TESTOSTERONE, CORTISOL, AND NOCTURNAL HEART RATE VARIABILITY IN RELATION TO TRAINING LOAD IN ENDURANCE TRAINING

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ABSTRACT

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The purpose of this master's thesis was to determine whether salivary cortisol and testosterone levels have a difference between low (LT) and high (HT) 4-day training period. In addition, heart rate variability (HRV) between the same training periods was examined, and whether there was a relationship between changes in hormonal values and HRV. The aim was to determine whether salivary testosterone and cortisol levels could be used to assess the training load of endurance athletes.

Testosterone and cortisol are hormones that have been shown to reflect anabolic/catabolic protein metabolism in the body. Testosterone and cortisol have been studied to respond to physical training both acutely and longer term. Heart rate variability has been found to decrease because of strenuous training and overtraining. The relationship between testosterone/cortisol and HRV are still unclear, even though suggestions of the connection between hypothalamic pituitary (HPA) axis and autonomic nervous system (ANS) have been. This master's thesis studied the differences in salivary testosterone (awakening: wTes, evening: eTes), cortisol (awakening: wCor, evening: eCor), testosterone/cortisol ratio (awakening: wT/C, evening: eT/C) and nocturnal HRV (RMSSD) on endurance athletes ($n = 20$) during a preparatory season between low (LT) and high (HT) 4-day period. The study was conducted by daily measurements of salivary hormone levels, heart rate variability and training load conducted during subject's everyday life. Training load was determined as TRIMP (training stress to training impulse) value based on the time spent in the heart rate zones during exercise ($TRIMP_{hr\_zone}$).

There were no statistically significant differences in salivary testosterone and cortisol values or HRV between the two training periods ($p > 0.05$). There were no correlations between changes in hormone levels and heart rate variability. Training load ($TRIMP_{hr\_zone}$) did have a significant difference between studied 4-day periods ($p < 0.001$). The results did not meet the hypothesis of differing hormonal and heart rate variability values between the LT and HT. Neither was the relationship between HPA axis and ANS confirmed. However, due to the nature of the study and assumed monotonic training, no conclusions can be done.

Key words: testosterone, cortisol, training load, HRV

TIIVISTELMÄ

Harjula, F. 2024. Testosterone, cortisol, and nocturnal heart rate variability in relation to training load in endurance training. Liikuntatieteellinen tiedekunta, Jyväskylän yliopisto, liikuntafysiologian pro gradu -tutkielma, 47 s, 2 liitettä.

Tämän pro gradu -tutkielman tarkoituksena oli selvittää muuttuvatko syljestä mitatut kortisoli- ja testosteroniarvot kevyellä ja kovalla neljän päivän mittaisella harjoitusjaksolla. Lisäksi tarkasteltiin sykevälivaihtelua samojen harjoitusjaksojen välillä, sekä oliko hormoniarvojen ja sykevälivaihtelun muutosten välillä yhteyttä. Tarkoituksena oli selvittää, voiko syljen testosteroni- ja kortisoliarvoja mittaamalla arvioida kestävyysurheilijoiden harjoituskuormituksen tilaa.

Testosteroni ja kortisoli ovat hormoneita, joiden on nähty kuvastavan elimistön anabolista/katabolista proteiiniaineenvaihduntaa. Testosteronin ja kortisolin on tutkittu reagoivan fyysiseen harjoitteluun niin akuutisti kuin pidemmällä aikavälillä. Sykevälivaihtelun on todettu laskevan kuormittavan harjoittelun ja ylikuormitustilojen seurauksena. Yhteydet testosteronin/kortisolin ja sykevälivaihtelun välillä ovat vielä epäselviä, vaikka hypotalamus-aivolisäke-lisämunuaiskuori akselin ja autonomisen hermoston yhteydestä on viitteitä. Tässä tutkimuksessa tarkasteltiin kestävyysurheilijoiden (n = 20) valmistavalla harjoituskaudella kevyen (LT) ja kovan (HT) 4 päivän tarkastelujakson välillä esiintyviä eroavaisuuksia syljen testosteronissa (aamu: wTes, ilta: eTes), kortisolissa (aamu: wCor, ilta: eCor), testosteroni/kortisoli suhteessa (aamu: wT/C, ilta eT/C) ja yönaikaisessa sykevälivaihtelussa (RMSSD). Tutkimus toteutettiin mittaamalla päivittäin urheilijoiden normaalin harjoittelun aikaisia syljen hormoniarvoja, sykevälivaihtelua ja harjoittelun kuormittavuutta. Harjoituskuormitus määritettiin sovellettuna TRIMP-arvona (training stress to training impulse) sykealueilla harjoituksen aikana vietetyn ajan perusteella ($TRIMP_{hr\_zone}$).

Syljen testosteroni- ja kortisoliarvot tai sykevälivaihtelu eivät eronneet tilastollisesti merkittävästi harjoitusjaksojen välillä ($p > 0.05$). Hormoniarvojen ja sykevälivaihtelun muutosten välillä ei esiintynyt korrelaatioita. Harjoituskuormitus ($TRIMP_{hr\_zone}$) muuttui tarkastelujaksojen välillä merkittävästi ($p < 0.001$). Tulokset eivät vastanneet hypoteesia hormonaalisten ja sykevaihteluarvojen eroista LT:n ja HT:n välillä. Myöskään aivolisäke-lisämunuaiskuori akselin ja autonomisen hermoston välistä yhteyttä ei voitu vahvistaa. Tutkimuksen luonteen ja oletettavasti melko monotonisen harjoittelun vuoksi johtopäätöksiä ei kuitenkaan voida tehdä.

Avainsanat: testosteroni, kortisoli, harjoituskuormitus, sykevälivaihtelu

ABBREVIATIONS

ANS	autonomic nervous system
CAR	cortisol awakening response
ECG	electrocardiogram
eCor	following day evening cortisol
eTes	following day evening testosterone
HIEE	high-intensity exercise endurance
HRV	heart rate variability
HPA	hypothalamic-pituitary-adrenal
IBI	interbeat intervals
LIEE	low-intensity exercise endurance
NN-interval	time between two normal R-peaks from ECG
LnHF	natural logarithm of the high-frequency power analysis of HRV
LnRMSSD	natural logarithm of the RMSSD
PNS	parasympathetic nervous system
PPG	photoplethysmography
RMSSD	root mean square of successive differences between normal heartbeats
RPE	rating of perceived exertion
RRI	time between two R-peaks from ECG
SNS	sympathetic nervous system
T/C ratio	testosterone to cortisol ratio
TRIMP	training impulse
wCor	following day awakening cortisol
wTes	following day awakening testosterone

CONTENTS

1 INTRODUCTION

Athlete's training aims for developing the skills and physiological mechanisms that increase the performance for the sport. Aiming for this development is a constant balancing between sufficient training, resting and nutrition. How to measure and evaluate what is the optimal amount of training for development but not an amount that would lead to overtraining, is a constant question the athlete and coaches need to evaluate. Different measures such as subjective, physiological, and external measurements have been used to track training load (Coutts et al. 2018). The problematics of which measures provide the best information and most clear results of overtraining or training induced changes in body are still unsolved (Carrard et al. 2022).

Heart rate variability (HRV) is a widely used method to track athletes and normal people recovery, stress and training state (Kinnunen et al. 2020). More specifically, HRV is seen as an indicator for measuring autonomic nervous system activity (Laborde et al. 2017). There are plenty of devices in the market that are accessible to almost everyone with their price and usability. HRV-guided training is used and found to be an effective training method to avoid overtraining and to optimize performance (Nuuttila et al. 2017). However, it is not clear if nocturnal heart rate variability will provide diagnostic tools for overtraining and sufficient information about training load (Hynynen et al. 2011). The autonomic nervous system and its changes are a mirror of many changes in body, and it is affected by plenty of factors (Kim et al. 2018)

Hormonal secretion and concentrations of cortisol and testosterone are regulating protein synthesis and muscles anabolic and catabolic reactions (Hall 2016, 972–973; McArdle et al. 2015, 425.). Measuring concentrations of cortisol and testosterone in saliva or blood is suggested as a possible method of evaluating athlete's recovery state (Anderson et al. 2016). The two hormones are presented to indicate the current anabolic/catabolic state of a body (Hall 2016, 927–928; McArdle et al. 2015, 426). Training and exercise have been studied to lead to changes in these hormones acutely (Hayes et al. 2015), so it is a good question, if the recovery state can be evaluated just by measuring the hormones.

The correlation of the two hormones and heart rate variability is unclear. Suggestions of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) being

connected and affected by each other are made. There were only a few available studies regarding the topic. The goal of this thesis was to study if change in training load affects heart rate variability and hormonal values of salivary testosterone and cortisol during endurance runner's preparatory season. In addition, associations between the differences in hormonal values and heart rate variability were studied.

2 ENDURANCE TRAINING IN RUNNING

Training for endurance in running aims for improving the running performance and physiological factors behind it. For high level endurance runners these main physical factors are maximal oxygen uptake and speed associated with it, lactate thresholds and the speeds associated with these thresholds (especially on threshold 2), running economy, and the ability to run at speed corresponding to high percentage of the maximal oxygen uptake. (Casado et al. 2022)

Reaching the optimal endurance exercise performance presents demands for the cardiovascular system of body. These demands are met by regulating the body's function by hormonal, neural and mechanical mechanisms. One of the most important regulators is the autonomic nervous system that presents changes in heart rate (HR) and further in HRV. (Martinmäki 2009) Endurance trained athletes body adapts to continuing training by metabolic, cardiovascular and pulmonary adaptations and with blood lactate concentration. Cardiovascular adaptations improve the oxygen delivery to active muscle. These adaptations include cardiac hypertrophy, increasing of plasma volume and therefore blood volume, adaptations in heart rate, increasing stroke volume, maximal cardiac output, more efficient oxygen extraction, blood flow distribution adaptations and changes in blood pressure. (McArdle et al. 2015, 464–477)

2.1 Periodization of training

Endurance performance and training can be classified into two categories: low-intensity exercise endurance (LIEE) and high-intensity exercise endurance (HIEE). The main energy supply to LIEE is offered by aerobic energy systems while HIEE relies on anaerobic systems. (Bompa & Haff 2009) The base for endurance training is agreed to be high amount of low intensity endurance training (80 % on low intensity, 20 % on high intensity) (Seller 2010).

Casado et al. (2022) concluded that endurance runners training on the preparatory season is based on a pyramidal training model, where on the bottom is the training under the speeds of first lactate/ventilatory threshold. After that is training between the first and second lactate/ventilatory threshold. The lowest amount of training occurs on a top of the pyramid, where is the training over the second lactate/ventilatory threshold. In addition, the training is

traditionally encouraged to be executed with a hard day–easy day basis (Casado et al. 2022) Usually training in the preparatory period is high volume with low intensities and high intensity training increases while transferring to the competitive period. However, the amount of low intensity training remains still relatively high. (Seller 2010) Typical duration of the preparatory season is 4 months, precompetitive from 2.5 to 4 months and competitive from 3 to 4 months. (Casado et al. 2022) Figure 1 presents an example of a training distribution for middle-distance and long-distance runners during on training period.

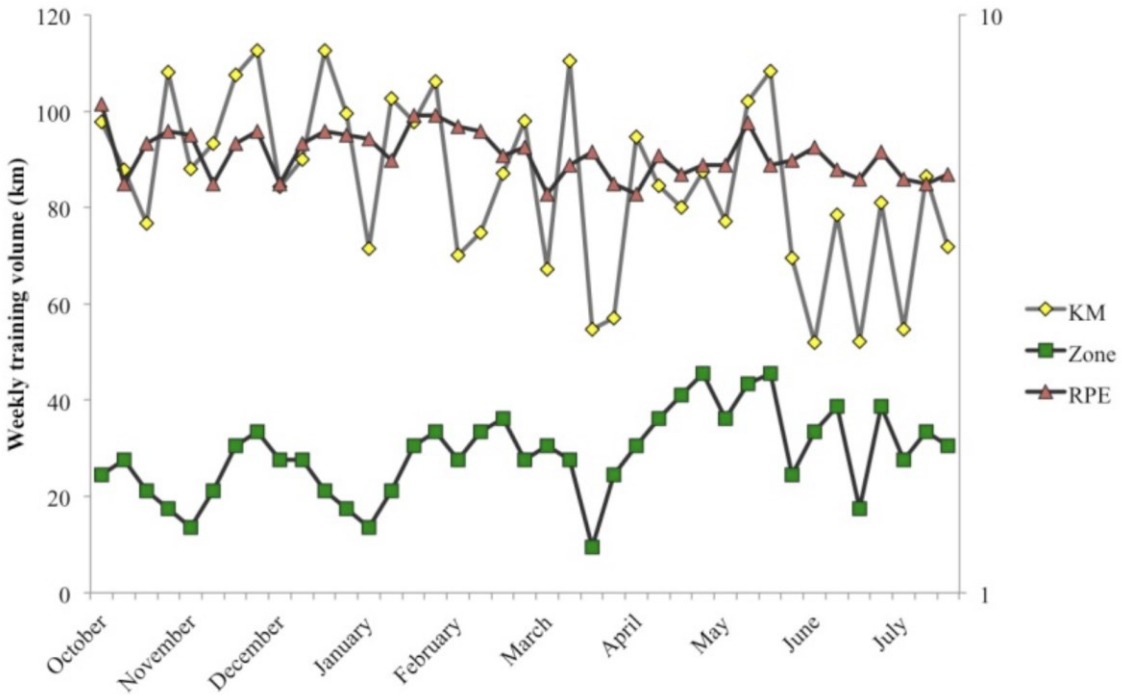


FIGURE 1. Weekly averages of training volume, training zone and session-RPE represented for high level middle and long-distance runners. Training volume is represented in kilometers (on the left Y-axis) and training zone and session-RPE with a logarithmic scale (right Y-axis). Training zone was distributed to three different zones, where 1 = long-distance continuous training or long intervals, 2 = middle-distance interval training (1-3 km) and 3 = short-distance and sprint interval training. (Balsalobre-Fernández et al. 2014).

2.2 Training load in endurance training

Casado et al (2022) discovered in their review that typical distance covered weekly for elite/high level runners competing on 1500 meters to marathon was 110–195 kilometers per

week. Training load is higher on the preparatory season compared to the competitive season (Casado et al. 2022). Another study following high-level middle and long-distance runners found that runners covered on average 85.4 ± 5.8 km per week during 10-month period of training (Balsalobre-Fernández et al. 2014).

Training load in endurance training is possible to measure with internal or external methods (Coutts et al. 2018). While measuring training load, it is important to view the methods contextually and dependent of the exercise type. To illustrate this, heart rate might be a good indicator of internal load in endurance running, but not in powerlifting. Gold standard for measuring internal or external training load cannot therefore be defined. (Impellizzeri et al. 2019) The training progress and its targets are concluded by the determinant factors of addressed sport, as presented in figure 2.

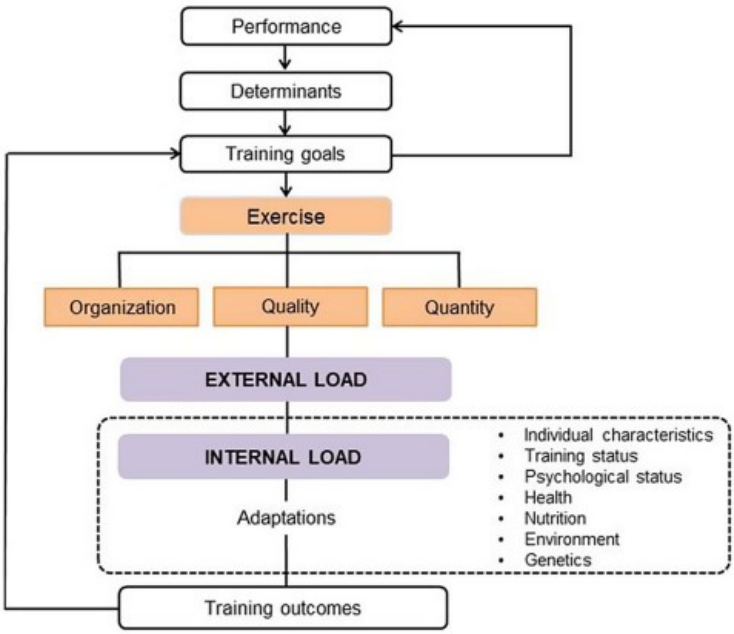


FIGURE 2. Theoretical framework of the training progress (Impellizzeri et al. 2019).

External training load is traditionally defined by work that is possible to view or measure from outside view of the athlete and how training is planned in the training program, as for example distance covered, speed or load carried (Impellizzeri et al. 2019). Various devices such as GPS make tracking of detailed changes in external load possible and provide information about it. External load can be viewed in many different methods, such as time spent in different speed

areas or number of accelerations. However, psychophysiological stress might not always be correlated directly and presented accurately by measuring external factors. (Coutts et al. 2018)

Internal measurements include physiological factors such as heart rate, oxygen consumption, blood lactate or samples from other bodily fluids or tissues, rating of perceived exertion (RPE) and critical power. These can be applied further to view training stress to training impulse (TRIMP), session RPE and summated heart rate zone score. (Borresen & Lambert 2009) Calculating training impulse with heart rate to a measure TRIMP was originally proposed by Banister in 1991 and used widely in studies and in commercial wearable devices (Kinnunen et al. 2020; Wallace et al. 2014) The method presents internal training load with following equations presented by Morton and colleagues (1990) and edited by Wallace and colleagues (2014):

$$TRIMP = D(\Delta HR\ ratio)e^{\,b(\Delta HR\ ratio)}$$

where D presents the duration of exercise, b is a weighting factor different for females and males and following equation for ΔHR ratio:

$$\Delta HR\ ratio = (HRex - HRrest) / (HRmax - HRrest)$$

where HRex = heart rate during exercise, HRrest = heart rate during rest, HRmax = maximal heart rate.

Criticism for Banister's TRIMP has been presented, since it is dependent on HR and has unclarities to track high-intensity training such as weight training because the strain is not always visible on HR. Training load values computed from duration of exercise and RPE versus summated HR zone method has been studied to give higher values (Foster et al. 2001) TRIMP has also been studied to overestimate the training load if the exercise time is spent on higher heart rate areas (Borresen & Lambert 2008).

Later, the TRIMP formula has been developed by researchers; Edwards used five different heart rate zones to summate time in different training intensities (Edwards 1993, as cited by Roos et al. 2013), Lucía and colleagues (1999) presented similar approach but with three intensity zones. Individual approach to Banister's formula was created by Manzi with his colleagues in

2009 when they used different weighting factor based on individual lactate curve for each athlete. Wallace and colleagues (2014) agreed that HR TRIMP correlations to training induced physical stress and recovery could be improved if individual physiological (lactate) thresholds are applied to the calculations.

3 TESTOSTERONE AND CORTISOL

Testosterone and cortisol are steroid hormones that have an important function in regulating metabolism of proteins, carbohydrates, and fats. These hormones are highly soluble and once secreted, they diffuse through the cell membrane and enter the blood via interstitial fluid. Cortisol and testosterone are not stored and the appearance of the hormones in bodily fluids can be viewed as markers of anabolic or catabolic reactions (Hall 2016, 927–928; McArdle et al. 2015, 426).

The secretion of testosterone and cortisol is controlled via negative feedback mechanisms for ensuring that the hormones are not oversecreted or the target tissue is not overactivated. (Hall 2016, 929) The receptors of cortisol and testosterone lie in the cell cytoplasm of the targeted cells (Hall 2016, 930).

3.1 Testosterone

Testosterone is a male-hormone that regulates the sperm production and male secondary sex characteristics. It has important anabolic muscle tissue building characteristics, making it an important hormone contributing the building of muscle and strength. (McArdle et al. 2015, 425) The secretion of testosterone is rapidly increased for men during the onset of puberty due to a stimulus of anterior pituitary gonadotropic hormones. Then the secretion remains mainly steady until the age of 50 years and then starts to decrease to as low as 20–50 % of the peak values by the age of 80 years. (Hall 2016, 1029) Due to differential hormonal secretion systems and hormonal behavior between sexes, all the following studies introduced include biological male only or only male results are presented.

Testosterone is secreted in the interstitial Leydig cells of the testes. It is catalyzed mainly by luteinizing hormone (LH) secreted from the gonadotropins in the anterior pituitary gland. The secretion of LH is controlled by gonadotropin-releasing hormone (GnHR), which is a 10-amino acid peptide secreted from the hypothalamus. Increases in testosterone will cause a direct effect on the secretion of GnHR leading to decrease in secretion of LH. Most of its effects on body are caused by enhanced protein formation in the target cells. (Hall 2016, 1031–1032) The

secretion of testosterone is regulated by a negative feedback mechanism, which is portrayed in figure 3 (Hall 2016, 1032).

Testosterone concentration in the blood plasma is commonly seen to serve as a marker for anabolic status (McArdle et al. 2015, 426) After secretion by the testes, 97 percent of the testosterone is moved to the blood, where it circulates for about 30 minutes (Hall 2016, 1028.) In the blood, testosterone is loosely bound to plasma albumin (38 %) or more tightly to steroid hormone-binding globulin (60 %). A small amount (2 %) of testosterone is also circulating in an unbound state, called free testosterone. (Schoenfeld 2010.) From the blood, testosterone is transferred to tissues or degraded into inactive products. (Hall 2016, 1028.)

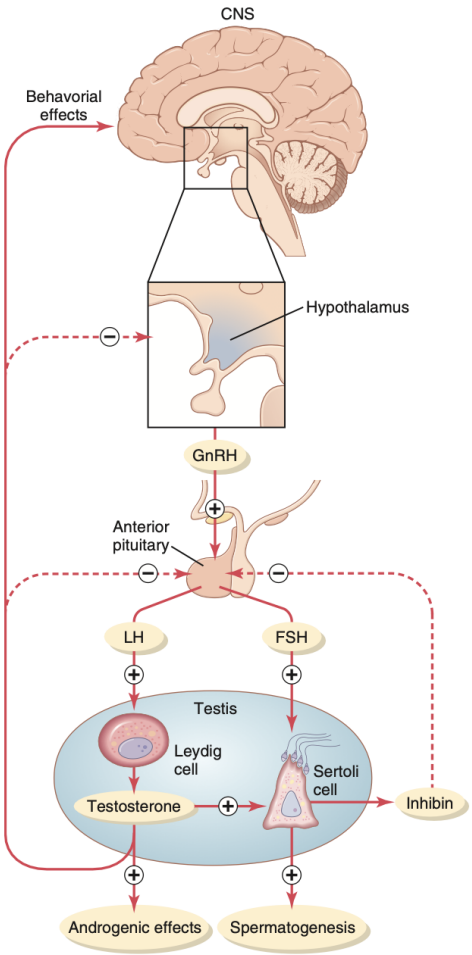


FIGURE 3. Feedback regulation of the hypothalamic-pituitary-testicular axis in males (Hall 2016, 1032).

Testosterone concentration has been found to peak in the morning and lower throughout the day. (Ahokoski et al. 1998; Doerr & Pirke 1976) There were no significant differences with the concentrations between timepoints 8:00 and 12:00 or between 16:00 and 20:00 (Ahokoski et al. 1998). Declines of testosterone from morning to evening have been found in measures from saliva and serum (figure 4) (Mezzullo et al. 2016).

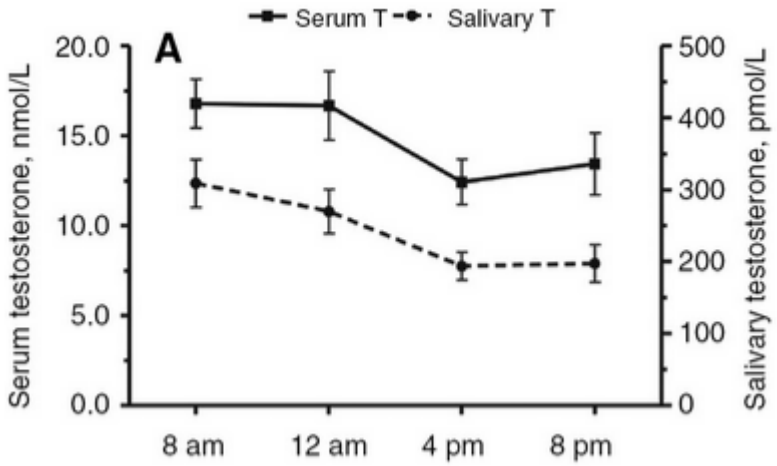


FIGURE 4. Salivary (dotted lines) and serum (bold lines) testosterone fluctuations during daytime for males (n=12). Values of serum testosterone are presented on the left Y-axis and salivary testosterone on the right Y-axis. (Mezzullo et al. 2016)

3.1.1 Testosterone and endurance training

Studies suggest that acute training increases salivary testosterone levels after the training session (Hayes et al. 2015), but on the contrary, single exhaustive endurance training may also decrease testosterone concentration after the exercise (Anderson et al. 2016; Lac & Berthon 2000). Sato and colleagues (2015) discovered an increase in serum free testosterone measured after aerobic training sessions independent of the intensity of the session in the sedentary male subjects. Only the high-intensity session did have a significant increase in endurance athletes. Measures were taken immediately after the exercise training session ended. (Sato et al. 2015) Supporting the theory of single exercise session leading to increases in blood serum free testosterone, Hough and colleagues (2021) found increased testosterone levels immediately and 30-minutes after cycle-ergometer HIIT-exercise for regularly active males. Similarly, sprint

exercise did increase the testosterone levels in both high volume and light training phase measured five and 60 minutes after the training. (Cook et al. 2021)

Contradictory results have also been found. In the study of Monje and colleagues (2020), HIIT-exercise done by endurance athletes did not lead to changes in salivary testosterone 20 minutes after the exercise session. Also, salivary testosterone levels decreased 10 minutes after a 5000-meter race compared to the values before the race (Li et al. 2015). On the other hand, when endurance trained males performed a single exhaustive endurance training session, it did evoke a decrease on serum free testosterone. Testosterone was studied to be decreased even 48 hours after the exercise session. (Anderson et al. 2016.) A long duration running race did also decrease the salivary testosterone during race and the race day evening. However, oppose to previous study, testosterone remained close to pre-values on the next morning and increased significantly from the race evening values to the next evening. (Lac & Berthon 2000)

Generally, endurance trained men present lower testosterone levels than non-endurance trained controls (McArdle et al. 2015. 442). Exercise induced serum testosterone reactions are larger in non-endurance athletes than endurance trained athletes (Sato et al. 2015). However, with shorter training periods the testosterone values might increase. (Ambroży et al. 2021; Nuuttila et al. 2017) After a controlled 8-week aerobic block training period with high and moderate intensity training, basal morning serum testosterone concentration decreased significantly from pre-values to the middle-phase of training intervention and increased significantly from middle-values to post-values. The training block structure was similar between weeks 1-4 and 5-8. Even though the subjects were regularly training endurance athletes, their endurance performance and maximal oxygen uptake improved. (Nuuttila et al. 2017) Also, 8-week HIIT-training period have been found to increase morning serum testosterone in 35–40-year-old males (Ambroży et al. 2021)

No clear effects on salivary testosterone were detected after a 10-day triathlon camp, where training load increased significantly. There were no clear responses on resting saliva testosterone in comparison to values before the training period. Increases in the training load were reported with higher amounts of time and distance covered on swimming, running and cycling. However, the responses to a high intensity cycling test were greater (increases in testosterone) before the training period. (Hough et al. 2015)

3.1.2 Testosterone and training load

Morning basal salivary testosterone concentration has been shown not to be correlated with training load (Gomes et al. 2013; Hough et al. 2015; Kamarauskas et al. 2022; Tiernan et al 2019). Internal training load (measured with RPE questionaries) did not either correlate with salivary morning testosterone concentrations when studying young male tennis players. During the 5-week training period training load did alternate significantly, but there were no changes in the testosterone concentrations. (Gomes et al. 2013) Kamarauskas and colleagues (2022) presented weekly salivary testosterone concentrations not correlating with internal training load. Like Gomes and colleagues (2013), Kamarauskas and colleagues (2022) presented the weekly salivary hormone concentrations based on one sample taken in rested and fasted states. They also mentioned that games and psychological factors might have an influence on hormones, but they were not recorded in the study. (Kamarauskas et al. 2022)

Evidence of training load affecting testosterone has been found (Coutts et al. 2007). Young rugby players presented lower total plasma testosterone during overload training period. During the training period, internal training load got higher simultaneously with lower testosterone concentrations. In the study, there were two different groups with different training loads. However, there were no significant differences between the groups. (Coutts et al. 2007) Filaire et al. (2001) discovered a significant decrease in salivary testosterone values after high intensity training period when testosterone was measured at daytime and in the afternoon (11:30 and 17:00), but no changes were noticed in the morning values. After a football match, the distance covered (during the match) was found to be correlated with the decrease of testosterone concentrations in saliva (Peñailillo et al. 2015).

3.1.3 Testosterone, recovery state and overtraining

A systematic review by Cadegiani and Kater (2017) suggested that basal testosterone levels are not altered from normal levels in overtrained athletes. Some suggestions of decreased basal testosterone levels have been found but no clear evidence has been presented (Carrard et al. 2022). Testosterone levels have been studied to increase after overtraining syndrome treatment and the levels became similar to healthy athletes (Cadegiani et al. 2021). Also, total testosterone has been studied to be one of the eligible diagnostic criteria for an overtraining syndrome

(Cadegiani et al. 2020). In addition, no clear relationship with performance (which has been seen to reduce due to overtraining) and testosterone have not been found (Filaire et al. 2001; Kraemer et al. 2004). For both testosterone and cortisol, it has been suggested that studying the hormonal reactions rather than basal levels might be more accurate for diagnosing overtraining (Carrard et al. 2022).

Perceived stress and recovery can affect the salivary testosterone levels (Rosa et al. 2020). General well-being, perceived sleep quality and social recovery did show clear relationships with salivary testosterone during several paralympic swimming training camps. Also, motivational factors, success, physical recovery, being in shape, personal accomplish, self-efficacy and self-regulation showed relationships with salivary testosterone. (Rosa et al. 2020.) It is good to acknowledge these factors while viewing the overall recovery of an athlete. Andre and colleagues (2018) created a Collegiate Basketball Demands score value, that measured the athletes complete physiological and psychological stressors (playing time, practice time, resistance-training volume, travel schedules and academic demands). Even though the score value changed through season, it did not change in relation to the testosterone levels. (Andre et al. 2018)

3.2 Cortisol

Cortisol is an adrenocortical hormone, that is secreted from the adrenal cortex's zona fasciculata. The secretion is controlled by the hypothalamic-pituitary axis via adrenocorticotropic hormone (ACTH). Cortisol, like all adrenocortical hormones, is synthesized from cholesterol. (Hall 2016, 965–966)

Cortisol is categorized as a catabolic hormone that reduces the protein stores in all the cells of the body, except for liver, where the proteins are increased. This is caused by the elevated catabolism of already existing proteins in the tissue and decreased protein synthesis mainly due to the decreased formation of RNA and, therefore, the protein synthesis especially in the muscle and lymphoid tissues. Cortisol also affects metabolism of carbohydrates and fats by stimulating gluconeogenesis, decreasing glucose utilization in cells, elevating blood glucose concentration and mobilizing fatty acids. (Hall 2016, 972–973)

Physical and neurogenic stress causes immediately a marked increase in ACTH secretion, which in turn causes a secretion of cortisol in a couple minutes. Even though the reasons why this mechanism is necessary for animals and humans are not completely clear, it might be due to the secured availability of amino acids and fats for synthesis of other compounds. Additionally, cortisol also works as a blocking factor to an inflammation caused by trauma in tissue preventing further damage. (Hall 2016, 974)

In normal circadian rhythm of cortisol, it peaks in the morning, lowers during the day and obtains its lowest values at midnight (Adam & Kumari 2009; Chan & Debono 2010; Ljubijankić et al. 2008). The cortisol awakening response (CAR) follows 30–45 minutes after awakening and the free cortisol levels in saliva have been found to increase by 50–75 % (figure 5) (Adam and Kumari 2009; Pruessner et al. 1997) After the morning surge the levels drop rapidly and decline until bedtime when they are the lowest (Adam & Kumari 2009).

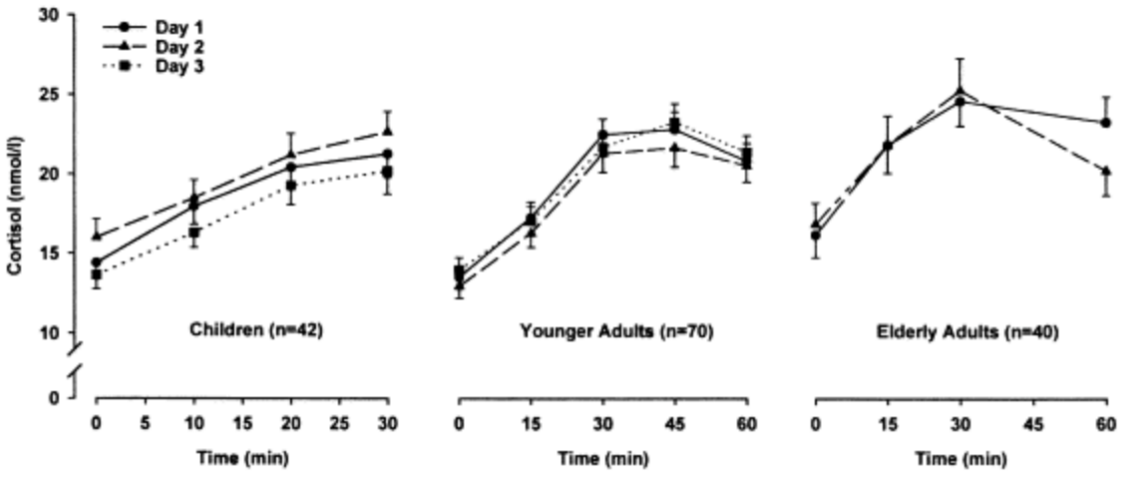


FIGURE 5. Salivary cortisol awakening response (CAR) in consecutive days (Pruessner et al. 1997).

3.2.1 Cortisol and endurance training

Salivary cortisol increases after an exercise session (Hayes et al. 2015; Hough et al. 2021; Li et al. 2015). However, all exercise does not necessarily lead to changes in salivary cortisol (Peñailillo et al. 2015; Wahl et al. 2013). Different intensities of aerobic endurance training did

lead to increases in serum cortisol concentration immediately after the exercise, but in the endurance athlete group only the maximal intensity was enough for significant increase. For the sedentary controls, all the intensities were enough to create an increase that was statistically significant. (Sato et al. 2015) For endurance athletes the all-out type or very high intensity training session increased the cortisol levels in serum significantly, but longer low-intensity training session did not have an effect on the cortisol. Interestingly, 180 minutes after all exercises, cortisol was significantly decreased from the pre-values. (Wahl et al. 2013) Decreased salivary cortisol levels have been studied even after 3 days of a marathon and long duration running race (Bobbert et al. 2005; Lac & Berthon 2000).

Likewise, Anderson and colleagues (2016) discovered that after an exhaustive aerobic exercise session the serum cortisol increased immediately after the exercise and then decreased under the pre-values after 24 hours. After 48 and 72 hours the cortisol values were returned to the baseline. (Anderson et al. 2016) Cycle-ergometer HIIT-exercise has also been studied to create an increase in salivary cortisol immediately after exercise and still increasing 30 minutes after (Hough et al. 2021). After running an HIIT-exercise saliva cortisol was elevated from pre-values to 20 minutes after the session for 41 ± 24 % in male participants (Monje et al. 2020).

Higher levels of physical activity are connected to a lower salivary cortisol concentration. In addition, the subjects reporting higher physical activity and lower sedentary behavior did have steeper decline on the salivary cortisol from awakening to daytime. (Gubelmann et al. 2018) Similar to changes in testosterone concentration, exercise induced reactions in serum cortisol are larger in non-endurance athletes than endurance trained athletes (Sato et al. 2015). Also, cortisol levels of plasma are increased less due to the same submaximal exercise in trained subjects than sedentary controls (McArdle et al. 2015, 443). Prior physical activity and endurance training period have been studied to diminish the increases in cortisol levels to a psychosocial stress test (Klaperski, et al. 2014; Wood et al. 2018).

Even though physical activity should decrease cortisol levels, endurance or HIIT training interventions have been studied not to have an effect on basal or exercise induced cortisol levels (Ambroży et al. 2021; Hough et al. 2015; Klaperski et al. 2014). When sedentary and close to sedentary study group performed a 12-week endurance training period, no changes in salivary cortisol were found between the beginning and the end of the test period (Klaperski et al. 2014).

Similarly, increasing values of salivary morning cortisol levels have been studied after a 4-week moderate aerobic exercise training (Alghadir et al. 2015).

3.2.2 Cortisol and training load

Training load measured with RPE has been studied to correlate positively with morning basal salivary cortisol during endurance runners prolonged training period while studying the season average. However, for weekly averages of salivary free cortisol, the training load did not have a relationship. (Balsalobre-Fernández et al. 2014) For young tennis players, internal training load (measured with RPE) did correlate significantly with resting salivary cortisol concentrations. Also, significant correlations between symptoms of stress to cortisol were found. (Gomes et al. 2013) In the study of Kamarauskas and colleagues (2022), there were no significant correlations between weekly internal training load and salivary cortisol in basketball players. Neither did Tiernan and colleagues (2019) find relationship between internal training load (RPE x duration of training) and salivary cortisol in longer periods of training seasons in young rugby players. (Tiernan et al. 2019) Previous days training load correlated positively with next morning CAR in endurance athlete group. The increase 30 minutes after awakening was positively correlated with previous days training load (TRIMP) in endurance athletes. However, individual values of after awakening or 30 minutes after awakening did not correlate with training load. (Anderson et al. 2018)

When studying the responses of salivary cortisol to ultra-exercises, Tauler and colleagues (2013) found that cortisol levels were negatively correlated with time spent on the track. Therefore, the intensity of the exercise was more determinant factor to the cortisol levels than kilometers. Also, Peñailillo and colleagues (2015) found that cortisol does not correlate with distance covered in football match. However, they did not find differences in salivary cortisol measured before and after match. Neither did Balsalobre-Fernández and colleagues (2014) found correlations between running distance and cortisol on endurance training season.

A triathlon camp that lasted for 10 days did not affect the salivary cortisol responses to HIIT-training session. There were no differences in morning resting salivary cortisol concentrations before and after the camp, even though the external training load was reported to be higher during the camp than during their normal practice. (Hough et al. 2015) There were no

differences in recovery-stress questionnaire after camp and Hough and colleagues (2015) suggested that the training load might not have been strenuous enough for the athletes to create responses in hormonal levels. The results might also suggest that basal cortisol measured from saliva does not correlate with training load or training volume. On the other hand, morning salivary cortisol did show correlation with harder training load during the shift of training to early competition season for young endurance athletes. (Mishica et al. 2021)

3.2.3 Cortisol, recovery state and overtraining

Some suggestions of increased basal cortisol and blunted exercise induced effects on cortisol have been suggested, but no compelling evidence have been found (Cadegiani & Kater 2017; Carrard et al. 2022). Hedelin and colleagues (2000) found that after high intensity training period blood cortisol levels were decreased even though there were signs of overtraining in performance. Neither there were no differences in overnight urinary cortisol in overtrained athletes and control group (Hynynen et al. 2006).

During the overload training period young rugby players did not present changes in cortisol levels. Groups with different training loads did not present different cortisol levels. Neither did the increase of training. (Coutts et al. 2007) Opposite effects for soccer players during preseason and competitive season have been found in cortisol levels, that were negatively correlated with vertical jump height during the season (Kraemer et al. 2004). Similar correlations between counter movement jump and salivary cortisol were found with middle- and long-distance runners (Balsalobre-Fernández et al. 2014). However, in the study of Filaire and colleagues (2001) among male soccer players, there were no significant changes in cortisol concentrations or relationship between individual or team performance. The cortisol measurements done during the day were increased after the high intensity training period, and therefore some effects could be seen. However, there were no clear evidence of morning measurements being a useful tool for assessing recovery state. (Filaire et al. 2001.)

Like testosterone, also cortisol was affected by recovery/stress factors in paralympic swimmers during training camp. Statistically significant differences were measured only in few parameters and during one camp. (Rosa et al. 2020.) Subjective fatigue has been found to correlate with resting blood cortisol after high intensity training period in rowers (Jürimäe et al. 2004). On the

other hand, for young rugby players salivary cortisol did not correlate with subjective markers of recovery (perceived fatigue, muscle soreness, stress level, energy and physical recovery) (Tiernan et al. 2019). For stress measurements, CAR has been thought to indicate basal HPA axis activity. While measuring the response, it is good to acknowledge that it is affected by several other factors such as age, gender, stress perception, health status and way of life. (Marques et al. 2010)

3.3 Testosterone to cortisol ratio as a metabolic marker

Negative correlations between internal and external training loads have been studied with testosterone to cortisol (T/C) ratio, but the results are not consistent (Andre et al. 2018; Filaire et al. 2001; Gomes et al. 2013; Kamarauskas et al. 2022). Internal training load (measured with RPE) did correlate negatively with T/C ratio. Also, significant correlations between symptoms of stress to T/C ratio were found. (Gomes et al. 2013). In the other study of Kamarauskas et al. (2022), T/C ratio did not, however, correlate with internal load. Neither did Filaire et al. (2001) find correlations with physical performance or competitive season changes with T/C ratio. Weekly recorded salivary hormones during basketball season were in relationship with game time. T/C ratio was significantly negatively correlative with played game minutes, suggesting that the combination of physical and physiological stress led to the highest responses in hormonal variables. Testosterone or cortisol itself did not show correlations with other variables. (Andre et al. 2018.)

Single exhaustive endurance exercise evoked a significant decrease in testosterone-cortisol ratio immediately after the session. Even though testosterone and cortisol values were different from the pre-values 24–48 hours after the exercise, T/C ratio returned to the baseline 24 hours after the exercise. The quicker recovery was probably due to the reverse effect of cortisol, that peaked first and then decreased. (Anderson et al. 2016) Running HIIT-exercise did not have a significant effect on T/C ratio, even though cortisol values were significantly increased after the exercise. However, in the male participants T/C ratio did have a minor decrease ($p = 0.09$). (Monje et al. 2020.)

There are still some unclarity of how overtraining affects T/C ratio. However, it might be better tool to assess recovery state than cortisol or testosterone itself (Andre et al. 2018). Cadegiani

and Kater (2017) discovered in their systematic review that even though basal testosterone, cortisol or T/C ratio did not show differences in overtrained athletes, T/C ratio responses were diminished more after overtraining period than both hormones itself. However, no clear evidence of T/C ratio being a reasonable marker for recovery or training load has not been found. (Cadegiani & Kater 2017)

When studying performance, male soccer players did show differences in cortisol, testosterone and T/C ratio during preseason and competitive season. T/C ratio correlated negatively with vertical jump, which might suggest that salivary hormone levels might be used to study athlete's recovery state and readiness for competition. (Kraemer et al. 2004) Overload training period did affect T/C ratio by decreasing it in young rugby players. The change was due to altered testosterone levels. (Coutts et al. 2007.)

Sleep efficiency measured by actigraphy has been found to have a negative relationship with salivary testosterone and T/C ratio upon awakening during a rugby camp. Surprisingly, sleep duration had a weak inverse relationship with salivary cortisol, testosterone and T/C ratio after waking. Also, muscle soreness was negatively in relation to testosterone and T/C ratio after waking up. (Serpell et al. 2019.)

4 HEART RATE VARIABILITY

Heart rate (HR) is regulated by the autonomic nervous system. The balancing of parasympathetic and sympathetic nervous system maintains the physiological homeostasis of the body and its systems. The cardiovascular regulation center in the medulla receives sensory information and regulates the activation of sympathetic and parasympathetic nervous systems. (Shaffer et al. 2014) Heart rate variability (HRV) is the variation of time between consecutive heart beats, measured from the time between consecutive R–R-intervals. (Task Force 1996) Measuring HRV is a non-invasive method of estimating the alterations of the autonomic nervous system and it is affected sensitively independent of individuals consciousness (Kaikkonen 2015). When simplified, parasympathetic activation increases HRV, while sympathetic activation decreases it (Ernst 2017).

4.1 Heart rate variability as physiological marker

Sinus node (SA) is responsible for starting the contraction of the heart by sending action potential to the atriums and ventricles by the cardiac conduction system. Without any regulation from the autonomic nervous system, HR is around 107 bpm for 20-year-old individuals and decreases with age. (Ernst 2017; Shaffer et al 2014) A parasympathetic nerve regulating the heart is called the vagus nerve, which slows down the firing of sinus node by releasing acetylcholine. (Hall 2016, 128) Heart rates lower than 107 are a sign of parasympathetic activation, which decreases HR normally to average of 75 bpm at rest. Both parts of the autonomic nervous system are, however, activated at rest. (Shaffer et al. 2014) HRV has been seen as an indicator of the vagus nerve activation by its increased values. (Laborde et al. 2017)

Epinephrine and norepinephrine mediate the sympathetic influences on HR. The effect on HR is the acceleration of the slow diastolic depolarization, which increases HR. (Task Force 1996) The mediating system for sympathetic stimulation is the intrinsic cardiac nervous system that targets the SA and atrioventricular (AV) nodes and the heart muscle. Due to the increased sympathetic activity, depolarization of the nodes increases accelerating HR and enhancing the contractility of the atria and ventricles. (Shaffer et al. 2014)

The parasympathetic and sympathetic effect dispatch and duration are altered dependent of their ways of influencing HR and HRV. Acetylcholine is hydrolyzed rapidly in SA, since it is rich in acetylcholinesterase and, therefore, vagal effects are brief. Parasympathetic responses are, however, likely to have dominant influence, in comparison to sympathetic, since release of norepinephrine is reduced in response to parasympathetic release of acetylcholine and the cholinergic attenuation of the response to an adrenergic stimulus. (Task Force 1996) Sympathetic effects to HR and HRV occur with a delay of approximately of 5 seconds, whereas parasympathetic effects take less than one second, but they can be observed for longer periods of time (Shaffer et al. 2014; Task Force 1996). The effect reaches its steady state in 20–30 seconds if the stimulus is conscious (Shaffer et al. 2014).

Age, genetics, gender, training background and posture have been found to cause differences in HRV. (Kaikkonen 2015) Overall health and HRV have been agreed to have connections between each other and decreased HRV is considered to be pathological and connected to decreased life expectancy (Ernst 2017). Heart rate variability and functioning of the autonomic nervous system is in relation to different psychological and physical stress factors. HRV-based stress factors have been studied to increase at the same time with subjective stress. (Föhr et al. 2017)

4.2 Measuring heart rate variability

Evaluation of heart rate variability can be made in different time periods, for example at rest, during exercise or during sleep. Parasympathetic activity is higher during sleep, and it has been recorded with HRV being increased during all sleep phases excluding the rapid eye movement (REM) sleep phase, in comparison to rest before falling asleep. Also, the outside stimulus has lesser of an effect on the autonomic nervous system. (Kontos et al. 2020) Recordings are often made with the first 4 hours of sleep, 30 minutes after falling asleep (Myllymäki et al. 2011; Nummela et al. 2010). However, averages of whole night or different segments down to 5 minutes can be observed as well. The beginning of sleep presents usually optimal data because of the higher rate of deep sleep, and the stress factors have decreased impact in comparison to hours awake. Lesser movements make HR and breathing cycle more stable. All in all, the parasympathetic nervous system is most active at this time and the changes to HRV are possible to be recorded. (Brandenberger et al. 2005)

Heart rate variability analysis can be divided to different measurement types: time and frequency domain methods are the most known ones (Shaffer et al. 2014). Time domain methods are based on determining HR at any point in time or the intervals between successive normal complexes (Task Force 1996). Time domain methods for analyzing HRV are simple to calculate and can be compared between different researchers, since it is always calculated similarly. However, frequency domain methods provide more accurate data to quantify autonomic dynamics or determine the rhythmic or oscillatory activity excited by different physiological control systems. (Shaffer et al. 2014) Measurements for heart rate variability are usually made in 5-minute segments, but to assess more diversely of the autonomic nervous responses in normal life, 24-hour recordings are applied (Kleiger et al. 2005; Task Force 1996).

Frequency domain method or power spectral density (PSD) analysis evaluates how power distributes as a function of frequency. The frequencies chosen or parameters are classified to total power, VLF (very low frequency, ≤ 0,04 Hz), LF (low frequency, 0,04 – 0,15 Hz), HF (high frequency, 0,15 – 0,4 Hz) and parameters derived from these. (Task Force 1996) Like time domain methods, different frequency parameters are studied to demonstrate different autonomic nervous system reactions (Shaffer et al. 2014). For example, LF has been suggested to reflect baroreflex signals or sympathetic excitation and HF parasympathetic or vagal activity (Maller et al. 1991; Shaffer et al. 2014).

Traditionally the simplest method of measuring HRV is the standard deviation of the time between two normal R-peaks in ECG (SDNN). One of the most common methods from the time domain methods is the square root of the mean squared differences of successive NN (RMSSD). Other traditionally used time domain methods derived from interval differences are SDANN (standard deviation of an average NN interval), NN50 (number of interval differences of successive NN intervals greater than 50 ms and pNN50 (proportion derived by dividing NN50 by the total number of NN intervals). It is also possible to convert NN intervals into geometric patterns. (Task Force 1996.)

Most of the carry-on commercial device report RMSSD to analyze and provide information about HRV, since it has been identified as an indicator for parasympathetic response to stress (Kinnunen et al. 2020; Stone et al. 2021). The steps to create the variable includes calculating each successive time difference between heartbeats in milliseconds first, then squaring each of

the value and averaging before the square root of the total value is achieved. From time domain methods RMMSD is seen as a method to estimate vagally mediated changes in HRV. (Shaffer et al. 2014) High correlation between RMSSD and HF has also been found (Kleiger et al. 2005).

Pulse rate variability. The golden standard for measuring HRV is traditionally electrocardiography (ECG). Oura ring (Ōura Health Oy, Oulu, Finland) measures HR and HRV with photoplethysmography (PPG) using infrared light from finger arteries (figure 6). It has been studied to correlate well with the ECG-measures during sleep and when there is no movement of the body, and it might present more reliable data than wrist PPG. It is lightweight, discreet and easy to use, since it appears like ordinary ring. The commercial use of Oura for athletes and non-athletes is popular and it is used in studies as well. (Kinnunen et al. 2020)

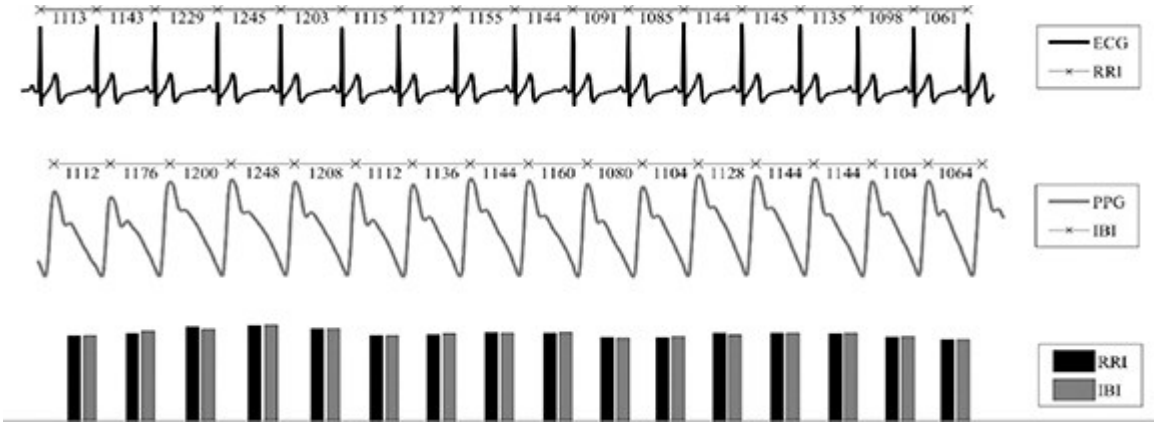


FIGURE 6. Differences of ECG and PPG information. Top panel presents beat-to-beat heart rate measured with ECG and RR-intervals (RRI), middle-panel PPG-signal and interbeat intervals (IBI) and bottom panel RRI and IBI tachograms. (Kinnunen et al. 2020)

4.3 Heart rate variability and endurance training

During exercise the metabolic demands of the tissues and cells in working muscles increases. Blood flow needs to increase as a response to this demand by enhancing cardiac output by higher heart rate and/or stroke volume. (McArdle et al. 2015, 344) While HR increases due to sympathetic nervous system activation, HRV decreases (Ernst 2017). HRV recovers after exercise dependent of the intensity of the exercise and the parameter in question. The recovery of HRV can be viewed to some point 5–30 minutes after exercise. (Kaikkonen et al. 2008)

Even though HRV recovers fully or partially during a 30 minute period after exercise (Kaikkonen et al. 2008), single training session have been found to decrease HRV on the following night or nights (Hynynen et al. 2010; Nuuttila, et al. 2022; Yoshida et al. 2018). Not all studies conclude the same: Kaikkonen (2015) presented constant values of nocturnal HRV (RMSSD) after two different interval and two different continuous exercise training sessions in comparison to previous night of the training.

Endurance athletes usually present lower HR and larger heart stroke volumes at rest. The reasons behind this are increased parasympathetic vagal tone and decreased sympathetic drive. In addition, the blood volume and the myocardial contractility and compliance in the left ventricle is increased. (McArdle et al. 2015, 344) Furthermore, endurance training has concluded to enhance vagal activity and heart rate variability (Nummela et al. 2010; Nummela et al. 2016; Pichot et al. 2000). Also, subjects with moderate level (4–6 h/week) of aerobic training have been found to present higher RMSSD and HF power in HRV compared to the subjects training less or higher amounts (<18 h/week) (Buchheit et al. 2004). Due to enhanced VO_{2max} HRV has been found to increase in sedentary training group (Nummela et al. 2016).

4.4 Heart rate variability and training load

It has been thought that the higher the total training load is, the more the nocturnal HRV is affected (Hynynen et al. 2010). With moderately training athletes HRV has agreed to be increased, whereas with high training loads HRV indices have been found to be decreased (Plews et al. 2013). Nocturnal HRV has been found to be more decreased after marathon than after moderate endurance training session. The intensity, duration and rating of perceived load (RPE) were higher in marathon than the training session. (Hynynen et al. 2010)

The intensity of exercise has been found to be important factor for acute decrease in HRV (Cottin et al. 2004; Martinmäki 2009; Nuuttila et al. 2022). The overall decrease of HRV is studied to be higher if exercise intensity is over the ventilatory threshold (Cottin et al. 2004). Shorter maximal tests of 3000-meter runs have also been found to decrease HRV and increase HR on the following night (Nuuttila et al. 2022).

However, also duration of exercise might present a role in changes in HRV. In a study done by Myllymäki and colleagues (2011), changes in different endurance exercises intensities did not show changes in nocturnal HRV variables. Instead, significant effect on nocturnal RMSSD variables was shown with different exercise durations. Nocturnal RMSSD was lower after 90-minutes exercise session than after a control day. On the other hand, change in duration between 30, 60 or 90-minutes showed no significant changes in nocturnal HRV parameters. (Myllymäki et al. 2011).

When training on higher loads for prolonged time, HRV is decreased, but after tapering period there is usually an increase in HRV (Pichot et al. 2000; Plews et al. 2013). If the higher load training continues and there is no tapering period or the deloading is not sufficient, overtraining can occur. Even though nocturnal HRV has been thought to decrease in response to overtraining (Baumert et al. 2006; Hynynen et al. 2011), not all studies conclude likewise (Hynynen et al. 2006). There are studies showing increased, decreased and no change in HRV due to overtraining (Plews et al. 2013). Hynynen and colleagues (2006) found no differences in HRV during sleep in overtrained athletes. Yet the overtrained showed lower HRV parameters after awakening than controls.

5 RELATIONSHIP BETWEEN TESTOSTERONE, CORTISOL AND HEART RATE VARIABILITY

The hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) are the two main pathways affecting the testosterone, cortisol levels and cardiovascular functions. These physiological pathway systems are highly coordinated and interconnected. Since HPA axis regulates the secretion of testosterone and cortisol and ANS regulates HR and therefore HRV, it may be justified to expect connections between these parameters. (Kim et al. 2018)

Supporting theory of vagal outflow fluctuations connecting with hormones controlled via hypothalamic-pituitary-adrenal axis Mishica and colleagues (2021) found that cortisol and HRV showed significant negative correlation in some time points in young male endurance athletes. Those points were the ones that did have the lowest nocturnal HRV and the highest morning salivary cortisol. They were in moderate relationship during other time points of the viewed training period. (Mishica et al. 2021) In addition, total power and HF have been studied to correlate positively with serum testosterone level and testosterone/cortisol ratio (Nuuttila et al. 2017).

Physical stress and training load have been suggested to lead to lower nocturnal HRV on following night(s) due to decreased parasympathetic activity (Hynynen et al. 2010; Nuuttila, et al. 2022; Yoshida et al. 2018). Also, stress has been found to increase cortisol levels (Hayes et al. 2015; Hough et al. 2021; Li et al. 2015). HRV while standing have been found to correlate negatively with T/C ratio (Huovinen et al. 2009). In addition, lower nocturnal heart rate variability might have a relationship to high morning salivary cortisol level (Mischica et al. 2021). However, opposite results of high salivary basal T/C correlating negatively with daytime resting HRV have been found (DeBlauw et al. 2021).

Huovinen and colleagues (2009) found out that testosterone to cortisol ratio was correlated with HRV indices (SDNN, absolute high frequency power and high frequency power measured in normalized units) measured at standing position after awakening. Heart rate did further correlate negatively with T/C ratio. This leads to suggestions of correlation between increased parasympathetic activity and T/C ratio. (Huovinen et al. 2009.)

Not suggesting to direct correlation, but still showing some connection between HRV and cortisol, Nuuttila and colleagues (2017) found more stable cortisol values with HRV-guided training. At the same time controls did decrease their cortisol levels during training period. Also, HRV guided group did increase their testosterone from the middle phase to the end of training period. Testosterone levels were correlated with nocturnal HRV indices in some time points. Also, higher HRV and serum testosterone concentration were found in the HRV-guided group. (Nuuttila et al. 2017.)

6 PURPOSE OF THE STUDY

The purpose of this thesis was to study if a change in training load affects hormonal values of salivary testosterone and cortisol during endurance runner's preparatory season. In addition, the possible differences in heart rate variability in comparison to changing training load was studied. Finally, associations between the differences in hormonal values and heart rate variability were studied.

Question 1. Are morning and evening salivary concentrations of testosterone and cortisol altered during the basic training period of male endurance runners when comparing light and hard 4-day periods of training load?

Hypothesis 1. Morning and evening values of testosterone and cortisol will not change unless the training load is very strenuous. In previous study, there have been no changes in morning salivary testosterone during training period (Filaire et al. 2001). Neither did morning testosterone correlate with training load (Gomes et al. 2013; Hough et al. 2015; Kamarauskas et al. 2022; Tiernan et al 2019). Evening values of testosterone might change with training load more likely than morning values since daytime and afternoon values have been found to react to high intensity training period even though morning values did not (Filaire et al. 2001). However, single exhaustive exercise has been found to have a decreasing effect on testosterone 48-hours after the exercise (Anderson et al. 2016).

In previous studies there have been no relationship with training load and resting cortisol values (Kamarauskas et al. 2022; Tiernan et al. 2019). However, if endurance training was exhaustive, the cortisol values were altered up to 24 hours after the exercise (Anderson et al. 2016). Also, differences in CAR have been recorded in relation to training load (Anderson et al. 2018). However, results of morning values of cortisol correlating with training load have been found (Gomes et al. 2013).

Question 2. Is nocturnal heart rate variability altered during the basic training period of male endurance runners when comparing light and hard 4-day periods of training load?

Hypothesis 2. Nocturnal HRV will decrease in the high training load week. Single training session have been found to decrease HRV on the following night/nights (Hynynen et al. 2010;

Nuuttila, et al. 2022; Yoshida et al. 2018) Also, in a study by Pichot and colleagues (2000) nocturnal HRV was decreased gradually during higher training load period and then increased during lighter training week. Therefore, if the high training week included more training sessions or more intense training it can be expected that HRV is decreased due to these factors.

Question 3. Is overnight heart rate variability difference between light and hard 4-day periods of training load correlated with testosterone and cortisol concentration differences?

Hypothesis 3. Heart rate variability correlates with testosterone positively and cortisol negatively. Serum testosterone and T/C ratio have been studied to correlate with HRV (Nuuttila et al. 2017). Nocturnal heart rate variability has been studied to correlate with morning cortisol if the values showed signs of low recovery (Mishica et al. 2021).

7 METHODS

The measurements and data analysis for the study were conducted in cooperation with Summa Labs and Finnish Athletics Federation in Pajulahti Olympic Training Center. Study measurements were completed between November 2022 – May 2023 as a part of a study assessing individual hormonal responses and heart rate variability for developing athletic training (SPORT2022**)**. The methods and protocol of this study received a statement by the Ethics Committee of Human Sciences of the University of Jyväskylä.

7.1 Study protocol

Participants completed their normal training mesocycle, which normally consists of three high load weeks followed by a recovery period. The measurements were performed during the preparatory training season of each athlete's personal training program.

Participants were measured daily throughout the training period for HRV, sleep and daily activity, salivary hormone samples (wakening and bedtime) and training load (TRIMP) (figure 7). They also filled training diary to phone app (Summa Labs, Oulu, Finland) or to paper as they used to do as a part of their normal training. A health questionnaire (appendix 1) was performed for screening of health issues before the training period. Running test was also performed before the first week and after the high loading period (after week 4). For practical reasons, the latter group of athletes did not perform running test.

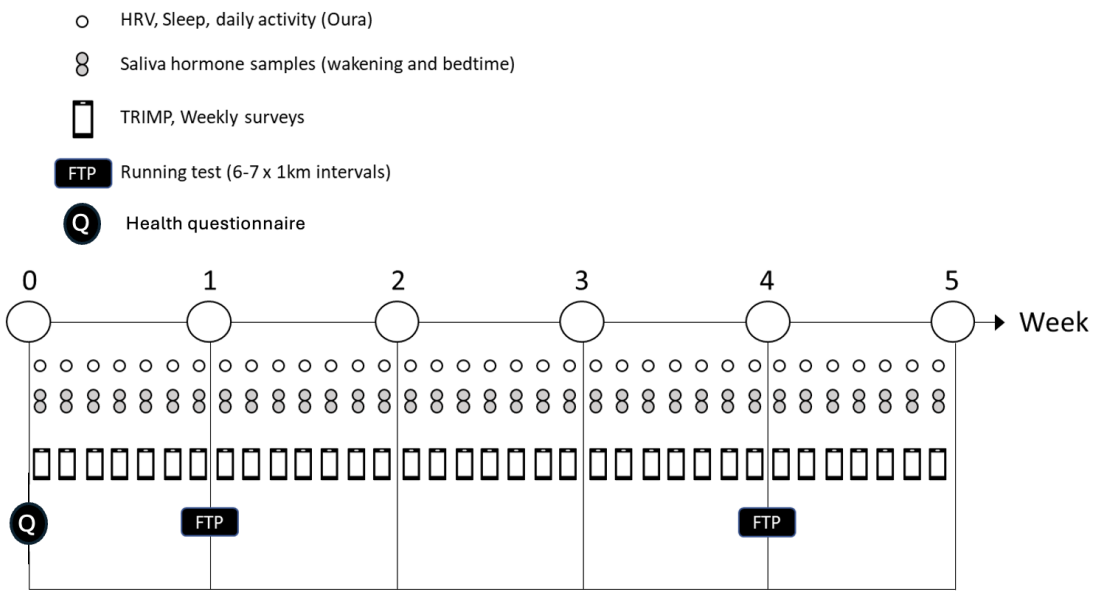


FIGURE 7. Study design in SPORT2022.

Post-hoc analysis included determining 4-day periods of high (HT) and low (LT) training load. Criteria for analysis were availability (at least one value of each) of heart rate data from training, evening hormones and overnight HRV (RMSSD). If there were more than one period with the same lowest or highest value of $TRIMP_{hr\_zone}$ the defining factor was HRV value from the period. Sick days were outlined if body temperature was increased 0.5 °C or more (measured with Oura ring (Ōura Health Oy, Oulu, Finland)).

Values for 4-day period were averaged. The HT and LT periods were then matched with averaged values of RMSSD, evening and morning hormones in the following order: first day of the period of high TRIMP average, following night HRV and hormones of the next day (figure 8).

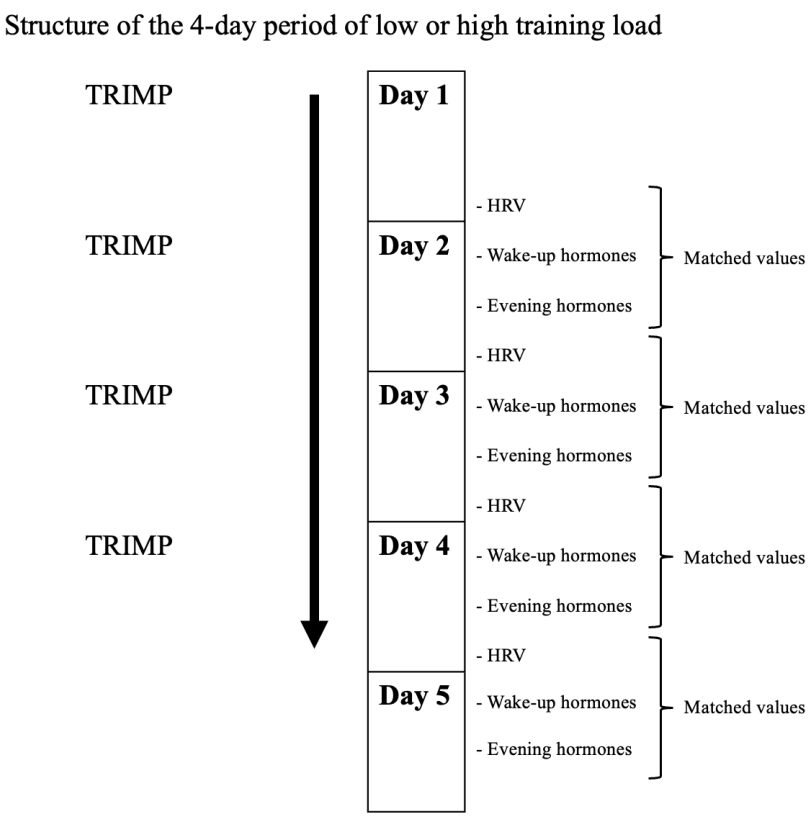


FIGURE 8. Visualized structure of the chosen 4-day periods for analysis. HRV = heart rate variability, TRIMP = training stress to training impulse.

7.2 Participants

Study subject group consisted of 20 male endurance runners (n = 20). Study participation was voluntary, and it was possible to stop participation at any point of the study. Participants were recruited as a part of the study of SPORT2022 conducted in the University of Jyväskylä. Twelve of the participants participated in the measurements between November 2022 and January 2023. Rest of the group (n = 8) were followed between March and May in 2023. The athletes were recruited with criteria of being elite level runners or triathlonists with approximately 600 yearly training hours and age of 35 ± 10 years.

The characteristics of participants are presented in table 1. All athletes were middle- or long-distance runners and participated in the national team training. The participants were aged between 17–33 years and had BMI (body mass index) between 18.6 – 22.3 kg/m^2.

TABLE 1. The anthropometrics of the participants (n = 20).

	Age (years)	Height (cm)	Body mass (kg)	BMI (kg/m^2)
Mean	22.7	181.0	66.8	20.4
SD	5.8	7.1	7.2	1.2

SD = standard deviation, BMI = body mass index

7.3 Hormone samples

Saliva hormone samples were collected each morning and evening. Samples were collected by the subjects themselves during the training period and by study personnel during running test protocol. The awakening sample was instructed to be taken 5 minutes after awakening and the evening sample 30 minutes before going to sleep. In addition, participants collected samples before exercise, after exercise and during the running test. The sample tubes were then scanned and registered to a phone app (Summa Labs, Oulu, Finland). Appendix 2 presents the instructions given to subjects for salivary measurements.

Subjects were informed to collect the sample from free-flowing saliva by a passive method, keeping an oral swab under the tongue until it is completely wetted. Before the sample collection the subjects were instructed to avoid teeth brushing, smoking, eating and drinking anything but water for 30 minutes. Also, teeth brushing, and hot beverages were instructed to be avoided two hours prior for preventing gum bleeding.

Samples were collected from the mouth cavity using Salimetrics Oral Swab (Demeditec Diagnostics, Kiel, German) and then refrigerated after collection. The samples were moved into the laboratory with iceboxes in sub-zero temperatures. Samples were assayed in duplicate using commercial Cortisol Free and Testosterone Free in Saliva ELISA -kits (Demeditec Diagnostics, Kiel, German). All sample preparations and manipulation were done according to the manufacturers kit protocol. Inter- and intra-assay coefficient variation for cortisol were < 10% and < 7%, respectively. Analytical sensitivity was 0.019 ng/ml. For testosterone inter- and intra-assay coefficient variation were < 9% and < 9%, respectively. Sensitivity was 6.1 pg/ml.

7.4 Heart rate variability

Nocturnal heart rate variability (HRV) was measured each night with third generation Oura ring (Ōura Health Oy, Oulu, Finland). The ring was instructed to be worn every night during the study period. The HRV data was collected and analyzed as RMSSD. The period of analysis constructs from whole night average RMSSD measured after 30-minutes from falling asleep. Sleeping time is determined from the accelerometer data of the ring.

Oura measures PPG signal from finger arteries and capillaries with infrared light sensors (900 nm) on 250 Hz frequency. The analysis contains following steps: Maximum and minimum values of the timing of each heartbeat is located using real-time moving average filter. Then each interbeat interval (IBI) is marked normal or abnormal by median filters. Each included IBI must have two previous and following normal IBI values. Then 5-minute samples are averaged if over 30% of the data is accepted by the previous criteria. After that the average for whole night can be constituted. (Kinnunen et al. 2020)

7.5 Training load

Training load was measured by using the training zones and time spent on them during training. For the athletes, who were using heart rate monitors, training zones were calculated directly from the available data. For the athletes, who were only tracking their practicing through training diaries, the zones were assumed based on the speeds and corresponding HR zones with the speed, determined by the running test. Heart rate data was collected with athlete's own devices. Eleven (11) of the athletes were using heart rate monitors by Polar (Polar Electro, Kempele, Finland) and one had Garmin (Garmin Nordic, Vantaa, Finland).

Training load was calculated as $TRIMP_{hr\_zone}$ that used Banisters (Wallace et al. 2014) calculations as a base. Due to the different input for training load, training zone specific TRIMP ($TRIMP_{hr\_zone}$) was calculated. The time spent in each of the five zones (51–60, 61–70, 71–80, 81–90, 91–100%) was defined. Individual factor was applied to each zone using average HR in the middle of the zone (except for the fifth zone, where it was assumed that the training was more on the lower area of the zone) (0.55, 0.65, 0.75, 0.85 and 0.92). Because all the subjects

were men, the sex related weighting factor 1.92 was applied (Wallace et al. 2014). $TRIMP_{hr\_zone}$ was presented as 4-day period weighted averages from the whole study period.

7.6 Running test

Running test was conducted for the first dataset with study personnel and for the second dataset test was instructed to be performed on their own. Test was conducted on a running track, and it included running 1000 meters with starting speed of 11 km/h and then increasing the speed every 1000 meters until the subject could not keep up with the speed. Salivary samples for hormones were taken 30-minutes before, before, after every 1000 meters, immediately after and 30 minutes after the end of the test. Lactate samples were taken before, after every 1000 meters and immediately after test.

7.7 Statistical analysis

Significant difference between two training load periods was analyzed with IBM SPSS Statistics 26 -program (International Business Machines Corporation, Armonk, USA). Normality of the $TRIMP_{hr\_zone}$ was tested using Kolmogorov-Smirnov and Shapiro-Wilk -test for normal distribution. Since the samples were not normally distributed, independent samples Mann-Whitney U-test was applied to test the differences between training load during LT and HT.

The rest of the data were analyzed using statistical program R **(**The R Foundation, Vienna, Austria) and the results are expressed as mean ± standard deviation. Mann-Whitney U-test was applied to test the differences between hormonal and HRV values in LT and HT. Same test was applied for the changes in hormones and HRV between HT and LT. Changes in RMSSD and hormones were calculated as relative changes ((HT RMSSD – LT RMSSD / LT RMSSD) x 100 %). Correlations between evening and morning hormones and HRV were analyzed with Pearson correlation analysis. Significance of correlation, means and standard deviations were calculated in Office 365 Excel (Microsoft Corporation, Redmont, WA, United States) with 2-way student's t-distribution test and 95 % confidence interval. Statistical significance was set at $p < 0.05$.

8 RESULTS

Low training load period (LT) and high training load period (HT) were successfully determined for all participants. Values for RMSSD and evening hormones (eTes, eCor, eT/C) were collected successfully from all participants (n = 20). For awakening values (wTes, wCor, wT/C), two participants had missing values (n = 18).

8.1 Training load

Training load ($TRIMP_{hr\_zone}$) (mean ± SD) was 160 ± 58 on HT and 39 ± 3 on LT. Training load was statistically significantly different between two selected 4-day periods ($p < 0.001$). Low training period included 1.6 ± 1.2 training sessions and 0.5 ± 0.8 recovery training sessions. High training period consisted of 3.5 ± 0.5 training sessions and 0.2 ± 0.4 recovery training sessions. Training was reported to be mostly aerobic training.

8.2 Hormones

Mean (± SD) salivary free testosterone values were 509 ± 125 pmol/l (wTes) and 255 ± 98 pmol/l (eTes) during the LT period and 501 ± 120 pmol/l (wTes) and 233 ± 70 pmol/l (eTes) during the HT period, respectively. Salivary free cortisol values were 34.8 ± 15.1 nmol/l (wCor) and 4.0 ± 2.2 (eCor) during the LT and 33.1 ± 11.2 nmol/l (wCor) and 3.9 ± 2.0 nmol/l (eCor), respectively. Responding values and T/C ratio are presented on table 2.

The relative difference between LT and HT for testosterone values were -0.5 ± 18.5 % (wTes) and -1.9 ± 30.0 % (eTes). For cortisol values the respective differences were -4.0 ± 33.5 % (wCor) and 19.9 ± 71.3 % (eCor). The values of T/C ratio are presented on table 2. Absolute individual values of evening cortisol are presented on figure 9. There were no statistically significant differences in the studied hormone levels between LT and HT for following day awakening or evening values ($p > 0.05$).

TABLE 2. Hormonal values, heart rate variability (HRV, RMSSD) and training load ($TRIMP_{hr\_zone}$) during low (LT) and high (HT) training load periods. All values are daily averages. * Statistically significant difference between the values ($p < 0.001$).

	LT		HT	
	Awakening	Evening	Awakening	Evening
Tes (pmol/l)	509 ± 125	255 ± 98	501 ± 120	233 ± 70
Cor (nmol/l)	34.8 ± 15.1	4.0 ± 2.2	33.1 ± 11.2	3.9 ± 2.0
T/C	18 ± 8	86 ± 56	19 ± 9	78 ± 37
RMSSD (ms)	87 ± 34		87 ± 35	
$TRIMP_{hr\ zone}$	39 ± 33 *		160 ± 58 *	

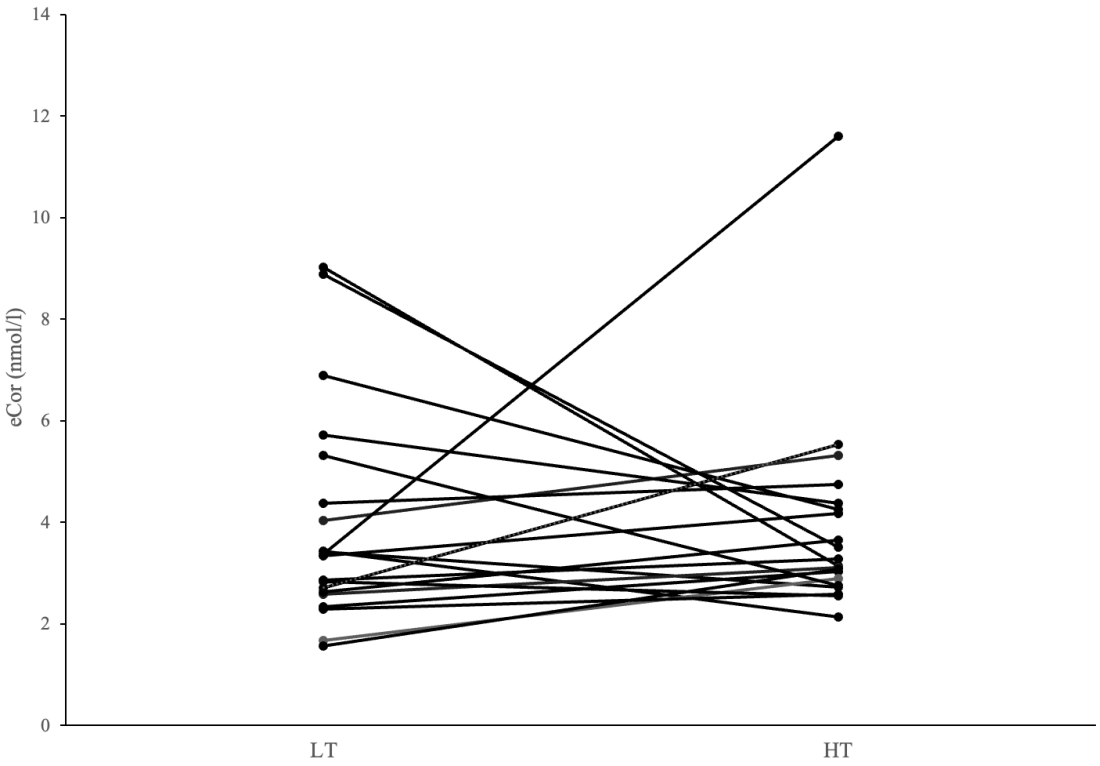


FIGURE 9. Individual differences in absolute evening cortisol (eCor) values during the low (LT) and high (HT) training load periods.

8.3 Heart rate variability

Mean (± SD) heart rate variability (RMSSD) was 87 ± 34 ms during LT and 87 ± 35 ms during HT. There was no statistically significant difference in the nocturnal RMSSD values between LT and HT ($p > 0.05$).

8.4 Relationships between hormone values and heart rate variability

There were no correlations between relative differences of heart rate variability (RMMSD) and hormonal values (wTes, eTes, wCor, eCor, wT/C & eT/C) between LT and HT. Differences between hormonal values and the correlations with RMSSD are presented in table 3.

Correlation coefficient between RMMSD and eTes was 0.345 ($r^2 = 0.119$), while RMSSD and eT/C ratio related with a correlation coefficient 0.248 ($r^2 = 0.062$). However, the correlations were not statistically significant ($p > 0.05$). Figures 10 and 11 present the correlations between these differences.

TABLE 3. Relative differences in heart rate variability (HRV, RMSSD) and hormonal values between low and high training load periods and correlations between HRV and hormonal value differences. Awakening testosterone (wTes), evening testosterone (eTes), awakening cortisol (wCor), evening cortisol (eCor), awakening testosterone to cortisol ratio (wT/C) and evening T/C ratio (eT/C).

	Difference in HRV (avg %)	Difference in hormone (avg %)	Correlations			
			Correlation	r^2	p	n
RMMSD & wTes	1.4 ± 20.2	-0.5 ± 18.5	0.157	0.025	0.53	18
RMMSD & eTes	1.4 ± 20.2	-1.9 ± 30.0	0.345	0.119	0.14	20
RMMSD & wCor	1.4 ± 20.2	-4.0 ± 33.5	-0.185	0.034	0.46	18
RMMSD & eCor	1.4 ± 20.2	19.9 ± 71.3	-0.061	0.004	0.80	20
RMMSD & wT/C	1.4 ± 20.2	13.9 ± 64.5	0.077	0.006	0.76	18
RMMSD & eT/C	1.4 ± 20.2	13.8 ± 69.6	0.248	0.062	0.29	20

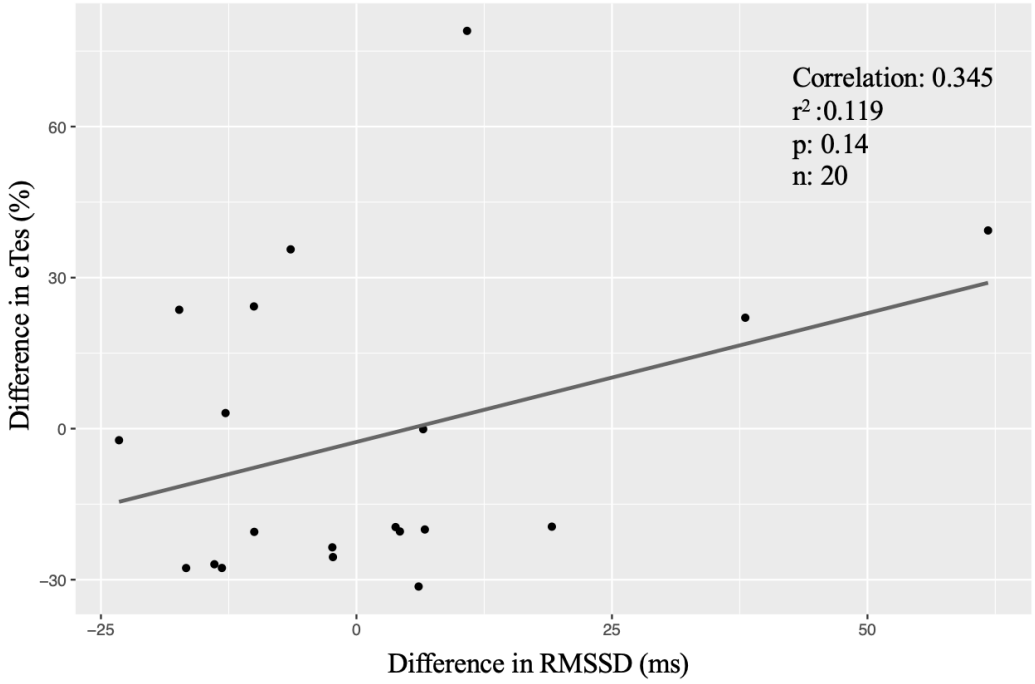


FIGURE 10. Correlation of differences between low (LT) and high (HT) training load periods in evening testosterone (eTes) and HRV (RMSSD). $p > 0.05$

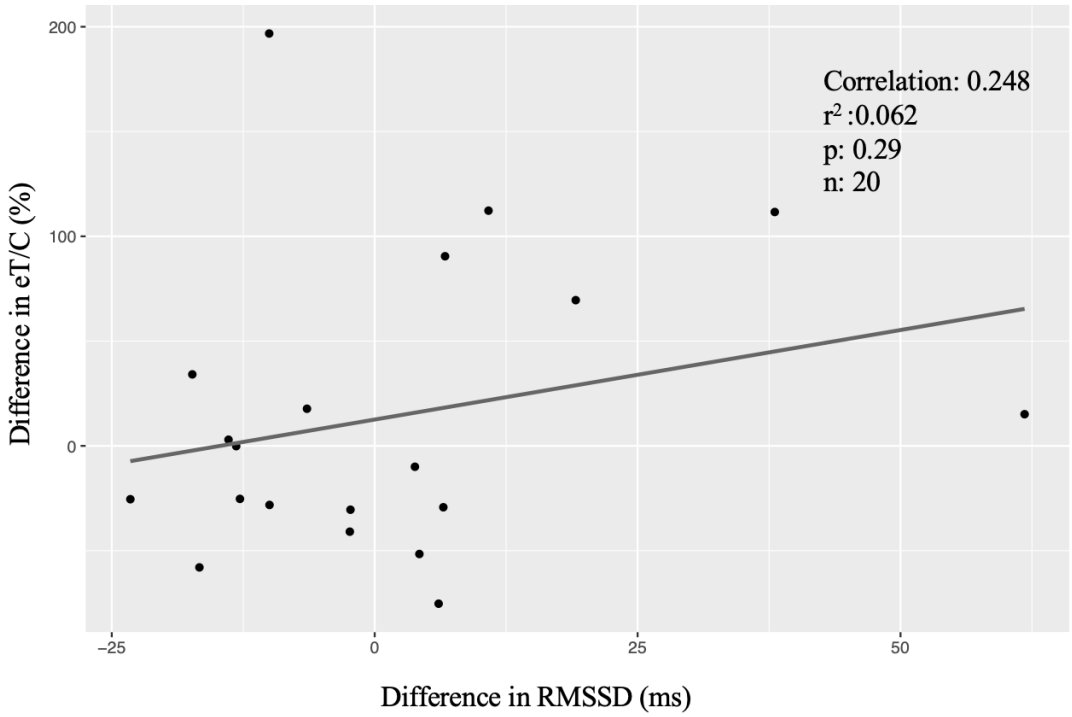


FIGURE 11. Correlation of differences between low (LT) and high (HT) training load periods in evening testosterone to cortisol ratio (eT/C) and HRV (RMSSD). $p > 0.05$

9 DISCUSSION

The main goal for this thesis was to study if a change in training load affects hormonal values of salivary testosterone and cortisol during endurance runner's preparatory season. The purpose was to evaluate if training load and recovery could be evaluated by measuring the anabolic/catabolic hormones. In addition, the possible differences in heart rate variability in comparison to changing training load was studied. Finally, associations between the differences in hormonal values and heart rate variability were studied.

There were no differences in awakening or evening testosterone or cortisol (wTes, eTes. wCor, eCor), or testosterone to cortisol ratio (wT/C, eT/C) between the training load periods. Neither there were differences in nocturnal heart rate variability (RMSSD). No correlations were found in between hormonal value or heart rate variability differences.

9.1 Hormonal differences between high and low training load period

There were no statistically significant differences between the salivary cortisol and testosterone between the high (HT) and low (LT) training 4-day periods. There was no difference between the values measured on awakening or in the evening. There was a decline in both hormones from morning to evening.

Previous studies have shown that volume of training might not be the most definitive factor for training induced responses in heart rate variability, testosterone and cortisol values (Cottin et al. 2004; Martinmäki 2009; Nuuttila et al. 2022; Sato et al. 2015; Tauler et al. 2013). Single exhaustive exercise has been found to influence hormonal values more likely in comparison to other types of exercises (Anderson et al. 2016; Lac & Berthon 2000; Sato et al. 2015). The earlier research of endurance training during preparatory season suggests that there was probably no high amount of training with high intensities or to exhaustion (Seller 2010). Due to the uncertainty and probability of no high intensity training during the study period, it is not that surprising that there were no differences in the hormonal values either.

Other possible explanation for indifferent hormonal values might be, that the individual responses were different between the study subjects. In a study by Nuuttila and colleagues

(2017) there were decreased testosterone values in the middle of training period including high and moderate intensity training in comparison to values before the training. Further on the training block, the values increased. (Nuuttila et al. 2017) Similarly, different exercises have been studied to have increasing and then decreasing cortisol values during the recovery from the exercise session (Bobbert et al. 2005; Lac & Berthon 2000; Wahl et al. 2013). Due to these effects, actual responses from individual training sessions might not be visible in this kind of study structure. Also, the delay for the hormonal value measurements were chosen as one day. Different kind of delays might present different results. It is however difficult to study more delayed periods since the following training might affect the latter hormonal values.

If some of the study participants were doing high intensity training leading to higher values and some exhaustive training causing lower values in both hormones, there would be no statistical differences and higher deviation in the hormonal values. Interestingly, the standard deviation (SD) was lowered for both hormones, especially in the evening values (table 2**,** figure 9). The reasoning for this might be other stress that caused especially the cortisol to have higher variation for the LT period (figure 9). Cortisol reactions to physical and psychosocial stress have been found to be diminished by more physical activity (Klaperski, et al. 2014; Sato et al. 2015; Wood et al. 2018). In table 3, the difference in evening cortisol (eCor) has presented to be 19.9 ± 71.3 %, but due to one subject that had very high values (shown in figure 9), the actual mean of differences with other subjects was not convergent. Neither there were statistically significant differences in the evening values of cortisol.

For measuring the awakening values, the timing of the measurement time has been studied to present important effects, since the cortisol awakening response (CAR) causes major changes in hormonal values in a short period of time (Adam and Kumari 2009; Pruessner et al. 1997). The subjects were instructed to take the samples 5 minutes after awakening, but the timing was not confirmed. In a previous study by Anderson and colleagues (2018), the actual response was found to have a relationship with training load more often than individual value in the morning. Using the CAR instead of individual values in the morning would be justified in the further research.

Suggestions of more valuable information on the metabolic state of the body is made by measuring the testosterone cortisol ratio instead of individual values **(**Cadegiani & Kater 2017). However, in this analysis, T/C did not present different results from the anabolic/catabolic state

of the body. Reasoning for this is probably due to the indifferent values of testosterone and cortisol during the study period. Therefore, verdict on the claim cannot be done either. Neither there were differences in the hormonal values between low and high training in none of the samples. Therefore, it was assumed that there were no differences in the daytime decline either. Further interesting follow up study question could be, however, studying the awakening to evening decline differences in between the low or high training periods.

9.2 Differences in heart rate variability between low and high training load periods

There were no statistically significant differences in the RMSSD values of heart rate variability in between low and high training load period. In the study hypothesis, there was a suggestion that heart rate variability would have a difference between the two training load periods if the training was strenuous enough.

It is hard to measure or define when training is too strenuous or too light for development of athlete's fitness (Carrard et al. 2022; Impellizzeri et al. 2019). In the analysis of this thesis, there were no subjective stress measures included, which might refer to different recovery state than internal or external measures itself (Foster et al. 2001). However, indifferent hormonal values might also suggest that the training intensity or volume was not high enough for responses in RMSSD. In addition, as mentioned in the earlier section, it is not typical to the preparatory period of training to have high intensity training (Seller 2010). The athletes were also all competing in national or higher level, and therefore have more tolerance for training in relation to effects on responses on the autonomic nervous or HPA axis **(**McArdle et al. 2015, 344**;** Sato et al. 2015)

Even though suggestions of training load effecting on autonomic nervous system function and, therefore, heart rate variability have been made, it is not concluded as reliable tool for diagnosing overtraining (Carrard et al. 2022). There are also studies finding no relationship between heart rate variability and high training load (Plews et al. 2013). In comparison to measurements from nocturnal values, the reaction values to awakening might present additional data to the research (Hynynen et al. 2006).

9.3 Correlations between differences in heart rate variability and hormonal values

Heart rate variability (RMSSD) differences between low and high training load periods did not have correlation with differences in morning or evening testosterone or cortisol values in the same follow-up periods. Two possible explanations for the lack of correlation are suggested. First one is that training load did not have significant effect on the HPA axis of the autonomic nervous system and the variation in values was between the normal life variation. The second suggestion is that the connection between the two pathways is not as strong as expected, or the connection cannot be recorded inside this kind of study structure.

The reasons why there were no statistically significant differences between the two training periods have been presented earlier in the discussion. The same reasons apply mostly to the discussion of why there were no correlations between the variables. When training load did not influence hormonal or HRV values, there are multiple other factors that apply effect on them. Normal life stress, possible recoveries from earlier sicknesses or underlying health factors might present more significant effect on the values.

However, with the suggestion of relationship between the HPA axis and autonomic nervous system, there could be a correlation with hormonal and HRV differences, even though the differences were not caused by training load (Kim et al. 2018). Changes in the autonomic nervous system can be recorded in relatively short amount of time. Especially, parasympathetic nervous system changes happen in a very short amount of time. RMSSD is viewed as an indicator of functions of the parasympathetic nervous system and activation of the vagus nerve and it can be recorded very quickly (Shaffer et al. 2014; Task Force 1996) When both HRV and hormonal values present some variation with increasing and lowering values after different exercises, it might present difficulties in matching the peaks and lows to the same time points that they would present correlations (Anderson et al. 2016; Bobbert et al. 2005; Lac & Berthon 2000; Pichot et al. 2000; Plews et al. 2013; Wahl et al. 2013)

The study structure applied a match of previous night RMSSD to following day hormonal values. The reasoning for the chosen analysis was the suggestion of longer reaction time from the exercise to hormonal values than for HRV. Longer delay was not applied due to the likeliness of interpretation of following exercise training to the hormonal and heart rate variability values. Other possible timing could have been the following order: evening

hormones, nocturnal HRV and awakening hormonal values. However, chosen structure was justified to enable further analysis of the daytime declines, even though it was not proceeded for this thesis.

9.4 Measuring hormonal values from saliva

Studies assessed in the previous review used both serum and salivary hormonal concentrations. However, it is not immaterial where the hormones are measured from. Hormones are secreted to the blood and are diffused into saliva (Hall 2016, 925). Both hormones, testosterone and cortisol diffuse passively from blood circulation to saliva through salivary glands and show a correlation with the free form serum concentration (Gatti & De Palo 2010). Hormonal measurements are traditionally made from serum and often descriptive of the total hormonal concentrations in the bloodstream (Mezzullo et al. 2016).

Measurement from blood serum is invasive and usually requires medical staff. Steroid hormones circulate in the blood mainly bound to plasma proteins and cannot diffuse across the capillaries easily (Hall 2016, 929). Free hormone hypothesis suggests that total hormonal concentrations in serum might not be the most descriptive measure when viewing the hormones available to cells (Narinx et al. 2022). Hypothesis may not hold its ground for all hormones, but proof of evidence for cortisol and testosterone has been found (Theocharidou et al. 2019; Narinx et al. 2022)

Correlations between serum free cortisol and saliva cortisol has been found (Galbois et al. 2010). For testosterone, a validation study for hormonal measures from saliva in comparison to serum concentrations found positive correlation ($r = 0.886$) for males (Mezzullo et al. 2016). Therefore, measurements from saliva offer an easier option and allow daily measurements in the daily life of individuals, since they can also be stored and transported to analysis later (Mezzullo et al. 2016). However, Ljubijankić and colleagues (2008) found cortisol concentrations over 20 times higher in the serum than in saliva. Also, correlation between salivary and serum cortisol and testosterone concentrations have been studied with conflicting results (Adebero et al. 2020; Mezzullo et al. 2016).

9.5 Strengths and weaknesses of the study

Strengths of the study was to study high national level athlete's training and responding hormones and HRV on the daily basis. Since there were no training intervention and measurements were conducted as a part of subject's normal life, the stress of the measurement situation was eliminated. In addition, information of the training load and physiological differences in preparatory season was provided.

In comparison to other studies conducted to study responses in hormonal values, the values were recorded daily or several times a day (Gomes et al. 2013; Kamarauskas et al. 2022). Therefore, the viewed values were conducted from several time points and had lesser interference from individual factors, like physiological stress. For example, this might have blunted the effects of one poorly slept night. However, for the averages of hormonal values and HRV from the 4-day period, only one value was sufficient for analysis. In addition, visual analysis and deleting the outliers in values were not done before the analysis. For example, the evening cortisol (figure 9**)** has one major outlier on the high training load period, that might influence the analysis and values presented.

The main problem of this study was the nature of the training during the study period. In the planning there was a suggestion that there would be a tapering period and high intensity training period. The measurements were conducted during the athlete's normal training and the differences in training intensity changes might have been smaller than expected. This also places uncertainty of measurements being conducted correctly. For example, if subjects went to sleep 30 minutes after saliva sampling or tracked their every training session reliably.

Due to the practical issues, HR monitors were not standardized for the study and there were different products used. Since there is no certainty, if all HR measurements were made with using chest-based ECG or for example with PPG from the wrist band, there might be errors in the training HR data. In addition, for almost half of the participants (n = 9) the HR zones were analyzed from running speeds reported in training. Due to different sources of training load, $TRIMP_{hr\_zone}$ was manually calculated and was not comparable to Banister's or other previously used TRIMP values.

The criteria for the selection of 4-day periods was the availability of evening hormones, TRIMP and HRV from the matching night. However, no morning values were demanded for the analysis, and there was a difference between the available values of morning and evening values (n = 18 & n = 20). The reasoning why this kind of criteria was selected, was the main interest in evening values, and to ensure, that the selection for certain training load period was based on the actual high TRIMP, not the availability of values.

9.6 Conclusions

There were no differences or correlations in testosterone and cortisol or heart rate variability (RMSSD) between low and high training load period. The results from this study suggest that measured values of cortisol and testosterone from saliva present no useful information on measuring endurance athlete's training load during the preparatory season of endurance training. However, the hormonal status and the nature of the training on the preparatory season rather implies on the training impact being too low, to cause effects on the homeostasis of the autonomic nervous system or hypothalamic pituitary axis.

Conclusions of the connections between the hypothalamic-pituitary axis and the autonomic nervous system to the training load cannot be done due to the problematics of the studied data and protocol of the study. Additional study on the topic with more standardized intervention period is regarded.

REFERENCES

Adam, E. K. & Kumari. M (2009). Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology 34 (19), 1423–1436.

Adebero, T., McKinlay, B. J., Theocharidis, A. & Root, Z. (2020). Salivary and Serum Concentrations of Cortisol and Testosterone at Rest and in Response to Intense Exercise in Boys Versus Men. Pediatric Exercise Science 32, 65–72.

Ahokoski, O., Virtanen, A., Huupponen, R., Scheinin, H., Salminen, E., Kairisto, V. & Irjala, K. (1998). Biological day-today variation and daytime changes of testosterone, follitropin, lutropin and oestradiol-17b in healthy men. Clinical Chemistry and Laboratory Medicine, 36 (7), 485–491.

Alghadir, A. H., Gabr, S. A. & Aly, F. A. (2015). The effects of four weeks aerobic training on saliva cortisol and testosterone in young healthy persons. Journal of Physical Therapy Science 27, (7), 2029–2033.

Ambroży, T., Rydzik, Ł., Obmiński, Z., Błach, W., Serafin, N., Błach, B., Jaszczur-Nowicki, J. & Ozimek, M. (2021). The effect of high-intensity interval training periods on morning serum testosterone and cortisol levels and physical fitness in men aged 35–40 years. Journal of Clinical Medicine 10 (10), 2143.

Anderson, T., Lane, A. R. & Hackney, A. C. (2016). Cortisol and testosterone dynamics following exhaustive endurance exercise. European Journal of Applied Physiology 116, 1503–1509.

Anderson, T., Lane, A. R. & Hackney, A. C. (2018). The cortisol awakening response: association with training load in endurance runners. International Journal of Sports Physiology and Performance 13, 1158–1163.

Andre, M. J., Fry, A. C., Luebbers, P. E., Hudy, A., Dietz, P. R. & Cain, G. J. (2018). Weekly salivary biomarkers across a season for elite men collegiate basketball players. International Journal of Exercise Science 11 (6), 439–451.

Balsalobre-Fernández, Tejero-González, C. M. & Campo-Vecino, J. (2014). Relationships between training load, salivary cortisol responses and performance during season training in middle and long distance runners. PLoS One 9 (8), e106066.

Baumert, M., Brechtel, L., Lock, J., Hermdorf, M., Wolff, R., Baier, V. & Voss, A. (2006). Heart rate variability, blood pressure variability, and baroreflex sensitivity in overtrained athletes. Clinical Journal of Sport Medicine 16 (5), 412–417.

Bobbert, T., Brechtel, L., Mai, K., Otto, B., Maser-Gluth, C., Pfeiffer, A. F. H., Spranger, J. & Diederich, S. (2005). Adaptation of the hypothalamic-pituitary hormones during intensive endurance training. Clinical Endocrinology 63 (5), 530–536.

Bompa, T. O. & Haff, G. G. (2009). Periodization: Theory and methodology of training. Fifth Edition. Champaign: Human Kinetics.

Borresen, J. & Lambert, M. I. (2008). Quantifying training load: A comparison of subjective and objective methods. International Journal of Sports Physiology and Performance 3 (1), 16–30.

Borresen, J. & Lambert, M. I. (2009). The quantification of training load, the training response and the effect on performance. Sports Medicine 39 (9), 779–795.

Brandenberger, G., Buchheit, M., Ehrhart, J., Simon, C. & Piquard, F. (2005). Is slow wave sleep an appropriate recording condition for heart rate variability analysis? Autonomic Neuroscience 121 (1-2), 81–86.

Buchheit, M., Simon, C., Piquard, F., Ehrhat, J. & Brandenberger, G. (2004). Effects of increased training load on vagal-related indexes of heart rate variability: a novel sleep approach. American Journal of Physiology: Heart and Circulatory Physiology 287 (6), 2813–2818.

Cadegiani, F. A. & Kater, C. E. (2017). Hormonal aspects of overtraining syndrome: a systematic review. BMC Sports Science, Medicine and Rehabilitation 9, 14.

Cadegiani, F. A., da Silva, P. H. L., Abrao, T. C. P. & Kater, C. E. (2020). Diagnosis of overtraining syndrome: Results of the endocrine and metabolic responses on overtraining syndrome study: EROS-DIAGNOSIS. Journal of Sports Medicine 937819.

Cadegiani, F. A., da Silva, P. H. L., Abrao, T. C. P. & Kater, C. E. (2021). Markers of recovery from overtraining syndrome: The EROS-LONGITUDINAL study. Sports Physiology and Performance (16), 1175–1184.

Carrard, J., Rigort, A-C., Appenzeller-Herzog, C., College, F., Königstein, K., Hinrichs, T. & Schmidt-Trucksäss, A. (2022). Diagnosing overtraining syndrome: A scoping review. Sports Health 14 (5), 665–673.

Casado, A., González-Mohíno, F., González-Ravé, J. M. & Foster, C. (2022). Training Periodization, Methods, Intensity Distribution, and Volume in Highly Trained and Elite Distance Runners: A systematic Review. International Journal of Sports Physiology and Performance (17), 820–833.

Chan, S. & Debono, M. (2010). Replication of cortisol circadian rhythm: new advances in hydrocortisone replacement therapy. Therapeutic Advances in Endocrinology and Metabolism 1 (3), 129–138.

Cook, C. J., Crewther, B. T., Kilduff, L. P., Agnew, L. L., Fourie, P. & Serpell, B. G. (2021). Testosterone and dihydrotestosterone changes in male and female athletes relative to training status. International Journal of Sports Physiology and Performance 16, 1700–1706.

Cottin, F., Médigue, C., Leprêtre, P-M., Papelier, Y., Koralsztein, J-P. & Billat, V. (2004). Heart rate variability during exercise performed below and above ventilatory threshold. Medicine & Science in Sports & Exercise 36 (4), 594–600.

Coutts, A.J., Crowcroft, S. & Kempton, T. (2018). Developing athlete monitoring systems: Theoretical basis and practical applications. In Kellman, M. & Beckmann, J. Sport, Recovery and Performance: Interdisciplinary Insights. Abingdon: Routledge, 19–32.

Coutts, A. J., Reaburn, P., Piva, T. J. & Rowsell, G. J. (2007). Monitoring for overreaching in. rugby league players. European Journal of Applied Physiology 99 (3), 313–324.

DeBlauw, J. A., Crawford, D. D., Kurtz, B. K., Drake, N. B. & Heinrich, K. M. (2021). Evaluating the clinical utility of daily heart rate variability assessment for classifying meaningful changes in testosterone-to-cortisol ratio: a preliminary study. International Journal of Exercise Science 14 (3), 260–273.

Doerr, P. & Pirke, K. M. (1976). Cortisol-induced suppression of plasma testosterone in normal adult males. The Journal of Clinical Endocrinology & Metabolism, 43 (3), 622–629.

Ernst, G. (2017). Heart rate variability – More than heart beats? Frontiers Public Health 5, 240.

Filaire, E., Bernain, X., Sagnol, M. & Lac, G. (2001). Preliminary results on mood state, salivary testosterone:cortisol ratio and team performance in a professional soccer team. European Journal of Applied Physiology 86, 179–184.

Foster, C., Florhaug, J. A:, Franklin, J., Gottschall, L., Hrovatin, L. A., Parker, S., Doleshal, P. & Dodge, C. (2001). A new approach to monitoring exercise training. Journal of Strength and Conditioning Research 15 (1), 109–115.

Föhr, T., Tolvanen, A., Myllymäki, T., Järvelä-Reijonen, E., Peuhkuri, K., Rantala, S., Kolehmainen, M., Korpela, R., Lappalainen, R., Ermes, M., Puttonen, S., Rusko, H. & Kujala, U. (2017). Physical activity, heart rate variability-based stress and recovery, and subjective stress during a 9-month study period. Scandinavian Journal of Medicine and Science in Sports, 27 (6), 612–621.

Galbois, A., Rudler, M., Massard, J., Fulla, Y., Bennani, A., Bonnefont-Rousselot, D., Thibault, V., Reignier, S., Bourrier, T., Poynard, T. & Thabut, D. (2010). Assessment of adrenal function in cirrhotic patients: Salivary cortisol should be preferred. Journal of Hepatology 52 (6), 839–845.

Gatti, R. & De Palo, E. F. (2010). An update: salivary hormones and physical exercise. Scandinavian Journal of Medicine & Science in Sports 21 (2), 157–169.

Gomes, R. V., Moreira, A., Lodo, L., Nosaka, K., Coutts, A. J. & Aoki, M. S. (2013). Monitoring training loads, stress, immune-endocrine responses and performance in tennis players. Biology of Sport 30 (3), 173–180.

Gubelmann, C., Kuehner, C., Vollenweider, P. & Marques-Vidal, P. (2018). Association of activity status and patterns with salivary cortisol: the population-based CoLaus study. European Journal of Applied Physiology 118, 1507–1514.

Hall, J. E. (2016). Guyton and Hall Textbook of Medical Physiology. 13th edition. Philadelphia: Elsevier

Hayes, L. D., Grace, F. M., Baker, J. S. & Sculthorpe, N. (2015). Exercise-induced responses in salivary testosterone, cortisol, and their ratios in men: a meta-analysis. Sports Medicine 45, 713–726.

Hedelin, R., Kenttä, G., Wiklund, U., Bjerle, P. & Henriksson-Larsén, K. (2000). Short-term overtraining: effects on performance, circulatory responses, and heart rate variability. Medicine & Science in Sports & Exercise 32 (8), 1480–1484.

Hough, J., Robertson, C. & Gleeson, M. (2015). Blunting of exercise-induced salivary testosterone in elite-level triathletes with a 10-day training camp. International Journal of Sports Physiology and Performance 10, 935–938.

Hough, J., Leal, D., Scott, G., Taylor, L., Townsend, D. & Gleeson, M. (2021). Reliability of salivary cortisol and testosterone to a high-intensity cycling protocol to highlight overtraining. Journal of Sport Sciences 39 (18), 2080–2086.

Huovinen, J., Tulppo, M., Nissilä, J., Linnamo, V., Häkkinen, K. & Kyröläinen, H. (2009). Relationship between heart rate variability and the serum testosterone-to-cortisol ratio during military service. European Journal of Sport Science 9 (5), 277–284.

Hynynen, E., Uusitalo, A., Konttinen, N. & Rusko, H. (2006). Heart rate variability during night sleep and after awakening in overtrained athletes. Medicine & Science in Sports & Exercise 38 (2), 313–317.

Hynynen, E., Vesterinen, V., Rusko H. & Nummela, A. (2010). Effects of moderate and heavy endurance exercise on nocturnal HRV. International Journal of Sports Medicine 31 (6), 428–432.

Hynynen, E. (2011). Heart rate variability in chronic and acute stress. University of Jyväskylä, Faculty of Sport and Health Sciences, Studies in sport, physical education and health 163. Doctoral thesis.

Impellizzeri, F. M., Marcora, S. M. & Coutts, A. J. (2019). Internal and external training load: 15 years on. International Journal of Sports Physiology and Performance 14, 270–273.

Jürimäe, J., Mäestu, J., Purge, P. & Jürimäe, T. (2004). Changes in stress and recovery after heavy training in rowers. Journal of Science and Medicine in Sport 7 (3), 335–339.

Kaikkonen, P., Rusko, H. & Martinmäki, K. (2008). Post-exercise heart rate variability of endurance athletes after different high-intensity exercise interventions. Scandinavian Journal of Medicine and Science in Sports 18 (4), 511–519.

Kaikkonen, P. (2015). Post-exercise heart rate variability. A new approach to evaluation of exercise-induced physiological training load. University of Jyväskylä, Faculty of Sport and Health Sciences, Studies in sport, physical education and health 224. Doctoral thesis.

Kamarauskas, P., Lukonaitiené, I., Scanlan, A. T., Ferioli, D., Paulauskas, H. & Conte, D. (2022). Weekly fluctuations in salivary hormone responses and their relationships with load and well-being in semiprofessional, male basketball players during a congested in-season phase. International Journal of Sports Physiology and Performance 17, 263–269.

Kim, H-G., Cheon, E-J., Bai, D-S., Lee, Y. H. & Koo, B-H. (2018). Stress and heart rate variability: A meta-analysis and review of the literature. Psychiatry Investigation 15 (3), 235–245.

Kinnunen, H., Rantanen, A., Kenttä, T. & Koskimäki, H. (2020). Feasible assessment of recovery and cardiovascular health: accuracy of nocturnal HR and HRV assessed via ring PPG in comparison to medical grade ECG. Physiological Measurement 41 (4). doi: 10.1088/1361-6579/ab840a

Klaperski, S., von Dawans. B., Heinrichs, M. & Fuchs, R. (2014). Effects of 12-week endurance training program on the physiological response to psychosocial stress in men: a randomized controlled trial. Journal of Behaviour Medicine 37, 1118–1133.

Kleiger, R. E., Stein, P. K. & Bigger, T. (2005). Heart rate variability: measurement and clinical utility. Electrocardiology 10 (1), 88–101.

Kontos, A., Baumert, M., Lushington, K., Kennedy, D., Kohler, M., Cicua-Navarro, D., Pamula, Y. & Martin, J. (2020). The inconsistent nature of heart rate variability during sleep in normal children and adolescents. Frontiers in Cardiovascular Medicine 7, 19.

Kraemer, W. J., French, D. N., Paxton, N. J., Häkkinen, K., Volek, J. S., Sebastianelli, W. J., Putukian, M., Newton, R. U., Rubin, M. R., Gómez, A. L., Vescovi, J. D., Ratamess, N. A., Fleck, S. J., Lynch, J. M. & Knuttgen, H. G. (2004). Changes in exercise performance and hormonal concentrations over a big ten soccer season in starters and nonstarters. Journal of Strength and Conditioning Research 18 (1), 121–128.

Laborde, S., Mosley, E. & Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research – Recommendations for experiment planning, data analysis, and data reporting. Frontiers in Psychology 8, 213.

Lac, G. & Berthon, P. (2000). Changes in cortisol and testosterone levels and T/C ratio during an endurance competition and recovery. The Journal of Sports Medicine and Physical Fitness 40 (2), 139–144.

Li, C-H., Hsu, G-S., Suzuki, K., Ko. & Fang, S-H. (2015). Salivary immune Factors, Cortisol and Testosterone Responses in Athletes of a Competitive 5,000 m Race. Chinese Journal of Physiology 58 (4), 263–269.

Ljubijankić, N., Popović-Javorić, R., Šćeta, S., Šapčanin, A., Tahirović, I. & Sofić, E. (2008). Daily fluctuation of cortisol in the saliva and serum of healthy persons. Bosnian Journal of Basic Medical Sciences 8 (2), 110–115.

Lucía, A., Hoyos, J., Carvajal, A. & Chicharro, J. L. (1999). Herat rate response to professional road cycling: the Tour de France. International Journal of Sports Medicine 20 (3), 167–172.

Maller, A., Pagani, M., Lombardi, F. & Cerutti, S. (1991). Cardiovascular neural regulation explored in the frequency domain. Circulation 84 (2), 482–492.

Manzi, V., Iellamo, F., Impellizzeri, F., D'Ottavio, S. Castagna, C. (2009). Relation between individualized training impulses and performance in distance runners. Medicine & Science in Sports & Sports Exercise 41 (11), 2090–2096.

Martinmäki, K. (2009). Transient changes in heart rate variability in response to orthostatic task, endurance exercise and training. University of Jyväskylä, Faculty of Sport and Health Sciences, Studies in sport, physical education and health 134. Doctoral thesis.

Marques, A. H., Silverman, M. N. & Sternberg, E. M. (2010). Evaluation of stress systems by applying noninvasive methodologies: Measurements of neuroimmune biomarkers in the

sweat, heart rate variability and salivary cortisol. Neuroimmunomodulation 17 (3), 205–208.

McArdle, W. D., Katch, F. I. & Katch, V. L. (2015). Exercise Physiology: Nutrition, Energy and Human Performance. Eight Edition. Baltimore: Wolters Kluwer Health.

Mezzullo, M., Fazzini, A., Gambineri, A., Di Dalmazi, G., Mazza, R., Pelusi, C., Vicennati, V., Paquali, R., Pagotto, U. & Fanelli, F. (2016). Parallel diurnal fluctuation of testosterone, androstenedione, dehydroepiandrosterone and 170Hprogesterone as assessed in serum and saliva: validation of a novel liquid chromatography-tandem mass spectrometry method for salivary steroid profiling. Clinical Chemistry and Laboratory Medicine 55 (9), 1315–1323.

Mishica, C., Kyröläinen, H., Hynynen, E., Nummela, A., Holmberg, H-C. & Linnamo, V. (2021). Relationships between heart rate variability, sleep duration, cortisol and physical training in young athletes. Journal of Sports Science and Medicine 20, 778–788.

Monje, C., Rada, I., Castro-Sepulveda, M., Peñailillo, L., Deldicque, L. & Zbinden-Foncea, H. (2020). Effects of a high intensity interval session on mucosal immune function and salivary hormones in male and female endurance athletes. Journal of Sport Science and Medicine 19, 436–443.

Morton, R. H., Fitz-Clarke, J. R. & Banister, E. W. (1990). Modeling human performance in running. journal of Applied Physiology 69 (3), 1171–1177.

Myllymäki, T., Rusko, H., Syväoja, H., Juuti, T., Kinnunen, M-L. & Kyröläinen, H. (2011). Effects of exercise intensity and duration on nocturnal heart rate variability and sleep quality. European Journal of Applied Physiology 112 (3), 801–809.

Narinx, N., David, K., Walravens, J., Vermeersch, P., Claessens, F., Fiers, T., Lapauw, B., Antonio, L. & Vanderschueren, D. (2022). Role of sex hormone-binding globulin in the free hormone hypothesis and the relevance of free testosterone in androgen physiology. Cellular and Molecular Life Sciences 79, 543.

Nummela, A., Hynynen, E., Kaikkonen, P. & Rusko, H. (2010). Endurance perormance and nocturnal HRV indices. International Journal of Sports Medicine 31, 154–159.

Nummela, A., Hynynen, E., Kaikkonen, P. & Rusko, H. (2016). High-intensity endurance training increases nocturnal heart rate variability in sedentary participants. Biology of Sport 33 (1), 7–13.

Nuuttila, O-P., Nikander, A., Polomoshnov, D., Laukkanen, J. A. & Häkkinen, K. (2017). Effects of HRV-guided vs. predetermined block training on performance, HRV and serum hormones. International Journal of Sports Medicine 38, 909–920.

Nuuttila, O-P., Seipäjärvi, S., Kyröläinen, H. & Nummela, A. (2022). Reliability and sensitivity of nocturnal heart rate and heart-rate variability in monitoring individual responses to training load. International Journal os Sports Physiology and Performance (17), 1296–1303.

Peñailillo, L., Maya, L., Niño, G., Torres, H. & Zbinden-Foncea, H. (2015). Salivary hormones and IgA in relation to physical performance in football. Journal of Sports Sciences 33 (20), 2080–2087.

Pichot, V., Roche, F., Gaspoz, J-M., Enjolras, F., Antoniadis, A., Minini, P., Costes, F., Busso, T., Lacour, J-R. & Barthélémy, J. C. (2000). Relation between heart rate variability and training load in middle distance runners. Medicine and Science in Sports and Exercise 32 (10), 1729–1736.

Plews, D. J., Laursen, P. B., Stanley, J., Kilding, A. E. & Buchheit, M. (2013). Training and heart rate variability in elite endurance athletes: Opening the door to effective monitoring. Sports Medicine 43, 773–781.

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., Kaspers, F. & Kirchbaum, C. (1997). Free cortisol levels after awakening: A reliable biological marker for the assessment of adrenocortical activity 61 (26), 2539–2549.

Roos, L., Taube, W., Brandt, M., Heyer, L. & Wyss, T. (2013). Monitoring of daily training load and training load responses in endurance sports: what do coaches want? Schweizerische Zeitschrift für Sportmedizin und Sporttraumatologie 61 (4), 30–36.

Rosa, J. P. P., Silva, A., Rodrigues, D. F., Menslin, R., Araújo, L. T., Vital, R., Tufik, S., Stieler, E. & de Mello, M. (2020). Association between hormonal status, stress, recovery, and motivation of paralympic swimmers. Research Quarterly for Exercise and Sport 91 (4), 652–661.

Sato, K., Iemitsu, M., Katayama, K., Ishida, Y. & Saito, M. (2015). Responses of sex steroid hormones to different intensities of exercise in endurance athletes. Experimental Physiology 101 (1), 168–175.

Schoenfeld, B. J. (2010). The mechanisms of muscle hypertrophy and their application to resistance training. Journal of Strength and Conditioning Research 24 (10), 2857–2872.

Seller, S. (2010.) What is Best Practice for Training Intensity and Duration Distribution in Endurance Athletes? International Journal of Sports Physiology (5), 276–291.

Serpell, B. G., Horgan, B. G., Colomer, C. M.E., Field, B., Halson, S. L. & Cook, C. J. (2019). Sleep and salivary testosterone and cortisol during a short preseason camp: a study in professional rugby union. International Journal of Sports Physiology and Performance (14), 796–804.

Shaffer, F., McCraty, R. & Zerr, C. L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. Frontiers Pshychology 5, 1040.

Stone, J. D., Ulman, H. K., Tran, K., Thompson, A. G., Halter, M. D., Ramadan, J. H., Stephenson, M., Finomore, V. S., Galster, S. M., Rezai, A. R. & Hagen, J. A. (2021). Assessing the accuracy of popular technologies that measure resting heart rate and heart rate variability. Frontiers in Sports and Active Living 3, 585870. doi: 10.3389/fspor.2021.585870.

Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. European Heart Journal (17), 354–381.

Tauler, P., Martinez, S., Moreno, C., Martínez, P. & Aguilo, A. (2013). Changes in salivary hormones, immunoglobulin A, and C-reactive protein in response to ultra-endurance exercises. Applied Physiology, Nutrition, and Metabolism 39, 560–565.

Theocharidou, E., Giouleme, O., Anastasiadis, S., Markopoulou, A., Pagourelias, E., Vassiliadis, T., Fotoglidis, A., Agorastou, P., Slavakis, A., Balaska, A., Kouskoura, M. G., GOssios, T. D., Karagiannis, A. & Mantzoros, C. S. (2019). Free cortisol is a more accurate marker for adrenal function and does not correlate with renal function in cirrhosis. Digestive Diseases and Sciences 64, 1686–1894.

Tiernan, C., Lyons, M., Comyns, T., Nevill, A. & Warrington, G. (2019). Investigation of the relationship between salivary cortisol, training load and subjective markers of recovery in elite rugby union players. International Journal of Sports Physiology and Performance 5 (1), 113–118.

Wahl, P., Mathes, S., Köhler, K., Achtzen, S., Bloch, W. & Mester, J. (2013). Acute metabolic. hormonal, and psychological responses to different endurance training protocols. Hormone and Metabolic Research 45 (11), 827–833.

Wallace, L. K., Slattery, K. M. & Coutts, A. J. (2014). A comparison of methods for quantifying training load: relationships between modelled and actual training responses. European Journal of Applied Physiology 114 (1), 11–20.

Wood, C. J., Clow, A., Hucklebridge, F., Law, R. & Smyth, N. (2018). Physical fitness and prior physical activity are both associated with less cortisol secretion during psychosocial stress. Anxiety, Stress and Coping 31 (2), 135–145.

Yoshida, Y., Yuda, E., Yokoyama, K. & Hayano, J. (2018). Evaluation of nocturnal heart rate variability for strenuous exercise day using wearable photoelectric pulse wave sensor. Journal of Exercise Rehabilitation 14 (4), 633–637.

APPENDICES

Appendix 1: Health questionnaire for athletes.

Liite 8a. Kyröläinen

Taustatiedot	Muuta huomioitavaa (sairaustelujaksot, lääkkeet, verestävät ikenet, loukkaantumiset
Sukupuoli (m/n)	
Pituus (cm)	
Paino (kg)	
Ikä (vuosia)	
Harjoittelumuodot, kilpailulaji	
Muut tiedot kuten lääkitys, verestävät ikenet	

Onko sinulla ollut viimeisen 6kk aikana	Kyllä	Ei	En osaa sanoa
Rintakipuja			
Hengenahdistusta rasituksessa			
Huimausoireita			
Rytmihäiriötuntemuksia			
Harjoittelua estäviä kipuja			
Ylikuormitus- tai stressioireita			

Onko sinulla tai onko ollut jokin seuraavista	Kyllä	Ei
Sepelvaltimotauti		
Sydäninfarkti		
Kohonnut verenpaine		
Sydänläppävika		
Aivohalvaus		
Aivoverenkierron häiriä		
Sydämen rytmihäiriö		
Sydämentahdistin		
Sydänlihassairaus		
Syvä laskimotukos		
Muu verisuonisairaus		
Krooninen bronkiitti		
Keuhkolaajentuma		
Astma		
Muu keuhkosairaus		
Allergia		
Kilpirauhasen toimintahäiriö		
Diabetes		

Anemia		
Korkea veren kolesteroli		
Nivelreuma		
Nivelrikko, -kuluma		
Krooninen selkäsairaus		
Mahahaava		
Pallea, nivus tai napatyrä		
Ruokatorven tulehdus		
Kasvain tai syöpä		
Leikkaus äskettäin		
Mielenterveyden ongelmia		
Tapaturma äskettäin		
Matala veren K tai Mg		
Kohonnut silmänpaine		
Näön tai kuulon heikkous		
Urheiluvamma äskettäin		
Muita Sairauksia tai oireita (mitä)		
Onko sinulla säännöllinen lääkitys (mikä)		
Tupakoitko tai käytätkö nuuskaa		
Onko sinulla todetty synnynnäinen sydänvika		
Onko lähisuvussa todettu sydänperäisiä äkkikuolemia		
Onko lähisuvussa perinnöllisiä sairauksia		

		Viikko 1
	SPORT2022 PROTOKOLLA, DATA	Päivä 1
Sykedata		
	Yön alin syke (bpm)	
	Yön alin syke (bpm)	
	Yön aikainen keskimääräinen sykevälivaihtelu (RMSSD)	
	Yön ylin sykevälivaihtelu (RMSSD)	
Sylkinäytteet		
	Aamunäyte 1kpl (0-5min, merkitse "X" tehdyksi)	
	FTP testi (merkitse "X" suorituspäivä)	
	Iltanäyte 1kpl (30min ennen nukkumaanmenoa, merkitse "X" tehdyksi)	
	Aamuverinäyte paaston jälkeen (laboratorio)	
Muu päivittäinen data		
	Alkoholi (annosta/päivä)	
	Kehon lämpötila unessa (Oura)	
	Päivittäinen kokonaisenergiankulutus (TEE, kcal)	
	Training load (TRIMP)	
Viikkokysely - valitse yksi vaihtoehto, joka kuvaa tunnetta viimeisimmän viikon aikana (POMS)		
	(vastaa A=ei lainkaan B=melko vähän C=jonkin verran D=melko paljon E=erittäin paljon)	
	Uupunut	
	Iloinen	
	Luottavainen	
	Elinvoimainen	
	Väsynyt	
	Kireä	
	Tarmokas	
Kuukausikysely - arvioi tuntemuksiasi viimeisimmän kuukauden aikana (PSS)		
	(vastaa A=en koskaan B=melko harvoin C=toisinaan D=melko usein E=hyvin usein)	
	Olet tuntenut olosi hermostuneeksi tai stressaantuneeksi	
	Olet tuntenut varmuutta kyvyistä ratkaista henkilökohtaisia ongelmiasi	
	Olet tuntenut että asiat ovat sujuneet kuten halusit	
	Olet pystynyt hallitsemaan harmeja elämässäsi	
	Olet ollut vihainen tapahtuneiden asioiden vuoksi, joihin et ole voinut vaikuttaa	
Suorituskykytestin tulokset		
	FTP testi,60min (Tulos, Wattia)	
	RPE - FTP testi 1-10 (1 = Lepo, 5 = Raskas, 10 = kuten raskain kilpailu)	

Appendix 2: Instructions for salivary samples.

How to collect saliva samples?

Saliva test kit use

01 **Clean mouth**

Make sure your mouth is in a natural, clean state.
Do not eat, drink or brush your teeth 30 minutes before you take the test.
Make sure you do not have bleeding gums or blood in your mouth.

02 **Preparations**

Remove the swab and the storage tube from the package.

03 **Collecting a saliva sample**

First, open the swab package and place the swab in your mouth, under the front of your tongue for 1-2 minutes. Next, place the swab in the storage tube, touch the swab as little as possible. Finally, close the tube properly.

04 **QR code**

Scan the QR code with your phone QR code reader and answer the questions.

05 **Storage**

Pay attention that the QR code sticker stays attached to its tube. Place the tube in the storage box. Keep the storage box in a freezer and aim to have samples frozen as soon as possible. Note – it is important that all collected samples are stored as uniformly as possible.

06 **Ship the saliva samples to Summa Labs**

Make sure the prepaid shipment stickers are placed on the return box.
Place the freezed cold gel pouches into the box with the tubes.
Close the return box properly and make sure the shipment stickers are visible.
The package can be dropped off at a nearby Matkahuolto service desk.

summa.bio orders@summa.bio

When to collect saliva samples?

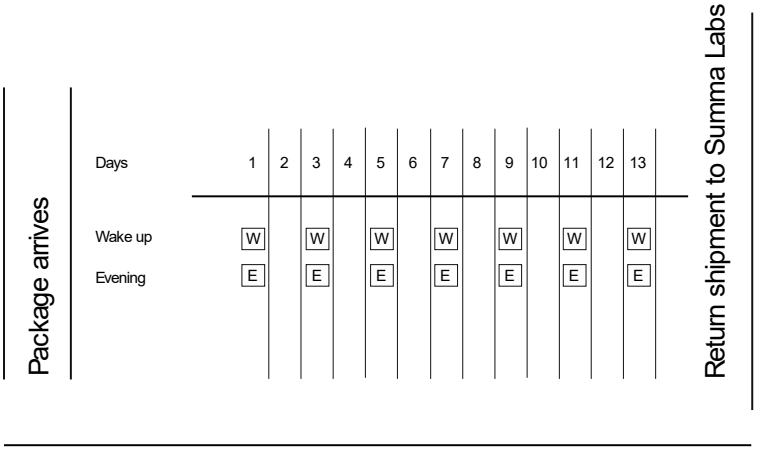
Sampling protocol

[28 samples]

Your coach will tell you when to take saliva tests

Example of sampling

W Wake up – take the sample 5 min after waking up.
B Before the exercise – take the sample 30 min before your exercise session.
A After the exercise – take the sample 0 min after the exercise.
E Evening – take the sample 30 min before you go to sleep.

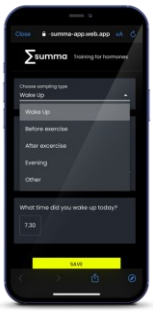


How to scan QR codes?

01.

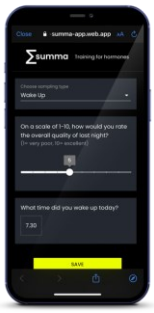
Read the QR code by your mobile device.

02.



Select a sample type from the pull-down menu.

03.



Wake Up
- Used to monitor the training status in the longer term
- Strive for regularity

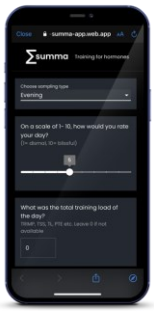
04.

Before exercise
- Used for acute training effect verifict iơn
- The sample is taken 30 minutes before the exercise

05.

After exercise
- Used for acute training effect verifict iơn
- The sample is taken immediately after the exercise

06.

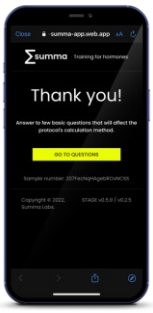


Evening
- Used to monitor the training status in the longer term
- Strive for regularity

07.

Other
- Used for accumulative stress assessment during exercise
- Write additional information in the fied

08.



Thank you
- Go to questions

09.

Questions
- Answer the questions