decline by itself by providing direct negative feedback effects to the hypothalamus and anterior pituitary gland to decrease formation of ACTH and CRF (see Figure 1) (Guyton & Hall 2006, 955). These self-regulatory effects of cortisol prevent the body from overexposure of cortisol which could eventually lead to adverse effects on various organ systems, leading to disease (McEwen 1998).

In addition to stress exposure, cortisol levels are also affected by circadian rhythm. Cortisol levels have large daily fluctuations and this diurnal rhythm is tied to sleep-wake and light-dark cycle (Bartels et al. 2003). Observations of the diurnal rhythm of plasma cortisol level have found that maximum level of cortisol appear in the morning, then decline throughout the day and the lowest concentrations are found around midnight (Bartels et al. 2003). The levels tend to raise in the early hours before awakening with further increases after waking up, peaking 20 to 45 minutes later (Chida & Steptoe 2008).

Age and gender have also been observed to have significant effects on cortisol concentrations. Van Cauter et al. (1996) studied the age and gender related changes in mean 24-hour cortisol levels and found that cortisol levels increase progressively with higher age. They also found, that in premenopausal women, mean cortisol levels are slightly lower than men in the same age, although after menopause the difference is no longer significant. Cortisol response to challenge in turn, seems to increase with higher age and this effect is almost three times stronger in women than in men (Otte et al. 2005).

3.3.1 Cortisol measurements

There is a large diversity in the literature in the methods to examine the connections between stress and cortisol secretion, including the different times of sampling and the various collection procedures. In the sampling times, elevated 24-hour cortisol, suppression of 24-hour cortisol, elevated evening cortisol, elevated overnight cortisol and elevated morning cortisol have all been identified as potential markers of stress (Powell et al. 2002). In order to determine the acute changes in cortisol secretion, cortisol measurements can be performed before and various times after a specific stress exposure (Daly et al. 2005; Hackney & Anderson 2016).

Different cortisol collection procedures include measuring cortisol concentrations from urine, plasma or saliva and these measurements have shown high intraclass correlation (Table 2) (Neary et al. 2002). Especially cortisol measurements from plasma and saliva have been observed to be highly correlated (Hellhammer et al. 2009; Neary et al. 2002; Putignano et al. 2001). Measuring cortisol levels from saliva has several advantages compared to the other two

methods: it is a simple, stress-free and non-invasive collection procedure which can be made without medical personnel and in different environments. Studies that require ambulatory assessments, large cohorts or children as subjects should especially prefer cortisol measurements from saliva. (Hellhammer et al. 2009.) According to Addison and the Association for Clinical Biochemistry (2012), reference intervals for morning cortisol levels in adults are 4–28 nmol/l when measured from saliva and 171-536 nmol/l measured from serum.

TABLE 2. Correlational matrix showing the relationship among serum (SER), saliva (SAL), overnight urine (ON) and 24 h urinary free (24 h) cortisol. Values are intraclass correlation coefficients (R). N = 8. (Neary et al. 2002.)

	SER	SAL	ON	24 h
SER	-	0.995*	0.994*	0.990*
SAL		-	0.927*	0.973*
ON			-	0.976*
24 h				-

*$p \leq 0.05$

Even though salivary cortisol has been found to be a reliable way to analyse cortisol levels, it should be noted that the cortisol concentrations are multiple times lower on saliva than they are on plasma serum or urine. Ljubijankić et al. (2008) evaluated cortisol in the saliva and serum and its daily fluctuations of healthy individuals and found that the concentrations were twenty times lower in the saliva in the morning and even twenty-seven times lower in the afternoon. They concluded that individual variability of cortisol concentration is evident during the day and because of the low salivary cortisol concentrations, sampling time of saliva should be taken into consideration. (Ljubijankić et al. 2008.) Due to these findings it can be concluded that in longitudinal studies, cortisol should always be measured at the same time of the day and when measuring from saliva, it could be beneficial to collect the cortisol samples after awakening when the concentration is high and easy to observe.

3.3.2 Effects of endurance training on cortisol secretion

Similarly to training induced changes in HRV, the changes in cortisol concentrations also differ when measured immediately after exercise and after long-term endurance training. Acutely after exercise, circulating cortisol levels tend to raise if the intensity of the exercise reaches so called threshold (Hill et al. 2008). Hill et al. (2008) examined how the cortisol levels changed in blood immediately after exercise at intensities of 20, 60 and 80 % of the subjects VO_{2max} on moderately trained men. The cortisol levels were significantly higher after the two most intense exercises (+ 39.9 ± 11.8 %, and + 83.1 ± 18.5 %, $P < 0.05$) supporting the view that moderate to high exercise causes a sudden increase in circulating cortisol levels. (Hill et al. 2008.) Hackney & Andersen (2016) ended up into similar results, noting that the changes can also be seen in salivary cortisol after moderate and high intensity exercises. Jacks et al. (2002) in turn, found that only high intensity exercise induced a significant change in plasma cortisol levels after exercise ($P = 0.042$) even though the intensity during the moderate intensity exercise was almost the same. They also found that cortisol levels were significantly higher at 59 minutes of high intensity exercise ($P = 0.004$), indicating that cortisol levels increase already during the exercise and that the increase is dependent form both exercise intensity and duration (Jacks et al. 2002).

After exercise during the recovery, cortisol concentrations have been observed to keep rising and reach its peak about 30 minutes after the exercise (Daly et al. 2004; VanBruggen et al. 2011). After that, cortisol levels decrease quite rapidly towards the pre-exercise values. Several studies have observed the recovery of cortisol levels after an exhaustive exercise and found that cortisol levels decline under the pre-exercise values and stay suppressed even 24-hours after the exercise (Anderson et al. 2016; Daly et al. 2004). These results indicate that it might take even several days for cortisol levels to return to resting levels.

There is a common belief that long-term endurance training causes hypercortisolism (Duclos et al. 2007). For example, Alghadir et al. (2015) found that four-weeks low intensity endurance training bout caused a significant increase in salivary cortisol in young healthy adults (+ 19.71 ± 4.66 pg/ml, $P < 0.01$). These higher cortisol levels have been noted to be associated with

better performance and lower session-RPE on endurance trained athletes (Balsalobre-Fernández et al. 2014). However, studies have found that in resting conditions, cortisol levels do not significantly differ between endurance trained athletes and control subjects. This is only a good thing, since prolonged elevated cortisol levels has several deleterious effects, including muscle catabolism (Duclos 2002; Duclos 2007). Due to these findings, it can be concluded that higher cortisol levels measured from endurance athletes are mainly caused by the repeated and prolonged exercise-induced increased cortisol secretion.

Cortisol is widely used as a marker of training induced fatigue and overtraining. Studies have observed associations between cortisol levels and training status and found increased serum cortisol levels in overtrained athletes (Bandyopadhyay et al. 2012; Roberts et al. 1993). Overtraining status has also been observed to cause a lower or absent cortisol response to exercise (Bandyopadhyay et al. 2012; Gleeson 2002).

Despite the many supportive findings in the literature, the use of cortisol in monitoring training should be made with cautions. For instance, conversely to earlier findings, Vesterinen et al. (2013) found no changes in basal cortisol levels after 28 weeks of endurance training with recreational endurance runners, even though the subjects endurance performance was significantly increased. Also, the increased resting cortisol levels during overtraining has not been observed by all investigators, some studies have even found associations between overtraining and decreased cortisol concentrations (Urhausen et al. 1995). Hormonal changes in response to training and overtraining are highly individual, which is why individual hormonal profiles should always be made in order to follow-up training effects (Uusitalo et al. 1998).

4 RESEARCH QUESTIONS AND HYPOTHESES

The purpose of this study was to examine how the values of recovery state and stress markers HRV and salivary cortisol vary in junior cross-country skiers and are there relationships between these two variables. The study included a seven-week long testing period where the nocturnal HRV and salivary cortisol were collected and analyzed every other week, each time for three consecutive days. The basic training characteristics (volume, intensity and training load) from each testing week were also recorded. Research questions and hypotheses were as follows:

Question 1. What changes and trends occur in HRV and morning cortisol levels in young endurance athletes?

Hypothesis 1. HRV will decrease when the training load increases and increase with decreasing training load.

Rationale. Many studies have shown that cumulative effects of heavy endurance training decrease HRV (Baumert et al. 2006; Hynynen et al. 2007; Pichot et al. 2000; Plews et al. 2014). The decreasing effect has also been observed in nocturnal HRV measurements (Hautala et al. 2001; Hynynen et al. 2010). The decrement of HRV will most likely occur progressively with increasing training load (Pichot et al. 2000) and with a sufficient resting period, HRV tends to rebound to higher resting values (Baumert et al. 2006; Pichot et al. 2000).

Hypothesis 2. Salivary cortisol levels will increase when the training load increases and decrease with decreasing training load.

Rationale. Cortisol has been used as a marker of stress and recovery in sports because of the tendency of the cortisol concentrations to raise when body encounters exercise-induced stress (Alghadir et al. 2015; Bandyopadhyay et al. 2012; Duclos et al. 2007; Gleeson 2002). Endurance training has been observed to increase cortisol concentrations and with adequate

resting period, the levels tend to return to resting levels (Alghadir et al. 2015). Studies have also found higher cortisol levels in fatigued overtrained athletes, indicating that cortisol reacts to high training loads. (Bandyopadhyay et al. 2012; Roberts et al. 1993).

Question 2. What are the relationships between nocturnal HRV values and morning salivary cortisol levels?

Hypothesis. Low HRV values will be associated with increased secretion of salivary cortisol.

Rationale. Although there are not many researches that have studied the relationships between HRV and salivary cortisol, both of those variables have been observed to react to exercise-induced stress, HRV by decreasing (Baumert et al. 2006; Hynynen et al. 2007; Pichot et al. 2000; Plews et al. 2014) and cortisol levels by increasing (Alghadir et al. 2015; Bandyopadhyay et al. 2012; Duclos et al. 2007; Gleeson 2002). That is why it can be concluded that there are associations between these two variables. Also, outside the exercise-field, relationships between salivary cortisol and HRV in response to stress have been observed (Michels et al. 2013).

5 METHODS

This bachelor's thesis was a part of a larger longitudinal study carried out over the course of three to four years testing and monitoring junior cross-country skiers. This particular study focused on measuring the stress levels and the recovery state of the athletes during the seven weeks testing period. The recovery state and the stress levels were measured using the nocturnal HRV and salivary cortisol measured immediately after awakening. Also, the relationships between these two variables were examined. The study was approved by the Ethical Committee at the University of Jyväskylä and the measurements were performed in accordance with the declaration of Helsinki.

5.1 Participants

Altogether 30 well-trained junior athletes that trained for cross-country skiing year-round participated in the study. Eventually, only 7 athletes, of which 5 were women and 2 were men, performed all the tests and were selected to this study. The participants were 16-18 years old first and second grade students in the sports high school of Vuokatti and members of Vuokatti-Ruka Sports Academy. At the beginning of the study, all the participants were fully informed of all details about the procedures and were told about their freedom to withdraw from the research at any point. Every participant, such as their parents, gave written consent to participation.

5.2 Study protocol

The study protocol included a seven weeks long measurement period that started at the end of October and ended at the beginning of December, so the tests were made at the very beginning of the athletes' race season. The tests were made every other week to allow for four testing weeks. During the testing weeks, the participants committed submaximal running tests which were not further examined in this study. However, the schedules of HRV and cortisol measurements were based on the running tests. The recovery state measurements were made in three consecutive days; one day before the submaximal running test, on the test day and one

day after the running test. The study protocol did not alter the athletes' training programmes but the training characteristics of each athlete were followed during the measurements.

5.3 Data collection and analysis

Heart rate variability. The nocturnal heart rate variability was measured with a contact-free sleep tracking Emfit QS-device (Emfit QS, Kuopio, FI). The measurement was done with a pressure sensor that was placed beneath the mattress under the participant's chest area and that started recording the HRV automatically when the participant went to sleep. The collected data was transferred and stored online via internet. Emfit QS reported the magnitude of HRV with a time-domain variable rMSSD with the sampling rate of 100 Hz.

HRV was measured from each participant every night during the seven weeks testing period although, the data was only analyzed every other week from the three days when the other tests were made too. The morning average rMSSD value, which describes the average of all three-minute window rMSSD values measured during last 90 minutes before waking up, was used in the analysis to describe nocturnal HRV. The validity and reliability of Emfit QS-device in measuring HRV has not been proven yet.

Salivary cortisol. The saliva samples were collected immediately after waking up for three consecutive days during the testing weeks from the same days the other tests were also made. The participants collected the saliva samples by themselves as they were instructed at the beginning of the testing period. The samples were collected using the Passive Drool-method: after washing their mouths with a certain amount of water, the participants collected at least 3 ml of saliva into the test tubes. They recorded the date, the time when the collection procedure started and the duration the procedure took. The samples were stored in the athletes' own freezers and collected from there at the end of the testing period.

The saliva samples were analyzed with IMMULITE 2000 XPi Analyzer (Siemens Healhcare Diagnostics Products Ltd., Glyn Rhonwy, Llanberis, UK) using the chemiluminescence

method. The sensitivity of the saliva assay for cortisol was 5.5 nmol/l with inter-assay precision 8.2 % at 12.5 nmol/l.

Training characteristics. The participants followed their own individual training plans throughout this study and they were supposed to log all trainings to their electronic training diaries (elogger.net, Espoo, FI). The training volume and intensity were analyzed form the training diaries and the training load was quantified using modified version of Lucia's simplified TRIMPS system (Cejuela Anta & Esteve-Lanao 2011). The training load was calculated using the equation:

$$TL = 1 \times ZONE1 + 2.5 \times ZONE2$$

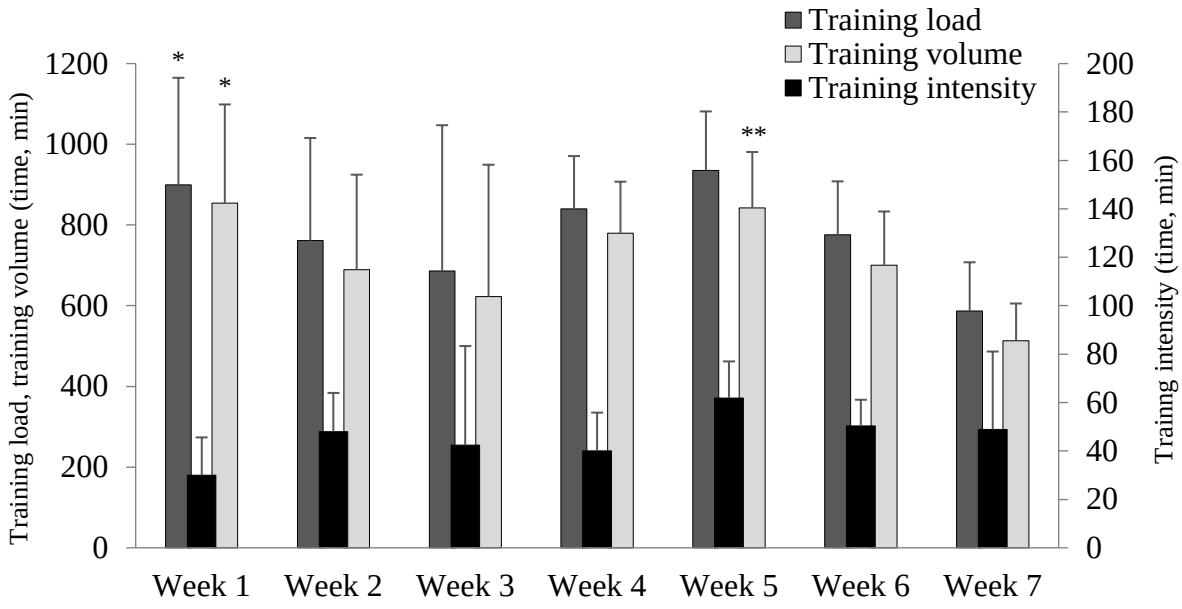
where TL = training load, ZONE1 = training time under aerobic threshold, ZONE2 = training time between thresholds + training time over anaerobic threshold. Training time was expressed in minutes. The training load, training volume and training intensity were calculated both in weekly values and from the three days when the other tests were made.

5.4 Statistical analysis

All the statistical analysis was performed with SPSS for Windows software (IBM SPSS Statistics 24 (SPSS, Inc., Chicago, Illinois, USA)). Due to small sample sizes, Friedman's non-parametric test for related samples was performed to analyse the changes in training, HRV and salivary cortisol values. The analysation of the correlations between HRV and cortisol, HRV and all training characteristics and cortisol and all training characteristics were made with the bivariate correlation test (Pearson's correlation) which is also suitable for small sample sizes. In addition, the correlation tests were made for computed variables. The results are presented as means ± standard deviations.

6 RESULTS

During the measurement period the athletes' training volume varied between 14 h 15 min ± 4 h 4 min and 8 h 33 min ± 1 h 32 min per week. The amount of intensive training in turn was 1 h ± 15 min as its highest peak and 30 min ± 16 min as its lowest. During the actual test weeks, the three-day average training volume decreased quite linearly from 1 h 49 min ± 58 min to 1 h 18 min ± 21 minutes while the amount of intensive training was lowest at the first (2.3 ± 2.8 min) and peaked at the third test week (6.7 ± 8.5 min). The calculated training load showed quite similar changes to training volume when expressed both in weekly and three-day average values. Instead, the amount of intensive training was not reflected from the training load as clearly. The amount training load, volume and intensity per week are presented in the figure 3. The same values for the test weeks three-day averages are shown in the figure 4.



* p < 0.05, ** p < 0.01, statistically significant difference from week 7.

FIGURE 3. Weekly training load, training volume and training intensity.

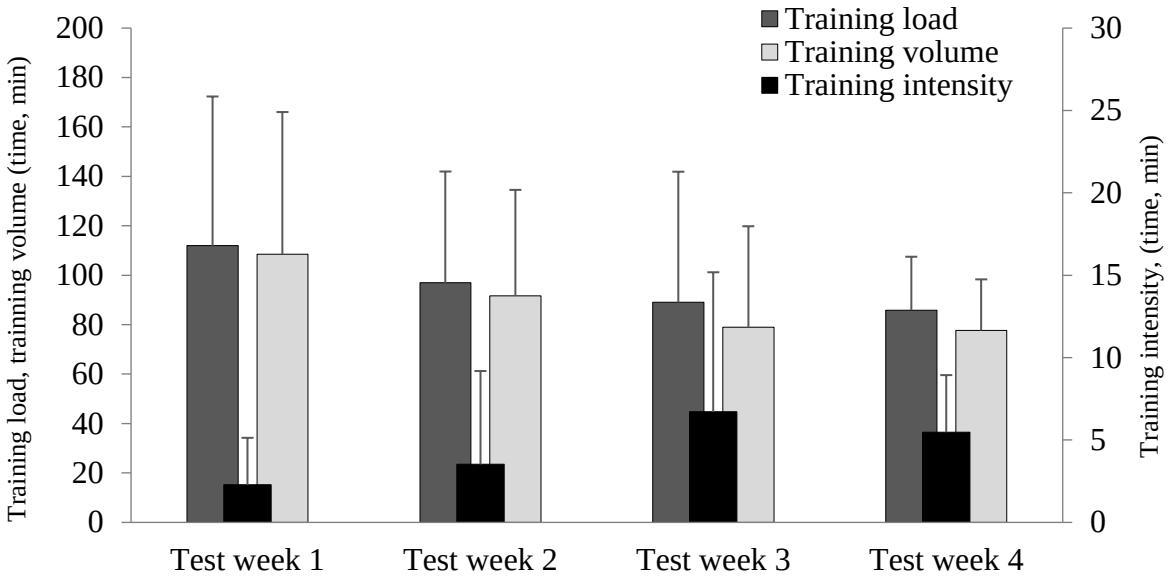


FIGURE 4. The test weeks three-day average values for training load, training volume and training intensity.

The HRV values increased during the first three testing weeks and then decreased for the last week. Cortisol in turn, showed reverse effect by increasing during the first three testing weeks and then decreased to the last one. However, there were no statistically significant differences in the changes of HRV or salivary cortisol values. The changes in HRV and salivary cortisol values are presented in table 3 and figures 5 and 6.

TABLE 3. Three-day average values of HRV and salivary cortisol during the test weeks.

	Test week 1	Test week 2	Test week 3	Test week 4
HRV, rMSSD (ms)	63.7 ± 16.8	71.4 ± 14.7	71.7 ± 32.0	69.0 ± 20.3
Cortisol (nmol/l)	68 ± 27	64 ± 34	58 ± 21	73 ± 25

HRV, heart rate variability; rMSSD, the square root of the mean squared differences of successive R-R intervals.

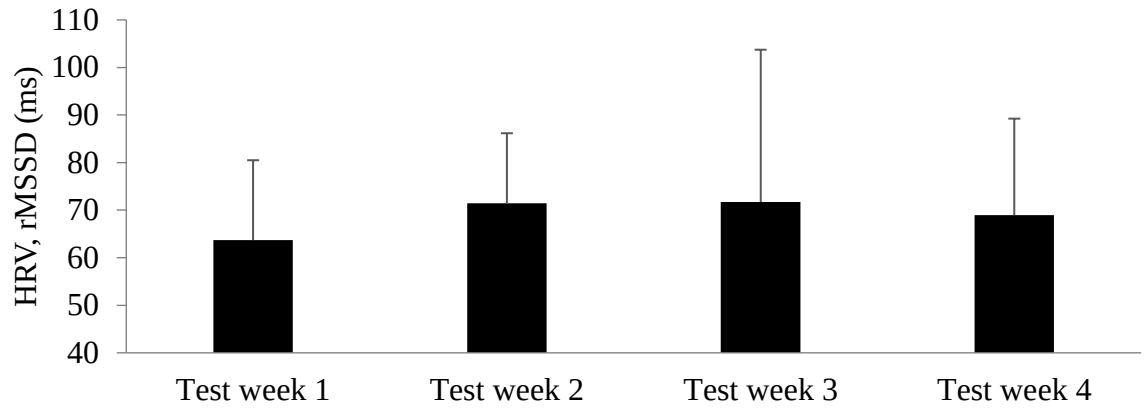


FIGURE 5. The three-day average values of nocturnal HRV during the test weeks.

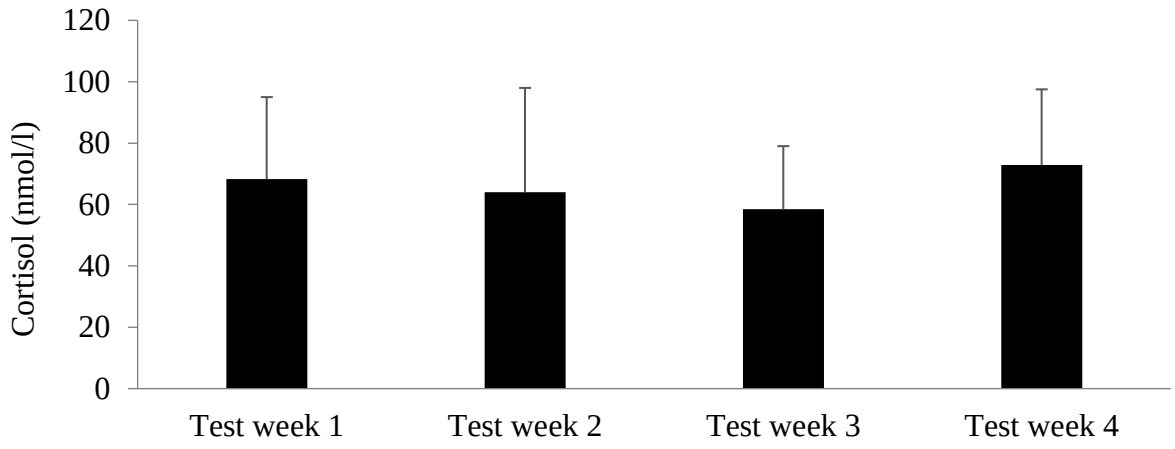


FIGURE 6. The three-day average values of salivary cortisol during the test weeks.

Training load, training volume and training intensity did not have any significant correlations with HRV or salivary cortisol levels when expressed in weekly averages. In turn, nocturnal HRV and morning salivary cortisol showed statistically significant negative correlation on the first and last test weeks (Table 3). Although the correlation coefficient was also relatively high on the second and third test weeks, it was not high enough to reach the statistical significance.

TABLE 3. Pearson's bivariate correlations between nocturnal HRV and morning salivary cortisol in the test weeks.

		Cortisol			
		Test week 1	Test week 2	Test week 3	Test week 4
HRV	Test week 1	-0,893**			
	Test week 2		-0,357		
	Test week 3			-0,464	
	Test week 4				-0,857*

* $p < 0.05$, ** $p < 0.01$, statistically significant correlation between HRV and salivary cortisol.
HRV, heart rate variability.

When the Pearson's correlation test was made for the computed variables, the significant correlation was only found between the salivary cortisol and the three-day average training intensity (Table 4). The correlation coefficients were also relatively high between HRV and cortisol, weekly training load, three-day average training load, three-day average training intensity and weekly training volume and between cortisol and weekly training intensity. However, the correlations between these variables were not high enough to reach the statistical significance.

TABLE 4. Pearson's bivariate correlations for computed variables.

		HRV	TL	TL TW	TI	TI TW	TV	TV TW
Cortisol	r	-0.643	0.214	0.000	0.643	0.811*	0.071	-0.214
HRV	r		-0.571	-0.536	-0.393	-0.667	-0.536	-0.321

* $p < 0.05$, statistically significant correlation between computed variables.
HRV, heart rate variability; TL, training load; TL TW, Training load during the test weeks; TI, training intensity; TI TW, training intensity during the test weeks; TV, training volume; TV TW, training volume during the test weeks.

7 DISCUSSION

The aims of this study were to examine how the values of recovery state and stress markers HRV and salivary cortisol vary in junior cross-country skiers in response to their training and to investigate the relationships between these two variables. The main findings of the study indicate that the changes in nocturnal HRV and morning salivary cortisol are highly associated. Also, the changes in training intensity seem to reflect salivary cortisol levels. Conversely, no other training variable showed associations with cortisol and there was no associations between HRV and training.

7.1 The changes in training characteristics, HRV and salivary cortisol levels

The first research question of the present study considered the changes that occur in HRV and salivary cortisol levels and the hypothesis was that the levels vary according to the volume and intensity of training. Against the expectations, the only variables that changed notably during the measurement period were calculated weekly training load and training volume while there were no significant changes in the salivary cortisol levels or HRV.

Training characteristics. The training load and training volume were significantly higher on the first week compared to the last one when expressed in weekly averages (figure 1). The training volume was significantly higher also on the fifth week when compared to the last one. In turn, the three-day average training values did not show any significant changes during the measurement period, even though the training intensity was almost three-folded on the third test week compared to the first one. There are two main reasons behind the non-significant changes in the training values, one of which is the excessively small number of subjects. When the number of subjects is this small, the statistical analysis requires enormous changes within the variables in order for the changes to be considered significant. The other reason is that the participants followed their own training plans during the measurement period, resulting in large deviation in the average training values. If the participants had followed the same training plan, the deviation in the volume and intensity of training would have happened simultaneously with every participant. This would have made the changes in the training characteristics more even

between the participants and probably leaded to more obvious and significant changes in average values.

Regardless of the fact that the training did not show many significant changes, some perceptions can still be made. The measurement period took place in the transition phase from training season to competition season and in cross-country skiing this means there is a shift towards lower training volume and higher training intensities (Sandbakk & Holmberg 2017). Looking at the results and graphs from the amounts of training (figures 1 and 2) it can be noted that training load and volume showed decrement and training intensity slightly increased towards the end of the measurement period, yet the changes were not linear. This shows that even though the athletes' training was not controlled during the measurements, the training seemed to follow the typical periodization model of the sport.

Heart rate variability. HRV increased during the first three test weeks and then decreased to the last one, although the changes were not considered significant. The change from the lowest HRV value to the highest was 12,6 %. The lack of significant changes in HRV can be explained due to the small number of subjects and with that HRV is a highly individual variable and both the absolute values and the changes in HRV show large diversity among individuals. This is why the changes in HRV do not follow any certain manner and the counted average changes in HRV values might stay suppressed.

Morning salivary cortisol. The deviations in the cortisol levels from the lowest to the highest value was 24,6 % (test weeks 3 and 4), yet the change was still not enough to be considered significant. With cortisol, the non-significance of the changes can again be explained due to the small number of participants. However, an almost 25 % change should not be completely ignored and as one of the main findings showed, the changes in cortisol were big enough to show relationships with the changes in training intensity. These relationships will be further discussed later in this chapter.

It should be noted that in every measurement point the morning salivary cortisol levels were relevantly higher than they should be for an average person. Reference interval for morning

salivary cortisol is 4-28 nmol/l (the Association for Clinical Biochemistry 2012) while in this study, the average values varied between 58 and 73 nmol/l. This finding indicates that endurance training causes hypercortisolism for young endurance athletes, which is not in line with earlier studies that have suggested that the cortisol levels do not differ between endurance athletes and control subjects in resting conditions (Duclos et al. 2002; Duclos et al. 2007). However, some studies have found that intensive endurance training can elevate the basal cortisol levels and it has been even been considered as a marker of impending overtraining (Bandyopadhyay et al. 2012). In the present study, the measurements occurred at the beginning of competition season and both the training intensity and volume were relatively high for each participant. High training loads added to the emotional stress caused by the beginning of a competition season and daily participation in high school has most likely caused the participants' stress levels to raise which has eventually led to an increment in resting cortisol levels. All in all, these remarkably high cortisol levels should be taken into considerations and the participants recovery state should be followed closely to avoid the unwanted state of overreaching or overtraining.

7.2 The relationships between the variables

HRV and salivary cortisol. As expected, nocturnal HRV and morning salivary cortisol showed significantly high negative correlation. However, this was only seen on the first and last test weeks when cortisol levels were the highest and HRV levels the lowest. High cortisol levels and low HRV values are both indicators of stress body experiences and since the correlations only existed with these values, it can be concluded that the associations between the two stress markers are the most remarkable in more stressful conditions. This makes sense noting that the training load was highest on the first test week (figure 4), indicating that the athletes had to handle more exercise-induced stress. On test week 4 in turn, the training load was the lowest, but the high stress levels can be explained with higher training intensity. All the subjects also participated in one of their first competitions of the season on the weekend before the tests, which might have also increased their stress levels.

Not many studies have studied the relationships between HRV and salivary cortisol and none of them in the field of exercise. The most similar research to our study, performed by Michels et al. (2013), tested these relationships with 5 to 10 years old children and found that salivary cortisol had associations with many parasympathetic activity markers, one of which was rMSSD. In the present study, rMSSD was used to illustrate the changes in the nocturnal HRV and similarly to Michels' study, it correlated negatively with the changes in the cortisol levels.

Huovinen et al. (2009) also studied the interactions between ANS and circulating hormones, but instead of cortisol, they used serum testosterone-to-cortisol levels as the other stress marker. Their study focused on conscripts during their first week of military service and the purpose was to detect the changes in stress markers in response to both physical and mental stress that the beginning of military service causes. They found that the cortisol levels increased during the measurement period and that the changes in HR and HF component of HRV were positively correlated with testosterone-to-cortisol ratio. In the present study, the changes in testosterone levels were not detected, but similarly to Huovinen's study, associations between circulating hormones and HRV were found.

Training intensity and the salivary cortisol. Another important finding in the present study was that the training intensity correlated with morning salivary cortisol levels on the days when the saliva samples were collected. Also, looking at the figures 4 and 6, it can be noted that the changes in cortisol and training intensity occurred in the same manner; cortisol concentrations increased when the training intensity increased and decreased with decreasing intensity, even though the changes within the variables were not considered significant.

Studies searching relationships between training intensity and cortisol have usually focused more on observing the relationships during or immediately after the exercise. These studies have concluded that the raise in cortisol concentrations is dependent on exercise intensity and that the concentrations increase remarkably only after moderate-to-high intensity exercise (Hackney & Andersen 2016; Hill et al. 2008; Jacks et al. 2002). These results are in line with the present study, since the only training characteristic that correlated significantly with cortisol was the training intensity.

Research investigating the long-term effects of endurance exercise and training to basal cortisol values have outlined that the resting cortisol levels slightly decrease or more often do not change at all after intensive training (Anderson et al. 2016; Daly et al. 2004; Vesterinen et al. 2013). For example, Vesterinen et al. (2013), found no significant changes in plasma cortisol concentrations in response to low- or high-intensity training in recreational endurance runners. In the study, Vesterinen et al. measured the basal cortisol levels after 14 weeks of basic training and after 14 weeks of training including exercises at higher intensities and the cortisol levels showed only slight decrement after the second 14 week training bout.

The results of the present study are not in line with the earlier studies since we found that the basal cortisol levels change in the same manner with changing training intensity, also by increasing with more intensive training. The reason behind these different results can be the different cortisol collection procedures. In the present study, cortisol samples were collected every other week during the athletes normal training and that way it was possible to detect the weekly variation in the cortisol levels. Earlier studies in turn, have measured the cortisol levels only after one intensive exercise (Anderson et al. 2016; Daly et al. 2004) or after a long training bout (Vesterinen et al. 2013). A single intensive exercise might not be hard enough to cause long-lasting increment in the cortisol levels that would be also perceptible on the morning after the exercise. In the case of longer training bout in turn, the participants usually follow a certain training program during the measurement period and the cortisol concentrations are measured only before and after the training bout. It is possible, that during the long training period, the participant get used to training and because of the adaptation the training does not affect the resting cortisol values anymore at the end of the training bout.

Other training characteristics and the stress markers. None of the other training characteristics had associations with either HRV or salivary cortisol. Many of the correlation coefficients between the stress markers and training characteristics were over ± 0.5, which usually is considered very high association between variables, but with the small number of participants they were not high enough to be considered significant. In the following section, the associations between the rest of the variables will be shortly discussed.

Training intensity and HRV. Earlier studies have found that the nocturnal HRV decreases remarkably after intensive training and returns to resting values with proper recovery (Hautala et al. 2001; Hynynen et al. 2010). In the present study, the associations between HRV and intensive training were not found even though the correlation between the variables was relatively high. The hypothesis of the study was that the HRV would have changed concurrently with changing training intensity. Looking at the figures 4 and 5, it can be noted that the HRV did decrease when the intensity increases and vice versa but since the average changes within the HRV values were so low, the statistical significance was not reached.

Training volume and the stress markers. The training volume did not show any correlations with the stress markers. This finding differs from the earlier studies because for example Alghadir et al. (2015) showed that 4-weeks low intensity endurance training had an increasing effect on basal cortisol levels. In contrast to their study, the participants in the present study were young athletes who had trained for cross-country skiing many years and were used to high training volumes. Also, the measurements were made at the end of training season, which means that the participants had been doing basic training already for more than six months before the tests took place. The training volume during the measurement period was most likely much less than it had been earlier in the season. Because of this, the participants were probably already used to relatively high training loads and it did affect to their stress levels as much as the increased intensity.

Training load and the stress markers. Similar to training volume, training load did not show any significant associations with HRV or salivary cortisol. The main reason behind this was that the equation that was used to calculate training load was more affected by the training volume than the intensity. Since there were no notable correlations between the training volume and the stress markers it is understandable that there were no associations between the training load and the stress markers either.

The original version of the Lucia's simplified TRIMP system separates the training time into three zones; the training time when the intensity is under the aerobic threshold, the time between the aerobic and anaerobic thresholds and the time over the anaerobic threshold. In the equation,

each training zone is multiplied with certain number to determine the training load because with more intensive training, the training load also increases. In the present study, the participants did not separate the training times that happened over the aerobic threshold into their training diaries which is why training on higher intensities had to be changed into one variable instead of two. It is possible that the modified equation underestimated the training load that came from training over the anaerobic threshold and eventually diminished the associations the training load and the stress markers. In the future research it would be good to find a better way to calculate the training load in order to find relationships between it and the stress markers.

Weekly training averages and the stress markers. The highest associations that occurred between the stress markers and the training characteristics were found between the stress markers and the three-day average training values. The three-day training values described the training that occurred during the days when the HRV and salivary cortisol were also measured. Weekly training load, volume and intensity were all reported and calculated to get the idea of the participants total training load, but according to the results they did not have any associations with the changes in the stress markers. The correlations were quite high for HRV and weekly training volume, HRV and weekly training load and salivary cortisol and weekly training intensity, but not high enough to be considered significant.

Since the highest correlations were found between the stress markers and the three-day average training intensity, it can be concluded that the stress markers provide the most information about the stressful effect of the intensive training that happens during the last few days before the morning cortisol or HRV levels are measured. The information it gives from the total load of the intensive training over a longer period of time does not seem to be as clear. In the case of salivary cortisol this would make sense, since for example Vesterinen et el (2013) could not find associations between 14-weeks of intensive training and resting cortisol levels, while in the present study strong correlations between the variables were found.

7.3 Limitations

There are several limitations in the study of which the most remarkable one was the small number of participants. When the number of subjects is this small, the statistical analysis underestimates both the changes within the variables and the relationships between the variables and it is hard to get results that would reach the significance level. Also, with small number of participants, any greater deviation from the mean has a large effect on the final results.

In the data collection the limitations focus on the ways the participants reported the data. The filling of the electronic training diary was not mandatory or anyhow controlled in the study which is why it is impossible to be sure that the participants have imported all their training into the training logs. The participants might also evaluate the intensity of the training differently which might have affected the amount of intensive training. Also, the collection of the saliva samples was done by the participants themselves and despite the instructions there might be differences in the ways the young participants gave the samples.

In the data analyzation as already mentioned, the equation used to calculate the training load might have underestimated the effect of training intensity, making the training load only follow the changes in training volume. In the future research it would be good to find another way to calculate the training load that would take also the intensity of the training into account.

7.4 Conclusions and practical applications

The results of the the present study authenticate the assumption that ANS and circulating hormones work together and are both responsible for the regulation of stress reactions. The associations between HRV and morning salivary cortisol, representing these two stress systems, were the most remarkable when the participants were experiencing the most stress. This finding indicates that the co-operation of these two stress systems is also stronger in more stressful conditions.

The present study also shows that the morning salivary cortisol levels are associated with the intensity of training that occurs during the last few days before the cortisol measurement. This finding confirms that cortisol levels can serve important information about the recovery state of body after hard training. Also, it addresses that the cortisol levels will not have to be measured during or immediately after the exercise, but can also be detected on the mornings after the intensive exercises. This makes it easier for the athletes to follow their cortisol concentrations, when they can do the testing in a more familiar environment at home.

One important notion in the study was that the participants' absolute cortisol values were notably higher that they should on average person. This finding indicates that endurance training might cause hypercortisolism for young endurance athletes, which differs from the discoveries of earlier studies. Another option is that the higher cortisol values were a sign of non-functional overreaching or overtraining and the recovery state of these athletes should be followed closely.

Since the changes in HRV and salivary cortisol were associated and the salivary cortisol was dependent on the changes occurring in the training intensity, it can be concluded that the training intensity somehow affects also to the HRV. However, because of the small number of subjects, the study did not find significant associations between HRV and training and consequently HRV cannot be recommended as a valid way to evaluate the recovery from training. In further research, it would be important to find more subjects to do this type of study in order to get more significant and valid findings.

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