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Christina Kuorelahti

Monitoring and Development of Junior Cross-Country Skiers During Sports High School

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF SPORT AND HEALTH SCIENCES

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*"You have brains in your head. You have feet in your shoes.
You can steer yourself in any direction you choose."*

Dr. Seuss

ABSTRACT

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This thesis followed young athletes attending a sports high school by evaluating various monitoring methods and athlete development. An eight-week longitudinal research period was implemented to examine changes in frequent variables of athlete monitoring (e.g. submaximal tests, nocturnal heart rate variability (HRV), cortisol, orthostatic test etc.) (I), as well as validate several monitoring outcome parameters of heart rate (HR) and HRV (II). Secondly, assessment of performance related outcome changes and associations between subjective stress, sleep and performance-related tests were examined to determine which factors have the greatest influence on performance (III). Finally, a continuous one-year training period was studied to provide developmental and performance-related details that coaches and athletes may encounter (IV). A total of 37 young athletes participated in the studies. Laboratory-based performance-related measures consisted of submaximal running tests, explosive jump tests, incremental maximal tests, and ski-specific double pole tests. A contactless ballistocardiography (BCG)-based sleep device was used to collect nocturnal HR indices and sleep data. Training load was reported in individual training diaries. Blood levels of ferritin, vitamin D and hemoglobin were monitored while subjective levels of perceived stress were assessed with a monthly questionnaire. Nocturnal HRV and salivary levels of cortisol were inversely related ($r = -0.552$, $p = 0.001$). Nocturnal HR and HRV, which were collected under real-life conditions with a BCG device, revealed to have a good relationship with morning values derived from orthostatic tests. At a group level, diminished sleep duration exerted a negative effect on perceived stress scores (PSS), with females displaying significantly higher PSS values. One-year analysis revealed a significant improvement in ski-specific double pole performance (DPP), but no other significant changes were observed. There were no significant associations between the changes in DPP and any other variable. Thus, the present results suggest that measures of nocturnal HRV indices, sleep duration, and perceived stress levels appear to be appropriate monitoring tools that may facilitate training and performance in young athletes, and one-year of endurance training induced significant improvements in ski-specific tests, but additional changes were minimal.

Keywords: cross-country skiing, heart rate variability, adolescents, perceived stress

TIIVISTELMÄ

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Tässä väitöskirjassa seurattiin nuoria urheilulukiossa opiskelevia urheilijoita ja heidän kehitystään erilaisia seurantamenetelmiä hyödyntämällä. Väitöskirja koostuu kahdesta osatutkimuksesta. Ensimmäisessä kahdeksan viikon pitkittäistutkimuksessa tarkasteltiin muutoksia erilaisten usein käytetyissä palautumista ja suorituskykyä arvioivissa muuttujissa (esim. submaksimaaliset testit, yöllinen sykevälivaihtelu (HRV), kortisoli, ortostaattinen koe jne.) (I) sekä validoitiin sykettä ja HRV:ta mittaavia parametreja (II). Lisäksi tutkimuksessa arvioitiin suorituskykyyn muutoksia sekä subjektiivisen stressin, unen ja suorituskykytestien välisiä yhteyksiä, joiden avulla pyrittiin selvittämään, millä tekijöillä on suurin vaikutus suorituskykyyn (III). Väitöskirjan toisessa osatutkimuksessa seurattiin urheilijoiden harjoittelua vuoden ajan, jonka avulla pyrittiin tarjoamaan valmentajille ja urheilijoille kehitykseen ja suorituskykyyn liittyviä tietoja (IV). Tutkimukseen osallistui yhteensä 37 nuorta urheilijaa. Suorituskykyä arvioitiin submaksimaalisten juoksutestien, räjähtävien hyppytestien, suoran maksimaalisen hapenottokyvyntestin sekä hiihtäjille suunnatun tasatyöntötestin avulla. Kontaktittomaan ballistokardiografiaan (BCG) perustuva unilaite keräsi yölliset sykkeen indeksit ja unitietoja. Harjoittelurasitusta raportoitiin yksilöllisissä harjoituspäiväkirjoissa. Tutkimuksessa seurattiin myös veren ferritiini-, D-vitamiini- ja hemoglobiinipitoisuuksia ja koettua stressitasoa arvioitiin kuukausittaisella kyselylomakkeella. Yöllisen HRV:n ja syljen kortisolipitoisuuksien havaittiin korreloivan käänteisesti ($r = -0{,}552$, $p = 0{,}001$). Yölliset syke- ja HRV-mittaukset, jotka kerättiin todellisissa olosuhteissa BCG-laitteella, olivat hyvin yhteydessä aamuarvoihin, jotka saatiin ortostaattisista testeistä. Ryhmätasolla, unen keston lyhentyminen vaikutti negatiivisesti koettuihin stressipisteisiin (PSS), ja naisilla oli merkittävästi korkeammat PSS-arvot. Vuoden mittaisessa seurannassa havaittiin merkittävä parannus hiihtäjille suunnatussa tasatyöntötestin suorituskyvyssä (DPP), mutta muita merkittäviä muutoksia ei havaittu. DPP-muutosten ja muiden muuttujien välillä ei ollut merkittäviä yhteyksiä. Tulokset viittaavat siihen, että yöllisten HRV parametrien, unen keston ja koetun stressitason mittaukset ovat sopivia seurantatyökaluja, jotka voivat helpottaa nuorten urheilijoiden harjoittelua ja kehittymistä. Vuoden mittainen kestävyysharjoittelu paransi tuloksia hiihtäjille suunnatuissa testeissä merkittävästi, mutta muut muutokset olivat vähäisiä.

Avainsanat: maastohiihto, sykevälivaihtelu, nuoret, koettu stressi

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Looking back, I realize my PhD journey was very similar to that of a running race. There were periods of ups and downs, excitement and frustration, expected and unexpected results and several moments where I whispered to myself, "*what in the world did I sign up for?*". Luckily, I kept proceeding one step at a time and as with most of life's endeavors I was never alone. A support team was essential to reach this finish line and therefore, I have many people I wish to express my gratitude towards.

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Vuokatti 30.08.2023
Christina Marie Kuorelahti

ORIGINAL PUBLICATIONS AND AUTHOR CONTRIBUTION

This thesis is based on the following original publications, which will be referred to by their Roman numerals.

I Mishica, C., Kyröläinen, H., Hynynen, E., Nummela, A., Holmberg, HC., & Linnamo V. (2021). Relationships between heart rate variability, sleep duration, cortisol and physical training in young athletes. *Journal of Sports Science and Medicine,* 20(4), 778-788. https://doi.org/10.52082/jssm.2021.778

II Mishica, C., Kyröläinen, H., Hynynen, E., Nummela, A., Holmberg, HC., & Linnamo V. (2022). Evaluation of nocturnal vs. morning measures of heart rate indices in young athletes. *PLoS One*, 17(1), e0262333. https://doi.org/10.1371/journal.pone.0262333

III Mishica, C., Kyröläinen, H., Taskinen, S., Hynynen, E., Nummela, A., Holmberg, HC., & Linnamo, V. (2023). Associations between objective measures of performance-related characteristics and perceived stress in young cross-country skiers during pre-season training. *Under review.*

IV Mishica, C., Kyröläinen, H., Valtonen, M., Holmberg, HC., & Linnamo, V. (2023) Performance-related physiological changes induced by one year of endurance training in young athletes. *Frontiers in Sports and Active Living*, 5, 1149968. https://doi.org/10.3389/fspor.2023.1149968

As the first author of the preceding original publications, with consideration of comments from the co-authors, I was responsible for drafting the study questions, collecting the data, and performing the statistical analysis independently or with the help of a statistician. I also took the primary responsibility for data interpretation and writing of the manuscripts.

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ABBREVIATIONS

XC	Cross-country
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Peak oxygen uptake
DP	Double pole
DPP	Double pole performance
MP	Maximal power
PP	Peak power
LT	Lactate threshold
MLSS	Maximal lactate steady state
LIT	Low-intensity training
HIT	High-intensity training
HR	Heart rate
HRmax	Maximal heart rate
$\%VO_{2max}$	Percentage of maximal oxygen uptake
CMJ	Counter movement jump
RMSSD	Root-mean squared difference between two successive RR intervals
PSG	Polysomnography
POMS	Profile of mood states
RESTQ-S	Recovery stress questionnaire for athletes
RPE	Rating of perceived exertion
PPS	Perceived stress score
HRV	Heart rate variability
BCG	Ballistocardiography
ECG	Electrocardiogram
ANS	Autonomic nervous system
RER	Respiratory exchange ratio
SRT	Submaximal running test
TTE	Time till exhaustion
HRV_{AM}	Morning average nocturnal RMSSD value
HRV_{PM}	Evening average nocturnal RMSSD values
LTT	Lactate threshold training
Fer	Ferritin
Hg	Hemoglobin
VitD	Vitamin D

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ORIGINAL PUBLICATIONS

1 INTRODUCTION

The challenge to become a top athlete requires several factors including time, commitment, and proper execution of training. Today, the pursuit to reach high-level competition in sport begins at a young age (Brenner, 2016). As a result, young athletes are a special population that is in demand of further investigation. Knowledge regarding the physical trainability of young athletes is limited (Naughton et al., 2000), and research in this area is difficult due to the high amount of physical growth and changes that occur. However, innovations in technology as well as the growing popularity of wearable technology (Düking et al., 2016) provide new non-invasive techniques that are suitable for young athletes and appear to be promising tools for long-term testing and/or monitoring in athletes. Therefore, the utilization of these advancements introduces new possibilities for research in exercise performance that may help expand knowledge about the development and optimal training methods that may benefit young athletes (Armstrong & McManus, 2011).

Endurance training is comprised of a structured program that maintains a sufficient balance of intensity, frequency, and exercise duration to induce an improvement in aerobic capacities. The implicit goal is to balance these variables in order to maximize performance while minimizing the risk of negative training outcomes or injuries (Seiler, 2010). Although research examining evidence-based dose-relationships between endurance training and aerobic fitness in young athletes is limited (Armstrong & McManus, 2011), it is now clear that the aerobic capacity of young athletes can be improved by training (Armstrong & McNarry, 2016). Therefore, the question that arises is how to effectively organize daily training to allow for the most significant improvements in adolescent athletes.

In order to assess an individual's response to training, a variety of psychological, physiological, performance-related and biochemical measures are commonly used (Meeusen et al., 2013; Saw et al., 2016; Taylor et al., 2012). Evaluation of psychological parameters are frequently assessed based on self-reported measures of perceived stress or mood, while physiological aspects are more frequently quantified on the basis of performance, e.g., in maximal or submaximal exercise tests (Saw et al., 2016). Biochemical and hematological measures are often utilized to indicate potential nutritional deficiencies that may exist, a prevalent occurrence amongst athletes, as well as help optimize the balance between training and recovery (Lee et al., 2017). Nowadays, tracking individual development and performance with a combination of various markers has become common practice, but it still remains unclear which measures are the most useful indicator(s) and/or valid parameters to monitor performance (Lee et al., 2017), especially in athletes during their adolescent years.

The purpose of this thesis was to observe various parameters that are commonly used to monitor training and investigate the development and influence of training on the results of performance-related tests in young endurance athletes specializing in cross-country (XC) skiing and biathlon. Furthermore, an emphasis was placed on assessing the potential relationships between variables which are used for athlete monitoring/recovery and performance-related tests. Insight into this can provide valuable information about which measures have the greatest implementation value and, therefore, could be best used for monitoring young adolescent athletes.

2 REVIEW OF LITERATURE

2.1 Endurance training for young athletes

2.1.1 Overview

An individual's ability to deliver and utilize oxygen to support muscle activity during exercise is defined as aerobic fitness. The maximal amount of oxygen that can be used at the cellular level for the entire body is known as maximal oxygen uptake (VO_{2max}). Beginning with the formative research performed by Robinson (Robinson, 1938) and Åstrand (Åstrand, 1952) in the mid 1900's, VO_{2max} during exercise in adolescents has been well documented (Armstrong & McManus, 2011). Thus, the highest oxygen uptake value obtained during an exercise test till exhaustion, known as peak oxygen uptake (VO_{2peak}), is widely known as the single best measure of aerobic fitness in both children and young adults (Armstrong & Welsman, 2001).

Displaying suitable physiological and/or physical characteristics, such as high VO_{2peak} values, is not the only significant factor needed to develop successful endurance athletes. Hard training appears to be the formula for both success and failure. If training stress is below the ideal load, the greatest performance gain will not be reached, and if exceeding the optimal level, the negative consequences of overtraining may arise (Kenttä & Hassmén, 1998). Thus, vital ingredients for athletic success include a training program that effectively combines a relatively high load of training with sufficient rest and recovery (FIGURE 1). Yet, physical stress and recovery are not the only factors that influence athletic balance, but psychological and social stress, in combination with physiological stress, determine an individual's magnitude of total stress (Kenttä & Hassmén, 1998). In turn, the cumulative stress, in conjunction with the athlete's ability to handle and manage stress, influences the recovery process and aids in determining whether performance improvements or decrements occur (FIGURE 2).

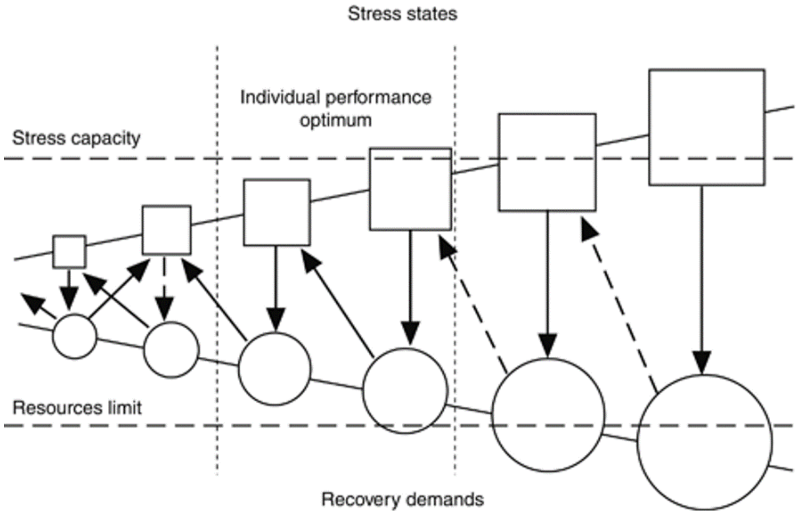

FIGURE 1 Model depicting the interrelation between stress-states and recovery demands. Reproduced with permission (Hanin, 2000).

When training for endurance performance, young athletes and adults alike are aiming to find this optimal balance. However, a young student-athlete must manage the demands of training with the additional task of studying. Young athletes attending a sports high school also have the added stress of living on

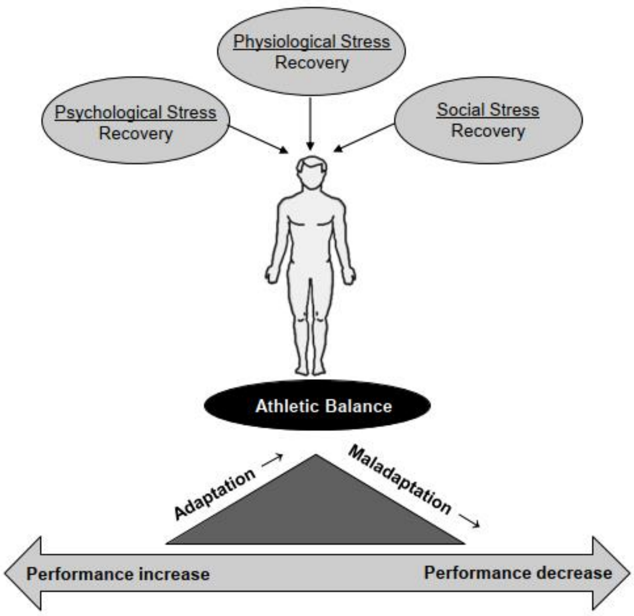

FIGURE 2 Model showing an overview of the recovery and stress process during endurance training. Modified and reproduced with permission (Kenttä & Hassmén, 1998).

their own. Clearly, longitudinal research concentrated on junior athletes engaging in intensive endurance training is an area that needs to be further investigated. In addition, effective monitoring and management of training load in young endurance athletes is crucial in order to develop resilient athletes that continue to participate in competitive sports into adulthood (Murray, 2017).

2.1.2 Development and age-related differences

Long-term athlete development, physiological monitoring and execution of training in young athletes are based on the development of aerobic and anaerobic fitness (Armstrong & Welsman, 2020). Evidence implies that both trained and untrained youth can benefit from endurance training (Hanin, 2000) but young athletes participating in endurance-related sports generally present higher VO_{2peak} values than untrained peers (Armstrong & McManus, 2011). Nonetheless, there are limited high-quality intervention studies in young, developing endurance athletes.

In both boys and girls, the most common measure of aerobic fitness, VO_{2peak} values (in $L \cdot min^{-1}$) increase with age (Armstrong & Welsman, 2020), with boys generally reporting higher values than girls of a similar age (Armstrong & Welsman, 2007). The effect of age begins to level-off between 16–18 years of age for boys and around 13–14 years of age for girls (FIGURE 3). Previous research has proven that age-related increases in VO_{2peak} are due to an increase in maximal stroke volume and maximal arteriovenous oxygen difference (Armstrong & Welsman, 2019). It is important to also recognize that VO_{2peak} is strongly correlated with body size in both adults and young athletes. However, a longitudinal data set of 11–17-year-olds investigating changes in VO_{2peak} revealed that when controlled for body mass and height, a positive effect was still present and hence, maturation appears to have a positive independent effect on VO_{2peak} values. This same study also demonstrated that changes in fat-free mass (muscle mass), in relation to maturity status, displayed the most prevalent positive influence on VO_{2peak}, indicating that the growth in muscle mass that occurs during the adolescent years likely influences aerobic fitness (Armstrong & Welsman, 2019). In addition, literature investigating active adolescents ages 12–15, also found that the development of VO_{2max} was largely influenced by fat-free mass (Landgraff et al., 2021). Yet, despite significant volumes of endurance training, VO_{2max} values remained similar in both boys and girls. However, improvement in performance was present in boys indicating that a high volume of training may contribute to an improved utilization and/or increase in aerobic capacity in adolescent boys (Landgraff et al., 2021). Although muscle mass increases through adolescence in both sex, boys generally have higher muscle mass than girls. Thus, when following adolescent growth, the physiological explanation for sex differences may be due to the greater increase in muscles mass that boys exhibit (Armstrong et al., 2011).

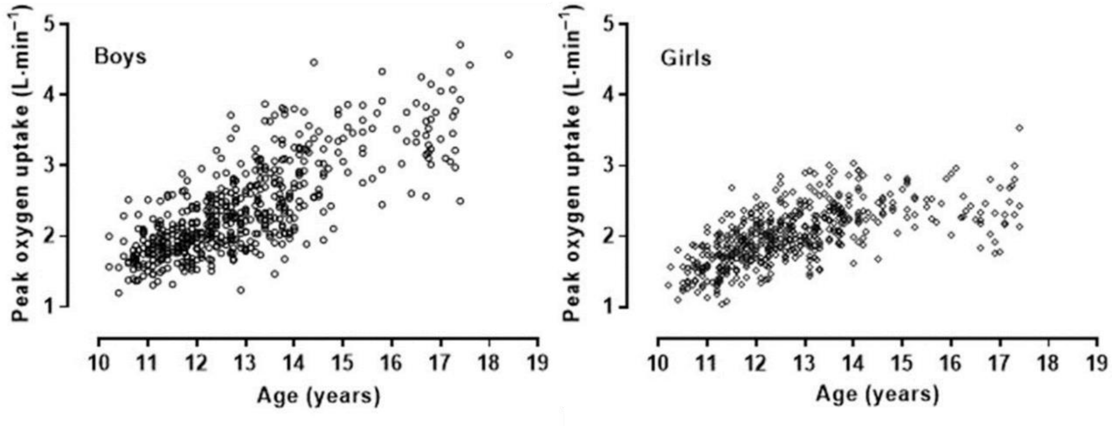

FIGURE 3 Changes in peak oxygen uptake in relation to age in 10–18-year-old boys and girls. Reproduced with permission (Armstrong & Welsman, 2019).

Anaerobic fitness may be identified as the capacity to maintain and produce energy to support the metabolic demands of maximal exercise through non-oxidative pathways. In contrast to aerobic fitness, direct measures during maximal exercise intensity are more challenging to conduct (Armstrong & Welsman, 2020), and knowledge depicting improvements in young athletes is unclear whether muscle size, fiber type or neurological/biochemical changes are the main influence of the progress seen in anaerobic fitness with training (Matos & Winsley, 2007). As a result, various measures of maximal power (MP) and peak power (PP) are often utilized. Longitudinal studies indicate that PP and MP significantly increase in both boys and girls with sex differences leveling off at 17 years of age when boys are displaying approximately 30% higher MP and PP than girls (Armstrong & Welsman, 2019). Therefore, it appears that increases in muscle strength and power are likely due to the growth that occurs during adolescence which also improves the ability to generate energy anaerobically (Armstrong & McManus, 2011). However, studies investigating anaerobic fitness in adolescents with training also report improvements in MP of 3–10% and in PP of 4–20%, suggesting training also has an influence on anaerobic fitness to some degree (Matos & Winsley, 2007).

In competitive sports, performance typically includes a combination of aerobic and anaerobic intensity and distribution is influenced by exercise intensity and duration as well as an individuals' physiology and current training status (Armstrong & Welsman, 2020). This balance between aerobic and anaerobic metabolism influences the production of lactate with higher aerobic capacities resulting in lower muscle lactate production (Armstrong & McManus, 2011). As the exercise intensity increases, the rate of diffusion of blood lactate from the muscle can no longer meet the rate of removal from the blood, and as a result, blood lactate accumulation occurs. The initial increase of blood lactate is commonly referred to as lactate threshold (LT), and the highest sustainable exercise intensity before a progressive increase occurs is referred to as maximal lactate steady state (MLSS) (Armstrong et al., 2011). In youth, the exact cause of changes in blood lactate accumulation is yet to be uncovered, but endurance training appears to influence blood lactate accumulation by reducing the accumulation of lactate despite exercising at the same pre-training percentage of VO_{2peak} (Barker & Armstrong, 2011). Therefore, monitoring blood lactate levels via standardized exercise tests is often implemented by coaches and young athletes to monitor the effects that training has on an individual's aerobic and anaerobic fitness (FIGURE 4).

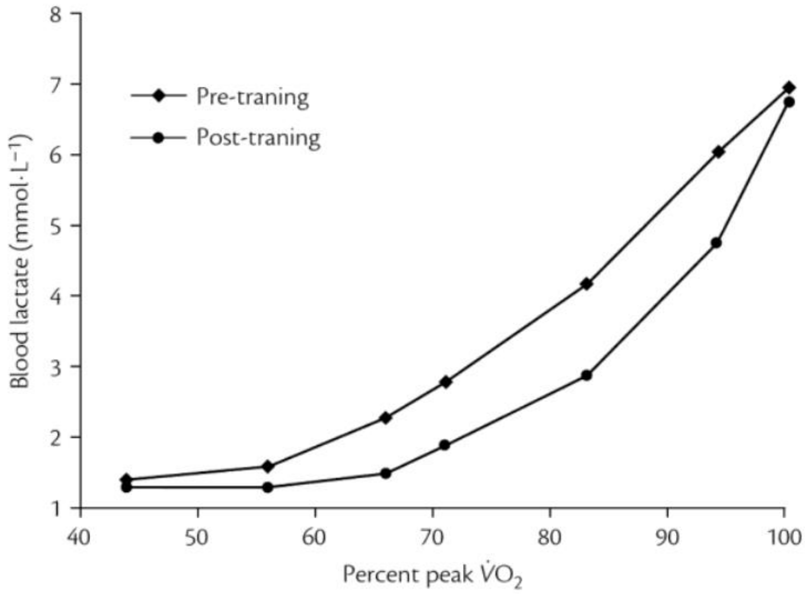

FIGURE 4 Blood lactates response to training and exercise. Reproduced with permission (Armstrong & Van Mechelen, 2008).

Following measures of aerobic, anaerobic and lactate thresholds are also common among adult athletes, and longitudinal data indicates that with adequate endurance training, aerobic, anaerobic and lactate threshold values will continue to develop into adulthood (FIGURE 5).

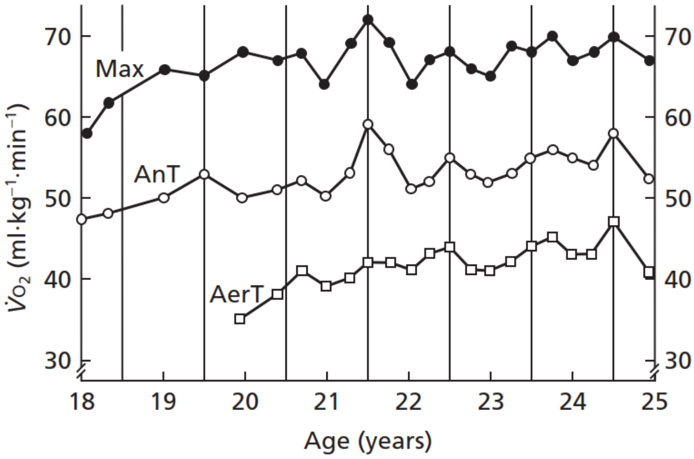

FIGURE 5 A world-class female XC skiers' longitudinal development of aerobic threshold (AerT), anaerobic threshold (AnT) and maximal oxygen uptake (VO_{2max}) while training during early adulthood. Reproduced with permission (Rusko, 2003).

2.1.3 Age of peak performance

Peak competitive performance occurs at many different ages depending on the sport-specific characteristics and skills needed for success. In endurance sports, linear treads typically depict the relationship that is found between age of peak performance and event duration, with the possibility of reaching peak performance at much older ages in endurance sports than sports that require powerful work (Allen & Hopkins, 2015). Previous research that examined the mean peak age in world-class track and field athletes found that women often display higher peak ages than males, and the typical range of peak performance varied in various events ranging between 20–30 years (FIGURE 6).

A study examining peak age in world-class XC skiers revealed that the mean peak age was around 26 years in both distance and sprint skiing with minimal to no significant differences between sex (Walther et al., 2022). In addition, when looking at peak age relative to performance, top performers displayed a higher peak age, and although they performed better as juniors, they found that junior performance only accounted for half of the variance in peak performance. Furthermore, recent research investigating if junior success is indicative of success as a senior revealed that around 89% of international level juniors of the U17/18 age category failed to achieve an equivalent level of success as senior athletes, and likewise, 82% of high level seniors had not previously reached an international level of success as junior athletes (Güllich et al., 2023). Thus, practitioners and coaches working with young XC skiers and/or endurance athletes should not place too much stress on competitive performance and should work to also motivate young athletes by placing a larger emphasis on the long-term development that occurs with consistent training.

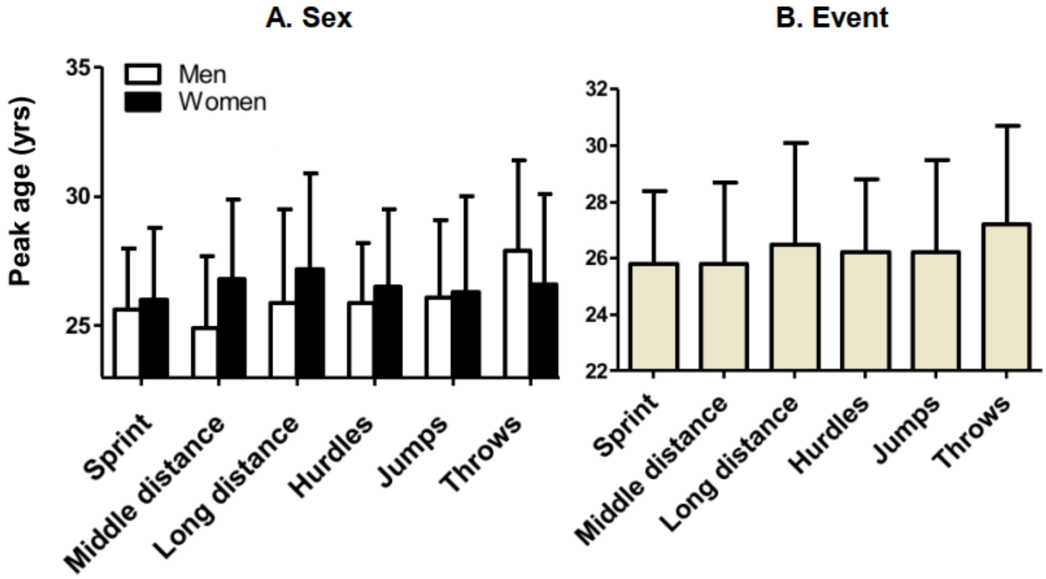

FIGURE 6 Peak performance age across sex (A) and event (B) in world class track and field athletes. Modified and reproduced with permission (Haugen et al., 2018).

2.1.4 Training volume and intensity distribution

To be successful in endurance sports, high volumes of training are required. As a result, year-round training begins at a young age and junior athletes (16–20 years of age) focused on the sport of XC ski are recommended to train 300–600 hours depending on their specific athletic background (Rusko, 2003). Quantification and measures of training volume are commonly measured by time (annual training hours), with elite endurance athletes following a systematic training plan for >11 months of the year (Seiler, 2010). This results in high training loads; however, exercise modality has a significant influence on the muscular load that a specific training session may exert and therefore, large variations between training volumes in different endurance sports are present (TABLE 1). For instance, weight-bearing exercises, such as running, have a substantial impact on muscles and tendons whereas swimming, which is a low-contraction exercise, allows for substantial volumes in swim-specific exercise modes (Sandbakk et al., 2021).

A more detailed analysis of the training routines of top XC skiers indicated that even at the elite level, the training foundation is built on endurance, with 85–86% of training occurring at low intensities (60–80% of maximal HR) and total training volume ranging from 750–950 hours. In addition, the low-intensity training is largely ski-specific, with 65–75% of low-intensity training being performed on skis (roller skiing and skiing) (Holmberg, 2015; Sandbakk & Holmberg, 2014, 2017). When investigating junior athletes, a group of elite XC skiers, around 16 years of age, report training volumes over 400 hours annually (410 ± 66 hours) (Sandbakk et al., 2011). In addition, during 16–22 years of age, when an athlete is transitioning from a junior to a senior athlete, the annual increase in volume of endurance training appears to follow a linear progression, increasing by approximately 55 hours each year. This increase in volume is primarily due to an increase low-intensity and ski-specific training (Karlsson et al., 2021). Thus, the training volumes of XC ski athletes at younger ages follow similar trends but the amount of annual training hours is significantly reduced.

TABLE 1 Typical durations for a low-intensity training session and annual training volume across different endurance sports. Modified and reproduced with permission (Sandbakk et al., 2021).

TYPE OF SPORT	TYPICAL DURATION OF LOW-INTENSITY TRAINING SESSION (H:MIN)	ANNUAL TRAINING VOLUME (H)
Long-distance running	0:45–1:30	5–600
Road cycling	3:00–5:00	9–1000
Rowing	1:00–2:00	6–800
Swimming	1:30–2:30	9–1000
Cross-country skiing	1:30–2:30	500–600

Nevertheless, training alone will not lead to success, and effective endurance training requires daily planning of training frequency, intensity and duration (Seiler, 2010). With regards to young athletes, one of the most important factors for improving aerobic endurance is training frequency and the training intensity is typically utilized to improve and/or change the utilization and supply of energy (Rusko, 2003). A common training intensity distribution in endurance sports includes around 80% of training sessions performed at low-intensity training (LIT) and 20% performed at around lactate threshold (LT) or as high-intensity training (HIT) (Seiler, 2010). XC skiing frequently follows this 80/20 intensity distribution, and since the end result of endurance competitions, such as XC skiing, are typically performed at intensities above 85% of $VO_{2max,}$ training is often designed to improve the fractional utilization of VO_{2max} (Joyner & Coyle, 2008). However, it is important to recognize that training intensity distribution is still a highly debated topic. Conflicting research following endurance-based runners, observed that a polarized training approach (80/20 intensity distribution) was only present during the competition period and that a pyramidal training intensity distribution, a distribution that is characterized by decreasing training volumes from low to high-intensity, was more prevalent during preparatory and precompetitive periods (Casado et al., 2022). In addition, previous research suggests that accurately following an 80/20 periodization of training intensity is seldom practiced by elite athletes and that individual needs and goals need to be highly considered before determining the most effective training approach (Burnley et al., 2022).

2.1.5 Demands of training and competition

The competitive demands of XC skiing differ greatly from those of other endurance sports. A competition weekend typically consists of races in two separate techniques (classic style and free style; known as ski skating) each with several sub-techniques (Losnegard, 2019). One unique aspect of XC competition is the non-steady work rate that is used throughout the race, as well as a wide variety of techniques (Losnegard, 2019; Sandbakk & Holmberg, 2014). This is largely due to variation in the competition terrain which is required to provide a technical, tactical, and physical assessment of the competitors' abilities, and therefore, consists of – one third of climbing, one third of rolling terrain, and one third downhills (Sandbakk & Holmberg, 2014; FIS, 2022). Furthermore, the speed of XC ski races has drastically increased overtime and the introduction of mass start and sprint races have resulted in tactics playing a major role in competition outcomes. As a result, athletes benefit not only from aerobic capacities but also their individual abilities in anaerobic capacity, upper-body power, high speed techniques and flexibility in their tactical approach (Sandbakk & Holmberg, 2014).

With regards to race distance, slight differences are present when comparing junior and senior athletes. In World Championship competitions, both boys and girls compete in distance races ranging from 10–20 kilometers and sprint races ranging from 1–1.8 kilometers, whereas senior athletes have slightly longer distance races (e.g., males: 15–50 km and females: 10–30 km) (FIS, 2022).

2.2 Important physiological factors in cross-country skiing

2.2.1 Maximal oxygen uptake

As previously indicated, one of the most important physiological factors that significantly influence endurance performance is the uptake and utilization of oxygen. Today, it is universally accepted that there is a physiological upper limit to the body's ability to consume oxygen (Bassett, 2000). Once the maximal amount of oxygen is reached, the value does not change regardless of an increase in workload and/or intensity. The integration of several physiological factors can limit VO_{2max} including: the pulmonary diffusing capacity, cardiac output, the oxygen-carrying capacity of the blood and the muscular system's ability to intake and utilize oxygen (skeletal muscle characteristics) (Bassett, 2000; Poole et al., 2008).

The sport of XC skiing, one of the most demanding endurance sports, requires athletes to utilize combined upper and lower-body effort and therefore, the demands for oxygen are extremely high in the whole-body (Holmberg, 2015). Thus, elite XC skiers have recorded some of the highest VO_{2max} values with men displaying values greater than 80 $mL \cdot kg^{-1} \cdot min^{-1}$ and females displaying values greater than 70 $mL \cdot kg^{-1} \cdot min^{-1}$ (Ingjer, 1992; Tønnessen et al., 2015). Nevertheless, endurance athletes do not perform at 100% of their VO_{2max}, and the mean value utilized during competition is more closely associated with the percentage of VO_{2max} ($\%VO_{2max}$) that is utilized at LT (Bassett, 2000).

The $\%VO_{2max}$ that can be maintained during an endurance event is also influenced by the adaptations that occur with consistent endurance training (Holloszy & Coyle, 1984). This includes skeletal muscle adaptations such as an increase in mitochondria and the respiratory capacity of muscle fibers. These changes decrease the disruption in homeostasis during exercise as well as increase fat utilization, which in turn, slows down the depletion of glycogen (Holloszy & Coyle, 1984). Thus, prolonged endurance training will likely result in an increase in both VO_{2max} and $\%VO_{2max}$ used during exercise (Bassett, 2000).

Unlike endurance running or biking, competitive XC skiing follows a non-steady work-rate and requires various sub-skiing techniques. These different techniques demand different levels of upper and lower-body muscles, and therefore, a unique characteristic of elite XC skiers is their ability to maintain high VO_{2peak}/VO_{2max} ratios, even when using techniques that require less muscle mass (Holmberg, 2015). Furthermore, high demands are placed on the interaction of aerobic and anaerobic systems during both competitive sprint and distance skiing. In competition, various segments (e.g. uphill) frequently require athletes' to perform at workloads above VO_{2peak} and therefore, performance may be highly influenced by an individual's ability to repeat high-intensity periods of work with a high rate of recovery (Losnegard, 2019). Hence, in order to reach success at the elite level, this ability to utilize a high $\%VO_{2max}$ and repeatedly obtain VO_{2peak} in all techniques is critical (Joyner & Coyle, 2008; Losnegard, 2019). Therefore, to reach success, young athletes should not only work at reducing the gap between their VO_{2max} and VO_{2peak} values but also assess individual technical abilities in various ski techniques, terrains, and during all levels of intensity.

2.2.2 Maximal and upper-body strength

Earlier research has shown that upper body endurance and strength/power play a significant role in XC ski performance (Børve et al., 2017; Fabre et al., 2010; Johansen et al., 2021; Sunde et al., 2019). In competitive XC skiing, this often refers to work performed in the technique that involves the highest requirement of upper body work, recognized as the double pole (DP) technique. When comparing pole forces against other sub-techniques, higher poling forces ($\approx$+50 Newtons) were generated in the DP technique (Millet et al., 1998) (FIGURE 7). Subsequently, various measures of maximal muscular strength are frequently used to analyze different DP characteristics. For instance, one study examining the impact of upper body strength on performance found that maximal strength in 1RM pull-down was associated with DP roller ski performance and that higher maximal upper body strength was linked to higher peak forces in the DP technique (Sunde et al., 2019). Additional research assessing upper body strength with bench-press and bench pull revealed that skiers who performed the highest power output in the upper-body measurements also reached higher speeds in the DP technique (Stöggl et al., 2011). Furthermore, when investigating upper body muscular endurance, training studies with as little as 6 weeks in well-trained adults resulted in an improved double pole performance (DPP), suggesting that upper body strength responds well to a proper training stimulus (Børve et al., 2017).

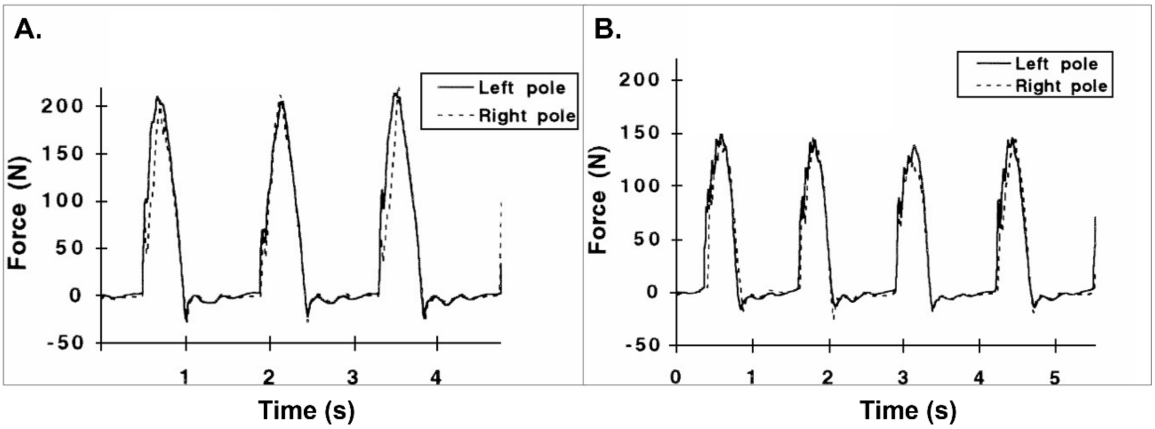

FIGURE 7 Pole forces in left and right pole in the double pole (A) and V2 skating (B) techniques. Modified and reproduced with permission (Millet et al., 1998).

When looking at sex-specific results, upper body strength has displayed a meaningful influence in both male and female skier's DPP (Sunde et al., 2019). However, an increase in maximal strength may not translate to improvements in performance, especially in young developing athletes. For example, one study with young female XC skiers assessed the effects of 10 weeks of upper body heavy strength training and although improvements in upper body strength were present no significant changes occurred in DPP (Skattebo et al., 2016). Alternatively, 12 weeks of heavy strength training in elite XC skiers showed a moderate correlation between muscle strength and ski-specific performance in women, while no correlation was reported for men (Losnegard et al., 2011). Together these findings indicate that a longer training period may be needed before improvements in strength are successfully applied to performance-related measures. Thus, incorporating maximal strength training into training plans may be of interest to skiers (Sunde et al., 2019) and an even greater interest to female skiers, who often display lower levels of upper body strength (Losnegard et al., 2011). However, it is also important to also recognize that XC skiing is a technically complex sport and therefore, regardless of an individual's level of strength, technical skill is needed in order for strength to be transferred into motion (Stöggl et al., 2011).

2.2.3 Other physiological factors in cross-country skiing

The developed complexity of both physiological and technical training needed to reach success in modern XC skiing results in a large variation in how individual athletes adapt and respond to training (Sandbakk & Holmberg, 2014). Furthermore, economy and gross efficiency are also of high importance and have a substantial impact on performance. Previous research comparing different performance levels found that elite skiers had a better skiing economy and higher gross efficiency (+ 4–5%) than junior skiers in both skate skiing technique (gear

3) and classical diagonal stride technique (Ainegren et al., 2013). Similarly, when investigating the effect of incline, skiers with higher ranking also displayed higher gross efficiency in the measured skate skiing techniques (Sandbakk et al., 2013). Interestingly, both studies found no differences between sex, indicating that male and female skiers appear to have a comparable ability to convert metabolic energy into work rate and speed (Ainegren et al., 2013; Sandbakk et al., 2013).

Since most ski-training is carried out during low-intensity training, it is also important to recognize that in order for performance to improve, technical developments need to be transferred into improved economy when skiing at race pace (Rusko, 2003). Young skiers' technique and economy will improve naturally with training, but certain techniques are more challenging and will require various levels of attention. However, in both sprint and distance XC skiing, the ability to convert metabolic power into speed are key determinants of performance (Sandbakk & Holmberg, 2017). Therefore, young athletes should work to strengthen their technical skills as well as improve endurance, so they are able to execute all techniques effectively during both easy and maximal velocities.

2.3 Training and recovery

Training plans and exercises are regularly discussed and evaluated among athletes and coaches, yet nearly all training adaptations take place during recovery. Regardless of the growing body of work in high-performance sports, there is a relatively poor understanding of what methods and tests provide the best insight into how individuals are coping with training (Kellmann & Beckmann, 2018). Although the training-recovery cycle seems simple, it appears one specific method will not achieve the correct balance. Instead, it requires a combination of applicable and comprehensive feedback that contribute to an increased understanding of training/recovery and thus assists in the development of an appropriate training program that also reduces the risk of injury, illness and overtraining (Halson, 2014; Murray, 2017).

2.3.1 Methods to monitor training responses

For optimization of training, both recovery and the training process itself must be regulated and a beneficial balance achieved. In this context performance-related tests are regularly employed to assess objective (physiological, biochemical) and subjective (perceived physical and psychological well-being/mood) factors (Lamberts et al., 2011b; Saw et al., 2016). Thus, effective monitoring of training and recovery is comprised of a combination of psychological, physiological, performance-related and biochemical measures that will be further discussed in the following chapter (Saw et al., 2016; Taylor et al., 2012).

2.3.1.1 Objective measures

In endurance sports monitoring, objective measures include external parameters that evaluate changes which may help facilitate the planning and execution of training. The quantification of performance-related capacities can be measured by monitoring physiological factors (Saw et al., 2016). This is often achieved by conducting standardized exercise tests in an exercise laboratory.

2.3.1.1.1 Submaximal and maximal performance tests

Practical objective measures of physical performance include submaximal, functional performance (jump tests) and maximal tests. Since maximal measures require an individual to reach an exhaustive state, submaximal tests and functional performance tests, i.e. tests with a reduction in overall effort, are more frequently conducted by coaches and athletes to help monitor athlete fatigue (Taylor et al., 2012). Regular monitoring of fatigue levels is valuable for athletes and coaches, and effective tests are able to help predict, monitor and fine-tune an individual's training plan (Capostagno et al., 2016; Lamberts et al., 2009). Applicable tests provide coaches and athletes with a quantifiable variable that displays a close relationship to actual performance and, therefore, is able to predict current performance levels.

Submaximal tests should be relatively short in duration, performed frequently and simple to administer (Capostagno et al., 2016). In addition, findings should be interpreted individually keeping the error of measurement and smallest worthwhile change in mind. When well-controlled testing protocols are utilized even small meaningful changes can be detected and used to help optimize performance (Lamberts et al., 2009). Since running is one of the most common training modalities in both HIT and LIT for XC skiers, submaximal running performance can be used to follow the performance/recovery status in skiers. A submaximal running test is typically designed using a standardized speed and inclination or based individually on heart rate (HR) and/or blood lactate concentrations. This provides an easily repeatable test that can be conducted in various training environments (e.g., training camps, post-travel). Previous literature has shown that higher blood lactate levels at controlled submaximal intensities are often indicative of a decrement in performance (Jacobs, 1986) and that submaximal HR is altered after both short and long-term increases in training load (Bosquet et al., 2008). Changes in HR at submaximal intensities suggest that increases in HR are related to a decline in fitness or parasympathetic overtraining syndrome, and decreases are associated with increases in fitness or a case of sympathetic overtraining (Kenttä & Hassmén, 1998; Kuipers & Keizer, 1988; R. P. Lamberts et al., 2004). However, fluctuations in HR should be interpreted with caution since variation in HR during the final stages of submaximal tests (around 90% maximal HR) has shown to vary by approximately 3 beats per minute (Lamberts et al., 2011a). Thus, a lack of individualization may reduce the reliability of a submaximal test. Intensities ranging from 86–93% of maximal HR (HRmax) are associated with the lowest day-to-day variation, and therefore, submaximal protocols should aim for the final stages of submaximal tests to elicit HR that is approximately 90% of maximal values (Lamberts et al., 2011a).

One of the most frequently used functional performance tests is vertical jump tests. Counter movement jumps (CMJ) are one of the most commonly performed vertical jumps and are a valid test for measuring fatigue and explosive power (Bosco et al., 1983; Taylor et al., 2012). Previous research on endurance athletes has revealed that season-best and season-worst running performances was associated to vertical jump height (Balsalobre-Fernández et al., 2014). This finding suggests that changes in jump height may be an important determinant for endurance athletes. Moreover, XC skiers displayed a positive relationship between maximal skiing speed and jump height (Stöggl et al., 2011). Although peak power, peak velocity, and peak force are all useful variables to monitor, the above results imply that jump height appears to be a valuable tool for monitoring neuromuscular function in XC skiers.

Maximal performance tests are laboratory exercise tests that are used to categorize and measure changes in performance in individuals and have been classified as the gold standard for detecting changes in performance capacity (Greenham et al., 2018). They are also valuable tools to aid in the classification of the various training zones used in endurance training as well as identify shifts in aerobic and anaerobic fitness. In addition, evaluation of an individual's physiological profile may help identify where a new training emphasis should be placed and/or detect the extent of decline in aerobic capacities following an injury or episode of over-training (Shephard, 2008). On the contrary, difficulties arise with the appropriate timing, and the performance of a maximal test may not accurately replicate an athlete's event/competition. In addition, maximal testing will induce an increase of fatigue, and therefore, although they have important applications in sport, implementation needs to consider an athlete's current motivation and upcoming competition schedule (Halson, 2014).

2.3.1.1.2 Salivary and hematological markers

Repetitive use of exercise-based tests may interfere with training and therefore, evaluation of hematological biomarkers that are associated with training adaptations/mal-adaptations are an advantageous method since they are measured under resting conditions (Greenham et al., 2018). Numerous biomarkers consisting of proteins, metabolites, electrolytes, and other small molecules may aid in identifying the balance between training and recovery but reliance on a single marker to provide a sensitive and accurate assessment of recovery is overly simplistic and even valuable biomarkers have limitations (Lee et al., 2017). Hence, to uncover meaningful applications of blood biomarker data, an assortment of various markers related to hydration, nutrition/metabolic health, cardiovascular endurance, muscle status, and inflammation can be monitored (Lee et al., 2017; Pedlar et al., 2019). Examples of the different biomarkers of the blood of which long-term assessment can aid in the detection and prevention of negative training effects or injury are presented in FIGURE 8. Since the insights gained with one single data point are minimal, an integrated and suitable course is necessary.

Recommendations suggest measuring hematological markers before and after the following time points: transitions in training, off-season, a heavy-training load and/or performance test, post-injury and major competitions. Application of blood biomarkers in elite sports is rapidly evolving, and following a comprehensive approach will enable athletes to establish their readiness to train based on a biochemically measurable level (Lee et al., 2017; Pedlar et al., 2019).

Saliva is a complex biological fluid whose compound and production is highly influenced by the sympathetic and parasympathetic nervous system activity (Khaustova et al., 2010; Nunes & Macedo, 2013). As a result, saliva contains several biomarkers that reveal the impact of stress and physical exercise and analysis of salivary composition is non-invasive and well tolerated by athletes compared to venous blood collection methods (Chicharro et al., 1998; Nunes & Macedo, 2013). Constituents of saliva that can be used as biomarkers for monitoring athletes include various proteins, enzymes, inorganic and organic compounds and hormones (Nunes & Macedo, 2013). Comparable to biomarkers of the blood, measurements of saliva should include several different parameters. Although standardization of several variables is still needed, it appears that analysis of salivary compositions' potential value is not only in the field of exercise testing and performance but also immunology, endocrinology and metabolomics (Chicharro et al., 1998).

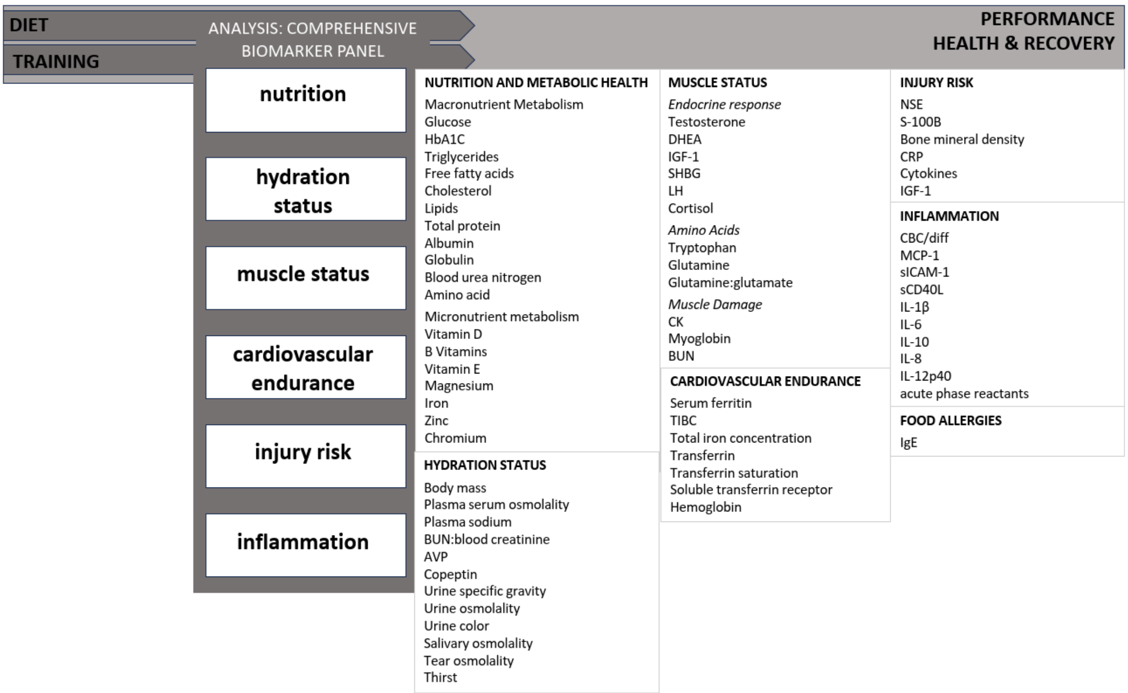

FIGURE 8 Comprehensive biomarker panel of various hematological markers to track performance, health and recovery using an individualized and practical approach. Reproduced with permission (Lee et al., 2017).

2.3.1.1.3 Heart rate measures

Various measures of HR, including resting, exercise, maximal and recovery HR, are of high interest for monitoring fitness, fatigue, and individual response to endurance performance. HR measures are non-invasive, time-efficient, low-cost and can be utilized on a daily basis and simultaneously with a wide range of athletes (Buchheit, 2014). The cardiovascular system plays a fundamental role in the recovery from endurance training. Recovery is a restoration process that is largely regulated by the ANS, and the time needed for autonomic recovery reflects the current state of cardiovascular homeostasis (Stanley et al., 2013). Assessment of autonomic cardiovascular function can be evaluated using measures of HR.

Although coaches and athletes have a high interest in measures of HR (Buchheit, 2014), surprisingly, only a few studies have explored the relationship between nocturnal HR and training status. During a period of intensified training, despite no changes in morning resting HR, a significant increase in nocturnal HR was present, suggesting that HR measures during sleep may present a measure of higher sensitivity (Jeukendrup et al., 1992). Differences in resting and nocturnal HR may be due to the fact that during sleep, there is a reduction in external influences and therefore, increased observability between sleep HR and training status is present (Waldeck & Lambert, 2003).

Assessment of numerous studies on athletes in a fatigued state reveals that when a state of 'overreaching' occurs, a decrease in HRmax is also present (Achten & Jeukendrup, 2003). Additional research found that HRmax was the only measure of HR that responded to both short and long-term increases in training load (Bosquet et al., 2008). In addition, to exercise HR, HRmax can be measured and followed by using a HR monitor. HR monitors have minimal to no interference with training and/or competition and are an affordable way to follow and detect changes in HR (Bosquet et al., 2008). Furthermore, the findings above emphasize the potential useful need that measures of HRmax may have for young athletes who are working to find an appropriate balance of training stress and rest.

Heart rate variability (HRV) signifies the variation of instantaneous HR and variation of time between two consecutive heartbeats. Advancements in technology have presented non-invasive, affordable and reliable means for monitoring HRV in response to daily stress (Giles et al., 2016). HRV is regulated by the sympathetic and parasympathetic activity of the ANS, and two common measures include using time via the root-mean-squared difference between successive RR intervals (RMSSD), and frequency domains (Aubert et al., 2003). When resting, the ANS favors parasympathetic activity (Stanley et al., 2013), increasing HRV and therefore, following resting RMSSD values is an effective way to monitor HRV (Buchheit et al., 2004). Procedures for measuring HRV include nocturnal measures during night sleep, morning and day-time values, as well as values pre, post and during exercise. Yet, despite its common application in both the field and in laboratory settings, mixed results in previous research suggest that HRV alone may not provide a comprehensive view of an athlete's overall well-being and recovery (Buchheit, 2014).

When examining changes in HR, it is critical to keep an athlete's individual training environment and personal training characteristics in mind. As with all monitoring tools, there is a danger of incorrectly interpreting results (Buchheit, 2014). TABLE 2 provides several guidelines that can be used to interpret changes that occur in various measures of HR.

TABLE 2 Guidelines to interpret changes in different measures of HR. Modified and reproduced with permission (Buchheit, 2014).

		Occurrence	Likely Mechanisms	Practical Interpretation
Resting HRV and HR				
Changes in RMSSD	Changes in HR			
↑	↓	Frequent in short-term training program of moderately trained athletes, and/or during the building-up phase of elite athletes (high-volume)	Increase in overall parasympathetic activity	Coping well with training
↑	↑	May occur at the beginning of a training block, likely observed in previously saturated athletes	Increased sympathetic activity which reverse the saturation phenomenon	1. If occurring in short training blocks, increased readiness to perform 2. If not, accumulated fatigue
↓	↓	Frequent during tapering	Increased sympathetic activity	1. If occurring during tapering, increased readiness to perform 2. If not, accumulated fatigue
↓	↑	Frequent in elite athletes or others with a long training history	Increase in overall parasympathetic activity that causes saturation	1. Elite athlete/ athlete with long training history coping well with training, likely high-volume and low-intensity training 2. If prolonged and not reversed with tapering, can inform on an overtraining state
Exercise HR				
	↑	Frequent, in relation to changes in training load	Decrease in relative exercise intensity, plasma volume expansion	Cardiorespiratory fitness improvements
	↓	Frequent, in relation to changes in training load	Likely increase in relative exercise intensity, or only reduction in plasma volume	Unclear; doesn't necessarily indicate decreased performance capacity

2.3.1.1.4 Sleep

Physiological processes that occur during sleep are a fundamental aspect of an athlete's recovery, and growing evidence has emerged addressing the positive relationship between sleep and optimal performance (Samuels, 2008; Simpson et al., 2017). Regardless of improvements in sleep appearing to aid in maximizing athletic success (Simpson et al., 2017), both the quality and quantity of sleep by young athletes have been declining (Samuels, 2008). As a result, it appears that monitoring and improving sleep may be an easy and attainable option for further enhancing training optimization. Currently, recommendations and guidelines regarding the best practice for monitoring sleep in athletes do not exist. As a result, there is a range of different methods to measure sleep as well as a series of steps that may be followed by athletes and coaches who wish to improve sleep (Halson, 2019) (FIGURE 9).

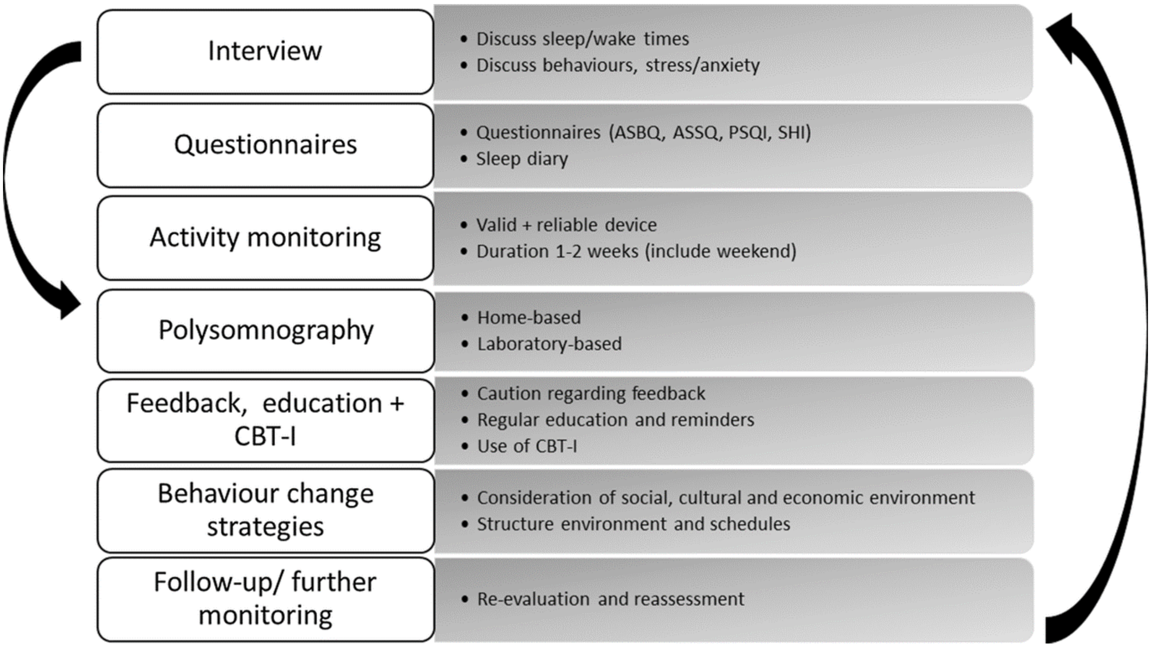

FIGURE 9 Process and procedure for monitoring sleep in athletes. Reproduced with permission, (Halson, 2019).

Polysomnography (PSG), the gold standard for measuring sleep, provides an evaluation of breathing and the different stages of sleep by using a combination of physiological measures, including eye movement, brain activity, muscle activity, heart rhythm, blood oxygen levels and breathing (Bianchi, 2018; Driller et al., 2023; Halson, 2019). However, PSG typically requires a laboratory, is expensive and a rather intrusive method (Halson, 2019). Therefore, in the field of athlete monitoring, where longitudinal measures of sleep are most valuable (e.g., during the competitive season, during training/off-season and when traveling), PSG does not add considerable value. As a result, even though PSG is considered the gold standard, sports scientists and practitioners rarely utilize this methodology in athletes (Driller et al., 2023).

Activity monitors may be a more suitable method for monitoring sleep via actigraphy. Previous research with elite athletes has shown that activity monitors are a valid alternative method to PSG for measuring sleep (Sargent et al., 2016). These devices are small, normally in the form of a watch or ring, and can be worn with little inconvenience. By utilizing accelerometers, sleep is assessed using movement and non-movement periods (Driller et al., 2023). Assessment of sleep/wake time can be easily attained over numerous days suggesting that actigraphy allows for longitudinal sleep monitoring (Halson, 2019).

Further advancing in the area of convenience and compliance when monitoring sleep are sleep devices. These devices can be placed on or near an individual's bed and generally use some form of non-contact monitoring such as ballistocardiography (BCG), light, sound, temperature and/or motion sensors to evaluate sleep (Bianchi, 2018; Driller et al., 2023). BCG-based sleep devices that are placed on the mattress can also measure thoracic movements of respiration and HR measures with their high-sensitivity pressure sensors. This provides similar data that PSG would obtain and shows additional physiological sleep data by providing estimated sleep-wake staging based off individual breathing patterns (Bianchi, 2018).

2.3.1.2 Subjective measures

Additional measures for athlete monitoring include subjective measures. Subjective measures are self-reported measures of perceived psychological and physical well-being that reflect current feelings of mental fatigue, effort, stress and motivation (Coyne et al., 2018; Saw et al., 2016). When compared to objective, subjective measures are easy to administer and a low-cost option that can be utilized routinely by athletes and coaches (Saw et al., 2016). They also enable a compression and/or integration between psychological, biochemical, and physiological information, which is highly relevant for the regulation of performance and recovery in athletes. In addition, facilitating an athlete's ability to transition from coach dependency to practicing their own self-regulation and awareness of health and performance is an important skill to develop, and application of subjective-based monitoring will aid in the advancement of this ability (Montull et al., 2022). As a result, subjective monitoring is beneficial and applicable to all athletes regardless of performance level, sport or economic status (Coyne et al., 2018), making it a good monitoring option also for young athletes.

Surveys and questionnaires

Research has validated a wide range of surveys and questionnaires that can be used to monitor subjective stress and recovery levels. Attraction and popularity of surveys and questionnaires largely result from their ease in their implementation and analysis process (Saw et al., 2015). Self-reported ratings monitored through a competition season revealed that well-being ratings appear to be an efficient monitoring option for overtraining and recovery (Hooper et al., 1995). An additional advantage is that context-specific surveys can help detect where the stress originated and enhance communication and feedback between athlete and coach. Typically, athletes will record training details such as sleep, recovery, nutrition, and data concerning ratings of subjective levels of perceived exertion. Ratings and/or data can then be used to determine the overall training load or help predict future performance (Saw et al., 2015).

One of the most common subjective questionnaires athletes use is the Profile of Mood States (POMS; (McNair et al., 1992)). The POMS is a 65-item self-reported questionnaire that assesses short-term mood in adults aged 18 and above (Searight & Montone, 2020). Research has shown that POMS is a useful tool to aid in the detection of mood disturbance before/after competition, during/following intense training and for longitudinal monitoring (Beedie et al., 2000).

Another frequent measure, known as the Recovery Stress Questionnaire for Athletes (RESTQ; (Kellmann & Kallus, 2001)) was designed with the objective of obtaining information about the stress and recovery process with a sport-specific focus. A 77-item questionnaire is used to assess current stress and recovery. Analysis of RESTQ displayed a dose-response relationship with endurance and training load, suggesting it may be a useful and practical monitoring tool and indicating that psychological stress is an important factor in relation to illness and/or recovery (Coutts & Reaburn, 2008).

Lastly, the 14-item Cohen Perceived Stress Scale (PSS) is an additional survey that was created to measure stress from a psychological perspective (Cohen et al., 1983). Research reveals that changes in individual perceptions of stress/recovery are positively associated to changes in performance, suggesting that communication about feelings and levels of stress have a high level of importance (Otter et al., 2015), especially with adolescent athletes.

As with many monitoring methods, compliance and appropriate evaluation of individual results is of high importance. Although the established questionnaires mentioned above are prevalent, the use of specific and individualized questionnaires are also used to investigate various ratings of recovery and fatigue. Thus, in order to maintain a high level of compliance, factors such as survey length and the type of questions and/or response (e.g. written response vs rating scale or checked box) required need to be considered in order to collect longitudinal data effectively (Robson-Ansley et al., 2009).

Rate of perceived exertion

One of the most widely used psychophysical tools to assess subjective perception of effort during exercise is Borg's 6–20 rating of perceived exertion scale (RPE) (Borg, Gunnar, 1970; Scherr et al., 2013). RPE is an assessment tool that provides immediate and highly usable training information that can be utilized in daily practice. In addition to sports and performance, RPE is commonly used in clinical settings to assess individual effort or exertion during exercise (Losnegard et al., 2021). Previous research reveals that the use of RPE in sport, exercise and rehabilitation has found strong associations with exercise intensity, as well as, additional physiological factors (e.g. HR, oxygen uptake, blood lactate), suggesting that RPE is a valid and practical monitoring tool (Eston, 2012; Scherr et al., 2013). As a result, RPE has been used for monitoring, prescribing and regulating training load and training intensity (Eston, 2012). Thus, it is a valuable option for the evaluation of training and provides coaches and athletes with an overview of differences in individual perceptions of various training sessions (Kellmann & Beckmann, 2018).

3 PURPOSE OF THIS THESIS

The development and training of young endurance athletes has a direct influence on the future of sport, and the current requirement for longitudinal research is high. However, proper execution of training is not the only factor that leads to success in sport, and physical changes due to growth and development and daily levels of stress also influence an individual's adaptation to training. Current advancements in technology have increased the ease and ability to follow and monitor various markers related to stress and training but substantial uncertainty remains on which measures are the most suitable, especially for young athletes. The main purpose of this thesis was to increase the understanding of how young student-athletes, specializing in cross-country skiing and biathlon develop and progress in both free-living and laboratory conditions while attending a sports high school. In addition, performance-related tests that are frequently utilized to monitor training were performed in combination with measures of sleep, nocturnal HR measures and perceived stress levels. Furthermore, associations between the various athlete monitoring/recovery parameters were investigated to determine which measures may provide the highest value for young athletes and coaches.

The specific aims and hypothesises for this study were:

1. To investigate how nocturnal HRV and cortisol vary individually and as a group and examine their association to training volume, intensity, and sleep.

   Hypothesis: Nocturnal HRV will exhibit a negative relationship to salivary cortisol levels in the morning (Stalder et al., 2011). In addition, a reduction in nocturnal HRV will be related to a decrease in sleep duration (Hall et al., 2004) alone or in combination with more intense and prolonged training (Pichot et al., 2000; Vesterinen et al., 2013) as well as an increase in morning salivary cortisol levels (Balsalobre-Fernández et al., 2014).

2. To evaluate the validity between HR and RMSSD (HRV measure) using a ballistocardiographic sensor during night sleep versus morning values obtained during an orthostatic test with a HR monitor. The validity of nocturnal HR and HRV measures would provide a practical way to follow nocturnal HR indices and sleep during long-term measurements.

   Hypothesis: Nocturnal and morning weekly HR and RMSSD values will show agreement, but morning orthostatic values will have a slightly lower RMSSD and slightly higher HR than nocturnal values due to additional thoughts and disturbances that may affect autonomic regulation when awake (Hynynen et al., 2011). Secondly, changes in RMSSD and HR will be related to fatigue (Pichot et al., 2000) and therefore, associated to performance changes in SRT and CMJ tests.

3. To examine the relationship and access changes between various objective measures of training and recovery and subjective stress in young athletes to determine which of these factors exert a significant impact on performance. Also of interest was the potential sex differences in these contexts.

   Hypothesis: Individual perceptions of stress/recovery (perceived stress) will be positively associated with meaningful changes in performance (Otter et al., 2015), and females will report higher levels of perceived stress than males (Brougham et al., 2009; Graves et al., 2021).

4. To investigate the changes that endurance training may induce on performance-related tests and other commonly utilized measures for monitoring training in young athletes. The objective would also be to provide details on these changes that coaches and athletes can apply to help optimize training and performance during this unique stage of development.

Hypothesis: Although adequate volumes of endurance training are present, minimal to no change in VO_{2max} values (Landgraff et al., 2021; Zoppirolli et al., 2020) and a moderate increase in fractional oxygen utilization will occur (Landgraff et al., 2021). In addition, changes in various performance-related tests will display an association with one another.

4 METHODS

4.1 Subjects

Altogether, 37 well-trained young endurance athletes participated in these studies comprising Articles I–IV (TABLE 3). All subjects were students at a sports high-school who trained and competed in cross-country skiing or biathlon all year round. The athletes had at least 3 years of competition experience at the national level. Several subjects participated in numerous studies of the project. All subjects were fully informed of the experimental procedures and provided written consent from a parent, or legal guardian before taking part in this study. A physician screened each subject's health, health history and electrocardiogram (ECG) prior to giving clearance to participate in the study. The ethics committee of the Central Finland Hospital approved the study, and measurements were performed in accordance with the declaration of Helsinki.

TABLE 3 Information of the subjects who participated in the study separated by study.

Study no.	Participants (N)	Age (years)	Height (cm)	Body Mass (kg)	Annual Training (hours)	Article
Study I						I
Males	3	16 ± 1	180 ± 3	69 ± 1	600 ± 71	
Females	5	16 ± 1	166 ± 10	60 ± 11	500 ± 76	
Study I						II
Males	7	16 ± 1	179 ± 2	68 ± 5		
Females	4	16 ± 1	167 ± 9	61 ± 10		
Study II						III
Males	15	17 ± 1	179 ± 7	71 ± 9	544 ± 65	
Females	14	17 ± 1	168 ± 6	63 ± 5	557 ± 54	
Study II						IV
Males	5	17 ± 1	180 ± 5	72 ± 10	587 ± 35	
Females	7	17 ± 1	170 ± 8	63 ± 6	562 ± 53	

Values are mean ± SD. Article I (6 XC skiers and 2 biathletes), Article II (10 XC skiers and 1 biathlete), Article III (22 XC skiers and 7 biathletes), and Article IV (9 XC skiers and 3 biathletes). Annual training hours include training of technical, tactical skills and endurance training. Shooting practice was not included.

4.1.1 General for all studies

All the studies took place during a period of racing and/or endurance training for XC skiing or biathlon while attending a sports high school. Throughout each study, sleep ANS state was assessed daily under free-living conditions during night sleep with nocturnal HRV and HR analysis, collected using a contactless sleep tracking device. Individual training and competition plans were followed throughout testing periods, and thus, measurement weeks were carefully considered with coaches to avoid weeks that included training camps or exams. In addition, the submaximal running and jump tests utilized during measurement periods were both exercises that were already incorporated into the tested individual's normal training period, so a preparatory training period was unnecessary.

4.1.2 Study I

A longitudinal study design was used to collect data during a 7-week training and competition period (FIGURE 10). During this 7-week period, HR and blood lactate during submaximal running tests, as well as salivary cortisol, sleep duration, training volumes and intensities, and nocturnal/morning measures of HRV and HR were monitored. Submaximal running tests were performed every other week, and morning salivary cortisol samples were taken on 3 consecutive days. Daily measures of nocturnal HR and HRV were collected automatically via the contactless sleep device, and morning HR and HRV values were collected with daily orthostatic tests upon waking up each morning. Individual training plans were followed throughout the testing period via electronic training diaries where training intensity, duration and exercise mode were recorded.

The relationships between HRV, salivary levels of cortisol, sleep duration, and blood lactate concentrations during submaximal running that were found during the whole 7-week period are examined in Article I. The agreement between nocturnal and morning values of HR and RMSSD values during a training period with additional performance-related tests to follow changes in performance are investigated in Article II.

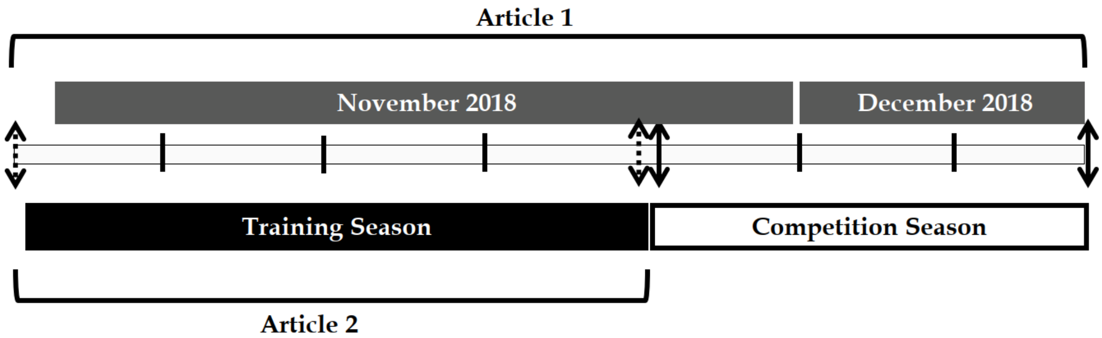

FIGURE 10 Details showing the data collection period of Article I and II during the 7-week longitudinal experiment in study I.

4.1.3 Study II

For study II, a longitudinal study design was implemented to monitor 29 young endurance athletes for a continuous 1-year period. Details concerning the various measurements that occurred throughout the 1-year period are shown in TABLE 4. Due to the longitudinal nature of the study, missing data occurred when subjects were sick or traveling and unable to make the scheduled measurements. As a result, Article III focuses on a 4-month pre-season period of endurance training that took place before the competition season began (FIGURE 11). During this time, subjects were preparing for the upcoming season and therefore, travel was minimal, subject attendance was high, and individual training goals were similar, providing valuable time to examine the various investigated factors related to performance and recovery.

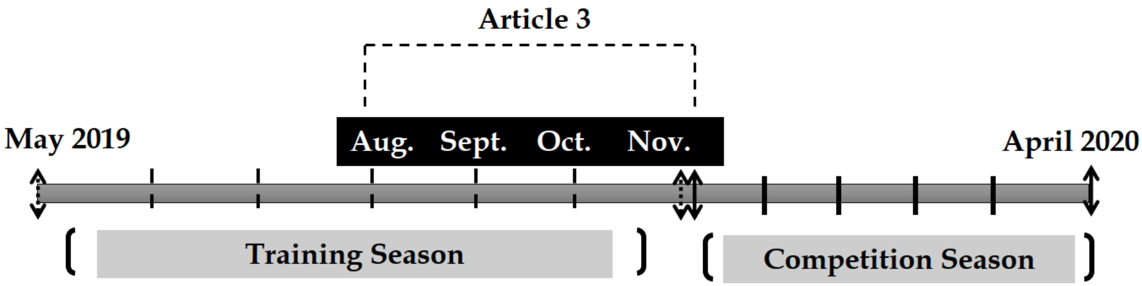

FIGURE 11 Details of showing the 4-month training period that was examined in Article III.

To examine the development and potential influence that endurance training may elicit on young athletes, a 12-month period was selected for further analysis and results are reported in Article IV. Various parameters that are commonly utilized to monitor training (e.g., sleep, HRV, ferritin, hemoglobin, etc.) as well as performance-related tests (e.g., submaximal, maximal, jump performance) were assessed as well as potential relationships involving any changes observed between the various investigated parameters.

TABLE 4 Rate of occurrence for the measurements that were used to monitor and follow development during study II.

	Daily	10x/yr.	3–4x/yr.	1–2x/yr.
Hematological measures				
Basic blood count			X	
Ferritin			X	
Vitamin D			X	
Performance-related tests				
Submaximal running test		X		
Countermovement jump test		X		
Maximal performance tests				
VO_{2max} Nordic walking test				X
Incremental double pole test			X	
Psychological measures				
Perceived stress survey		X		
Nocturnal measures				
HR indices (HR and HRV)	X			
Sleep duration	X			
Sleep latency	X			

4.2 Data collecting and recording methods

4.2.1 Measures of maximal oxygen uptake

Maximal oxygen uptake was measured using a Nordic walking test. Subjects performed a continuous, incremental, maximal test by walking or running with poles on a large motor-driven treadmill (Telineyhtymä, Kotka, Finland). The test started at an inclination of 2.8° with a speed of 5.5 km $\cdot h^{-1}$ for males and at an inclination of 3.5° and speed of 5.0 km $\cdot h^{-1}$ for females. Every three minutes, the workload increased so that the predicted oxygen uptake, calculated using the equation by Balke & Ware (1959), was raised by 6 mL $\cdot kg^{-1} \cdot min^{-1}$ in every stage. This testing protocol is a classic method used as a maximal test by athletes and coaches in Finland and therefore, subjects were familiar with this testing protocol. The test was considered maximal when the subject demonstrated clear signs of maximal effort, such as, unsteady gait and/or an inability to continue, despite strong verbal encouragement. To ensure safety was met, participants wore a harness that was attached to a rope which hung from a frame in the ceiling above the treadmill.

Prior to each test, resting values of HR and blood lactate were recorded and volume and gas calibration of the ergospirometer was performed. Throughout the test, breathing gases were measured using a mixed chamber system (Medikro 919 Ergospirometer, Medikro Oy, Kuopio, Finland). During the last 60 seconds of each stage, the VO_{2max} (highest 60 s average) and respiratory exchange ratio (RER) were recorded. Heart rate was continuously observed using a Polar H10 heart rate belt (Polar Electro Oy, Kempele, Finland) and the average HR from the last 60 s of each stage was recorded. Blood lactate samples were collected from the fingertip and directly placed into a capillary tube (20 µL) that contained 1-mL of a hemolyzing solution. Collection occurred during the last 10 seconds of each stage, and after completion of the test, samples were immediately analyzed using the Biosen C-line analyzer (EKF diagnostics, Barleben, Germany). The time to exhaustion (TTE) was defined as the total time (in minutes) that the subject was walking and/or running on the treadmill.

4.2.2 Measures of lactate threshold

Determination of LT was identified by utilizing the data obtained from the VO_{2max} tests. Previous research has suggested that a continuous incremental running treadmill protocol that uses three-minute stages is a reliable and valid method to assess LT (Medbø et al., 2000). LT was determined by adding 1.5 mmol $\cdot L^{-1}$ to the warm-up lactate value (i.e., measured after the first stage). This method is in accordance with and recommended (Medbø et al., 2000) by previous research (Helgerud el al., 2007, Helgerud, 1994). Additionally, further analysis of the percentage of oxygen utilization ($\%VO_2$) at the defined LT was calculated based off the highest VO_2 value (VO_2peak) that occurred during the maximal test.

4.2.3 Measures of submaximal running

Submaximal running tests (SRT) were used to evaluate aerobic fitness and to monitor athletes' performance-related characteristics and/or recovery. The SRT included 4 incremental stages and was a total of 16 minutes in length. All tests were performed on a Tunturi GO RUN 50 motorized treadmill (Tunturi Fitness, Flevoland, Netherlands). Throughout the test, speed was standardized (females: 10.0 km $\cdot h^{-1}$, males: 11.7 km $\cdot h^{-1}$), and every 4 minutes, the inclination increased, starting at 2%, then 4%, 7% and 9%. The following protocol was designed to elicit HR values at approximately 90% of HRmax, so meaningful changes in HR variation were measurable (Lamberts et al., 2004). One familiarization test was provided to all subjects. However, most subjects had previous exposure, so a familiarization was not necessary. Although we do not have validation of this current protocol, it was designed for junior athletes, and it is a classic method utilized as a control test by XC skiers and biathlon athletes and coaches in Finland. Therefore, with the highly homogenous nature of the researched group, the utilized protocol was appropriate, and submaximal intensities were reached for all subjects.

Prior to arrival, subjects were instructed to complete a 15-minute warm-up running at a self-selected submaximal speed. Before the test began and during the last 30 seconds of each stage, subjects were shown short descriptions of the Borg-Scale (Borg, Gunnar, 1970) to aid in determining their current overall rating of perceived exertion. HR was followed during the whole test with a HR-monitor (Polar V800, Polar Electro Oy, Kempele, Finland) and recorded when 15 seconds of each load remained. At the end of each stage, blood lactate samples were obtained from the fingertip and placed into capillary tubes (20 µL) which included 1-mL hemolyzing solution. Analysis of samples was processed once the test was complete following the manufacturer's instructions (EKF diagnostic, C-line system, Biosen, Germany).

4.2.4 Measures of countermovement jumps

Performance of CMJ (Bosco et al., 1983) was used to evaluate athletes' current level of explosive power and/or recovery. A warm-up comprised of 15-minutes of running at a self-selected speed was conducted prior to the testing of jump performance. Subjects were directed to jump as high as possible while keeping their hands fixed to their hips, feet shoulder-width apart and bending their knees to a 90-degree angle. Each subject performed three jumps with about 1-minute of recovery between the jumps, which were conducted on a force plate (HUR FP8, HUR Oy, Kokkola, Finland). Jump height was calculated from impulse (Linthorne, 2001) using the Coachtech system (Vuokatti Sports Technology Unit, University of Jyväskylä, Finland, (Ohtonen et al., 2015)). The highest jump was recorded as the current measure of performance. CMJ is a commonly used (Taylor et al., 2012) and valid test for measuring the effect of fatigue on explosive power (Bosco et al., 1983).

4.2.5 Measures of ski-specific performance

Measures of ski-specific performance were evaluated using a maximal DPP incremental treadmill test, which was performed on a roller ski treadmill (Rodby Innovation AB, Vänge, Sweden) with Marwe 800 XC roller skis (Marwe Oy, Hyvinkää, Finland) equipped with prolink bindings (Salomon Group, Annecy, France) and standard 6C6 wheels (Marwe Oy, Hyvinkää, Finland). Each subject was instructed to use their own classic ski boots and poles. A customized tip, specific for roller skiing on a treadmill, was then placed onto the subject's ski poles. Following an individual self-selected warm-up outside the laboratory, subjects performed a 10-minute warm-up on the roller ski treadmill at a workload equal to the first stage of the test. During the final 3-5 minutes of the warm-up, each subject performed two 12–15 s sprints at a workload equal to the fourth through sixth stage of the test. This ensured that the poles and additional equipment were working adequately, and the subject felt ready for a hard effort.
Ski-specific performance was then evaluated using an incremental treadmill test in the double pole technique at an inclination of 2°. Males started the test at 13 km $\cdot h^{-1}$ and females at 10 km $\cdot h^{-1}$. During the maximal text, inclination was constant, and speed increased by one km $\cdot h^{-1}$ every minute until volitional exhaustion. A harness was attached to a rope which hung from a frame in the ceiling above the treadmill for safety. Time to exhaustion (TTE) and HR (during each stage) were recorded. This testing protocol was familiar for all subjects, and verbal encouragement was used to help individuals obtain their best effort.

4.2.6 Measures of HRV and sleep

Measures of nocturnal HR, HRV, sleep latency and sleep duration were measured using a portable bed sensor. This device (EMFIT QS, Emfit OY, Jyväskylä, Finland) consists of a contactless pressure sensor (542mm x 70mm x 1.4mm) that utilizes BCG to numerically and graphically depict repeated movements, such as heartbeat (E. Pinheiro et al., 2010) (FIGURE 12). Evaluation of this device has shown that clinically it is an acceptable method for continuous monitoring of sleep (Hendriks et al., 2021) and measures of onset sleep latency (Kholghi et al., 2022). In addition, assessment in a free-living environment revealed good agreement to the validated reference values for both nocturnal HR and HRV (Vesterinen et al., 2020). Hence, it appears to be an effective and convenient method for long-term monitoring of young endurance athletes.

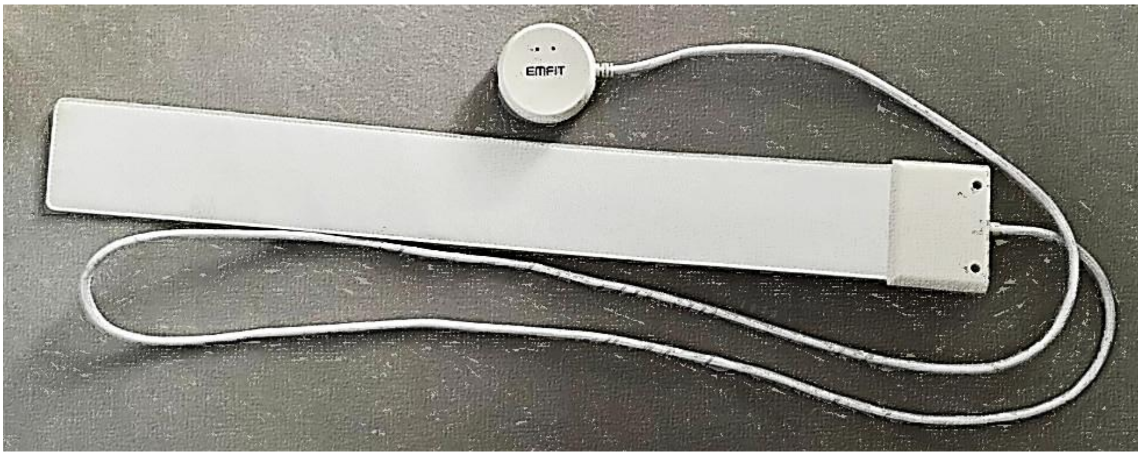

FIGURE 12 Contactless sleep device that was used to collect nocturnal measures of HR and sleep.

Subjects were instructed to place the sleep tracking devices under their mattress near the chest area. The recording process began automatically once body weight was sensed and stopped when the subject exited the bed each morning. The subjects were unable to detect any presence of the device, and the device recorded at a sampling rate of 100 Hz during continuous periods consisting of 3-minutes. The user interface of the sleep device automatically presents nocturnal HR and sleep values. As a result, in order to increase the likelihood of real-life application, automated values were used for analysis. Values for nocturnal HR were determined by calculating the average HR value during the entire night of sleep. Since nocturnal HRV values can significantly change during the night, nocturnal HRV were presented as two different measures of HRV, evening and morning values. To determine morning (HRVam) and evening (HRVpm) values, a linear line of best fit was applied to the graphical representation (relative to time) of all RMSSD values and the endpoints of the line were selected to represent the average RMSSD values for the evening (HRVpm) and morning sleep (HRVam) (Mishica et al., 2022) (FIGURE 13). Weekly averages were established for all measures of HR and HRV, and average values were used for further analysis. Criteria for the calculation of weekly values followed the recommendation of previous literature that suggested a minimum of three days is required in order to obtain weekly HRV values with accuracy (Plews et al., 2014).

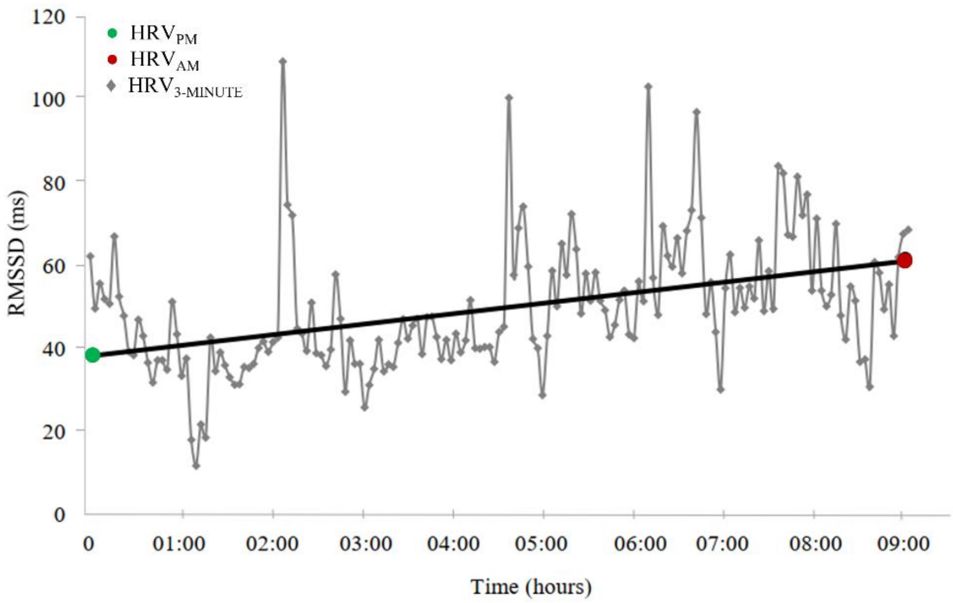

FIGURE 13 Representative graph of the 3-minute sampling of the root mean square of successive differences between RR intervals (RMSSD) of heart rate variability during sleep.

4.2.7 Measures of blood variables

Basic blood count

Measures of basic blood count were taken in the morning during a fasted state in a seated position with the arm extended. Venous blood samples were obtained from the antecubital vein by a qualified lab technician. For analysis of basic blood count, samples were drawn using sterilized needles into EDTA tubes (Greiner-Bio-One GmbH, Kremsmünster, Austria), and further analysis was instantly performed using the Sysmex XP300 analyzer (SysmexCo., Kobe, Japan).

Serum blood variables

Serum blood samples were collected in the morning during a fasted state in a seated position with the arm extended. Venous blood samples were obtained by a qualified lab technician from the antecubital vein. For measures of blood serum, blood samples were drawn into Vacuette gel serum tubes (Greiner-Bio-One GmbH, Kremsmünster, Austria) and after 10 minutes of centrifuging at a rate of 3,600 rpm serum was collected. Serum samples were immediately frozen to −20°C for further analysis. For analysis of serum ferritin levels (Fer), serum samples were analyzed with Siemens Immulite 2000 XPI analyzer (Siemens HealthcareLianberis, United Kingdom), where the serum ferritin was determined by using the immunometric chemiluminescence method. The sensitivity of the assay for ferritin was 0.4 $\mu \cdot L^{-1}$, and the precision (CV%) for the assay was 4.6%. For the determination of serum vitamin D levels (Vit D), analysis of serum total 25-hydroxyvitamin D (e.g. serum 25(OH)D) was obtained using electrochemiluminescence immunoassays (ECLIA).

Blood lactate
Blood lactate samples were obtained from the subject's fingertip and placed into capillary tubes (20 µL), which included 1-mL hemolyzing solution. Analysis of blood lactate was performed using a Biosen C_line lactate analyzer (EKF diagnostic, C-line system, Biosen, Germany).

4.2.8 Measures of salivary cortisol

Measures of cortisol were determined with an analysis of morning saliva samples. Prior to the first testing period, subjects were informed on how to correctly handle and collect saliva samples. After waking up, subjects were instructed to immediately collect saliva samples prior to drinking, eating or brushing their teeth. Small cups were provided so if needed, subjects could take 100 mL of water to wash out their mouth before beginning the saliva collection process. Salivary collection occurred using the passive drool-method, and subjects were instructed to provide at least 3 mL of saliva in each sample. The time of day, date, and length of collection time were all recorded. Once samples were complete, they were immediately placed into the subject's freezer. The collection process occurred every other week for 3 consecutive days during a 7-week period (Article I).

Analysis of saliva samples was performed using the chemiluminescence method with the IMMULITE 2000 XPi Analyzer (Siemens Healthcare Diagnostics Products, Ltd., Glyn Rhonwy, Llanberis, UK). The sensitivity of the saliva assay for cortisol was 5.5 $nmol \cdot L^{-1}$. Analysis of salivary cortisol included three-day average values to coincide with remaining testing values and/or periods.

4.2.9 Measures of perceived stress

Measures of perceived stress were evaluated using a stress survey. The 14-item Cohen Perceived Stress Scale (PSS) was implemented monthly to assess individual levels of perceived stress. The PSS is a reliable and validated psychological tool that was developed to determine individual levels of perceived stress from a psychological perspective (Cohen et al., 1983; Hynynen et al., 2006). It consists of various negative and positive items, with the negative component intended to evaluate the lack of control and the positive element focused on the individual's ability to cope with existing stressors (Walvekar, 2015). Assessment of each item was performed on the five-point Likert-type scale, ranging from 0, "Never" to 4, "Very Often". Total scores range from 0–56, with higher scores indicating that a higher level of perceived stress is present. Total scores may be further categorized into three different levels: scores 0–13 indicating low stress, scores 14–26 indicating moderate stress and scores 27–40 indicating high stress (Hrozanova et al., 2019). Upon arrival to the testing laboratory, subjects were instructed to sit down and fill out the stress survey. To ensure that subjects were well rested and able to properly answer all questions without any external influence, stress surveys were conducted prior to the performance of additional testing.

4.2.10 Measures of training volume

Throughout the whole research period, subjects followed and reported their individual training plans. All participants recorded training data in electronic training diaries (elogger.net, Espoo, Finland). The subjects were familiar with recording training electronically and had previously reported training in this manner for at least one year prior to the start of this research. In addition, individually determined training zones, based on prior maximal exercise tests, were utilized to guide training intensity and physiological monitoring of training occurred with the aid of individual HR monitors (additional monitoring of blood lactate occurred on occasion).

Due to the longitudinal design of this work, variations in how training intensity was reported were present. For study 1, endurance training intensities were based on a 5-zone training zone distribution with zone 1 and 2 representing basic training (estimated: ≤ 2 mmol blood lactate) and zone 3–5 representing intensity training. Easy training (zone 1 and 2) was defined as all training below aerobic threshold and hard training (zone 3–5) was defined as all training above aerobic threshold. For study 2, training intensity distribution was separated into three different training intensities: low-intensity training (LIT, blood lactate < 2.0 $mmol \cdot L^{-1}$), lactate threshold training (LTT, blood lactate < 4.0 $mmol \cdot L^{-1}$) and high-intensity training (HIT, blood lactate < 4.0 $mmol \cdot L^{-1}$). The mode of training was also evaluated, and the three most frequent training modes were reported, including: running, skiing and roller skiing. Daily training and/or competitions were recorded throughout the study period, and training was analyzed according to the electronic training diaries.

4.3 Data and analysis and processing

In all articles, standard statistical methods were used to check the normality of the data and descriptive statistics were calculated and reported as means and standard deviations (SD). Unless otherwise stated, all statistical analyses were performed using SPSS version 26 (IBM SPSS Statistics 26, IBM GmbH, Munich, Germany), or the previous version of it. Statistical significance was set at $p < 0.05$.

4.4 Statistical methods

In Article I, where the subject number was relatively low, changes in training, HRV and salivary cortisol levels were analyzed using Friedman's non-parametric test for related samples. Post-hoc analyses were performed with the Wilcox signed rank test and relationships, as well as relative changes, between HRV, cortisol, sleep duration and training characteristics were assessed with Spearman's correlation coefficients.

Article II applied a within subject approach by utilizing paired t-tests to examine the differences between nocturnal and morning measurements. Agreement was assessed using Pearson's correlation coefficient, and the difference between measures made in the morning vs. during the night were assessed by determining the coefficient of variance and 95% confidence intervals.

Reliability between the various nocturnal HRV measures was measured using Intra-class correlation coefficients (ICC), and ICC estimated were calculated with a single-rating, constancy-agreement, 2-way mixed-effects model (Koo & Li, 2016).

For Article III, linear mixed-effect models (Pinheiro & Bates, 2000) were used to test the significance of within-sex changes in variables over time. Separate of sex, linear mixed-effect models were fitted using PSS scores, jump, and running variables as a response with time as a fixed covariate. Due to the longitudinal nature of the data, random subject variables were added in mixed-effect models. Analyses were carried out using the nlme package (Pinheiro et al., 2022). Sex differences and time-sex interactions were examined with a combined dataset with sex and sex-time interactions as covariates. Standardized effect size (Hedge's *g*) was calculated to interpret the magnitude of differences over time within sex. In addition, the effects of PSS scores on various variables of sleep, running and jump performance were also examined with several linear mixed-effect models. Sex was added to each model to account for changes between males and females, and the pooled estimate was obtained using the R package broom.mixed (Bolker & Robinson, 2022).

In Article IV, a Shapiro-Wilk test was performed following a linear mixed model to determine the effects of one-year endurance training in young athletes. In longitudinal data, the use of linear mixed-models for analysis has widely been used when measurements of the same subjects are repeated overtime. This provides an assessment of within-subject and between-subject changes over time. The mixed-model analysis was comprised of test (test 1 and test 2), sex (male or female), and interaction terms between treatment and tie as fixed effects with subject as a random effect. Hedge's *g* effect size (ES) was calculated to represent the magnitude of differences between tests and were interpreted as 0.2 small, 0.5 medium, and 0.8 large. Descriptive analysis was used to evaluate differences, independent of sex and Pearson's correlation coefficients were used to determine the associations between various variables.

5 RESULTS

The main results of the thesis are presented here, while the complete results can be found in the original Articles I–IV.

5.1 Heart rate variability, cortisol, sleep and training in young athletes (Article I)

A summary of weekly values during the 7-week research period (study I) is presented in TABLE 5. With regards to daily values, values for HRV and cortisol are shown in FIGURE 14. Throughout the study, HRV and salivary levels of cortisol were inversely related ($r = -0.552$, $p = 0.001$). The most pronounced correlation on a weekly basis between HRV and cortisol occurred during week 1 and week 7 ($r = -0.833$, $p = 0.010$), with moderately negative correlations for all remaining test weeks.

TABLE 5 HRV, morning salivary levels of cortisol, nocturnal heart rate and sleep, amounts of hard and easy training, overall training load, and blood level of lactate and heart rate during submaximal running tests (SRT) at different time-points during the 7-week study period (means ± SD).

	T1, week 1	T1, week 3	T2, week 5	T2, week 7
RMSSD (ms)[a]	66.9 ± 6.3	75.0 ± 6.0*	75.6 ± 10.1	67.0 ± 8.8*
Cortisol ($\mu g \cdot L^{-1}$)[a]	6.7 ± 0.9	6.3 ± 1.1	5.6 ± 0.7	7.0 ± 0.9
Hard training (min)[b]	44 ± 30	64 ± 28	45 ± 17	53 ± 18*
Easy training (min)[b]	508 ± 190	555 ± 151	743 ± 204	577 ± 250
Training load[b]	616 ± 246	714 ± 209	856 ± 283	710 ± 132*
Blood lactate ($mmol \cdot L^{-1}$)[c]	3.8 ± 1.0	3.6 ± 0.6	3.5 ± 0.6	3.7 ± 1.1
Heart rate (bpm)[c]	183 ± 7	184 ± 8	180 ± 9	182 ± 9
Sleep (min)[a]	482 ± 29**	513 ± 36	513 ± 55	527 ± 54
Nocturnal heart rate (bpm)[a]	58 ± 5**	55 ± 4	55 ± 3	54 ± 5

[a]Average 3-day values for the nocturnal root mean square of successive differences between RR intervals (RMSSD), nocturnal heart rate, night sleep and saliva levels of cortisol. [b]Weekly values. [c]Average during the final stage of the SRT. *$p < 0.05$ compared to the previous week. ** $p < 0.05$ when compared to week 7.

During the training period (weeks 1–3), changes in sleep and cortisol demonstrated a negative correlation (r = –0.762, p = 0.028) and the same relationship was displayed between the amount of hard training and sleep (r = –0.762, p = 0.028). Once the training period shifted to the early competition phase (weeks 3–5), changes in the amount of hard training displayed a strong positive relationship with changes in salivary cortisol (r = 0.810, p = 0.015). At the same time, changes in HRV during the transition to the competition phase (weeks 3–7) displayed a positive relationship with both hard training (r = 0.738, p = 0.037) and training load (r = 0.810, p = 0.015).

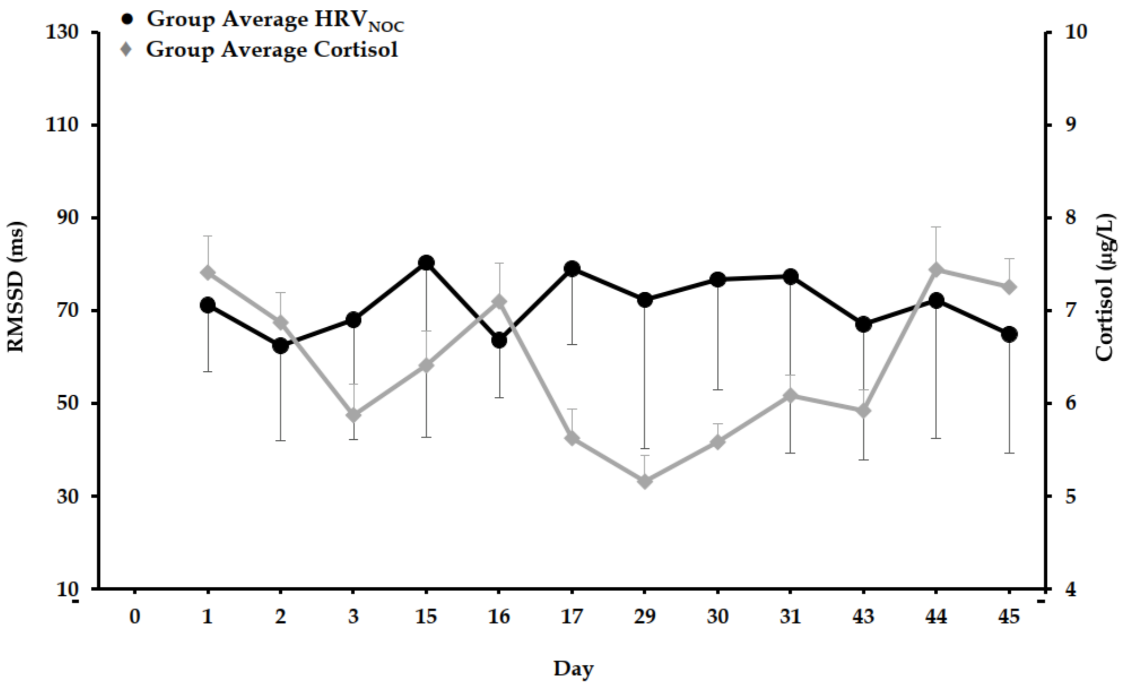

FIGURE 14 Mean (±SD) daily values during the 7-week testing period of average root mean square differences between RR intervals (RMSSD, ms) and morning salivary cortisol. Modified and reproduced (Mishica et al., 2021).

The response in cortisol to changes in submaximal running tests altered throughout the investigated period. The initial differences (during week 3–7) revealed a strong negative association between submaximal HR ($r = -0.929$, $p = 0.003$) and blood lactate concentrations ($r = -0.857$, $p = 0.014$). However, during the final weeks of the testing period (weeks 5–7), cortisol levels increased and displayed a strong positive relationship with submaximal running HR ($r = 0.929$, $p = 0.001$).

5.2 Validation of nocturnal measures for HR and HRV (Article II)

Results presented in Article II are from a 3-week training period that was included in the data that was obtained during study I (FIGURE 10). Results indicate that nocturnal HR and HRV values, collected under real-life conditions with a BCG device, reveal good agreement with morning values derived from orthostatic tests (FIGURE 15).

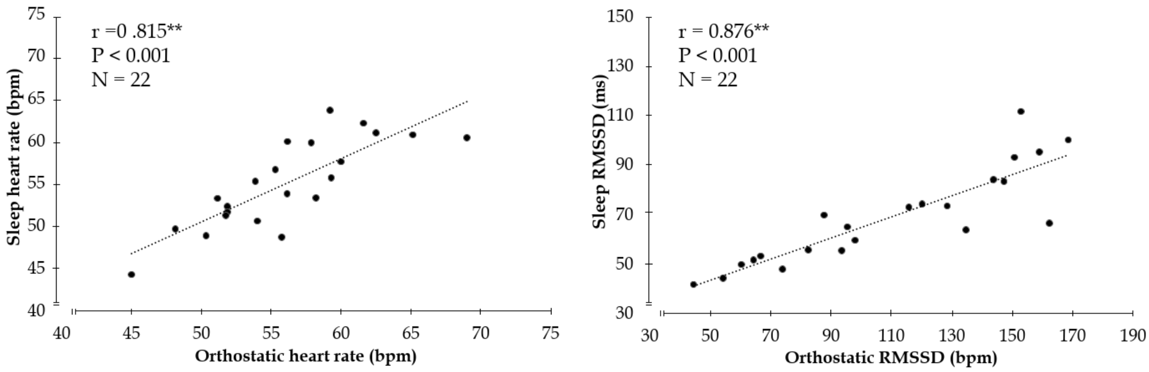

FIGURE 15 The relationship between nocturnal (sleep) and morning measurements for HR and RMSSD during a 3-week training period in young athletes. Modified and reproduced (Mishica et al., 2022).

No significant differences were found in HR values between the measurements obtained during night sleep vs. morning orthostatic tests. In contrast, significant differences were found for HRV values between devices ($p < 0.008$), but very high correlations were also observed during both week 1 ($r = 0.895$, $p < 0.001$) and week 3 ($r = 0.878$, $p < 0.001$) for whole night average HRV measures. This resulted in moderate to good reliability for all HRV measures.

No significant differences were found for performance-related tests. Paired samples t-tests revealed minimal differences between week 1 and week 3 for nocturnal HRV (mean difference 6.8%, $p = 0.344$), HRV_{AM} (mean difference 13.4%, $p = 0.151$), morning HR (mean difference –3.9%, $p = 0.063$), SRT HR (mean difference –0.7%, $p = 0.447$), SRT blood lactate (mean difference 4.9%, $p = 0.781$), CMJ (mean difference –4.2%, $p = 0.122$) and training volume (mean difference 16%, $p = 0.499$). FIGURE 16 shows a visual comparison of changes that occurred in two subjects during the 3-week study period.

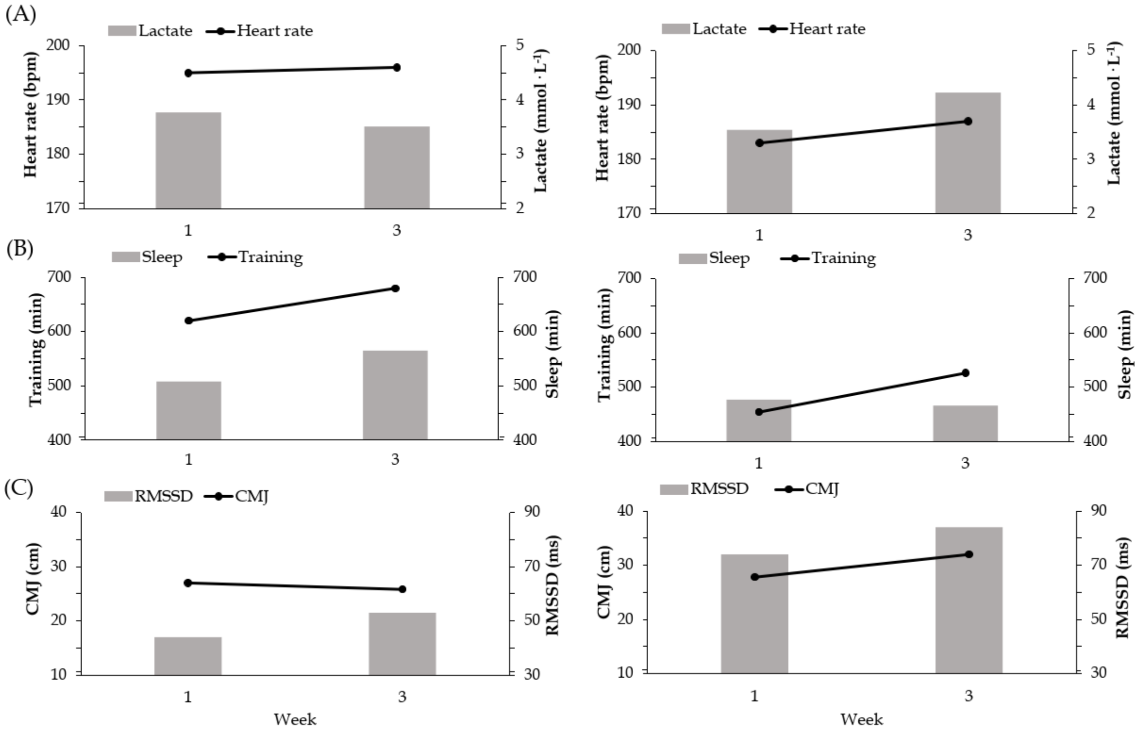

FIGURE 16 A comparison of performance-related tests, training and nocturnal measures of two subjects during the study period. (A) Submaximal running test blood lactate and heart rate values during week 1 and week 3 for two different subjects. (B) Average values for night sleep and training during week 1 and week 3 for two different subjects. (C) Counter movement jump height and nocturnal RMSSD values during week 1 and week 3 for two different subjects. Modified and reproduced (Mishica et al., 2022).

5.3 Performance-related characteristics and stress in young athletes (Article III)

Results presented in Article III are from a 4-month training period that took place during study II. Analysis of the total study population indicated that for every 1 hour increase in sleep duration, the PSS score is estimated to decrease by 2.8 points (B = –2.789, p = 0.013) and blood lactate concentrations during submaximal running are estimated to decrease by 0.62 mmol · L^{-1} (B = –0.623, p = 0.044) (TABLE 6). An additional negative relationship was found between SL and CMJ (B = –0.092, p = 0.087) as well as HR during the low-intensity running of SRT (B = –0.004, p = 0.025).

TABLE 6 Pooled regression estimates of perceived stress scores and variables associated with jump performance and their 95% confidence intervals.

	PSS score		Jump	
	B	95% CI	*B*	95% CI
Intercept	28.58*	(0.29, 56.88)	43.49***	(30.44, 56.55)
Sex (female)	5.93*	(1.09, 10.77)	–6.05***	(–8.40, –3.69)
PSS score			–0.04	(–0.16, 0.09)
Sleep HR	0.17	(–0.25, 0.59)	–0.14	(–0.32, 0.04)
Sleep duration	–2.79**	(–4.95, –0.62)	0.40	(–0.78, 1.58)
Sleep latency	–0.03	(–0.29, 0.23)	–0.09	(–0.20, 0.01)
HRV_{AM}	0.01	(–0.07, 0.09)	0.00	(–0.03, 0.04)

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, HR = heart rate, HRV_{AM} = Morning nocturnal heart rate variability, PSS = perceived stress score

Assessment of jump performance revealed a slight increase in both males (6.1%) and females (1.2%), with a significant time-sex interaction in males (p = 0.005). Sleep HR and perceived stress levels increased significantly (6.3%, p = 0.004 and 31.3%, p = 0.015, respectively), whereas the respective changes were much smaller in females (sleep HR: < 1%, p = 0.231 and PSS: 15.5%, p = 0.431). An effect of sex was present for PSS and jump performance, with males obtaining a higher jump height and lower PSS than females. When comparing sex differences, significant differences were found in several items of PSS responses (FIGURE 17).

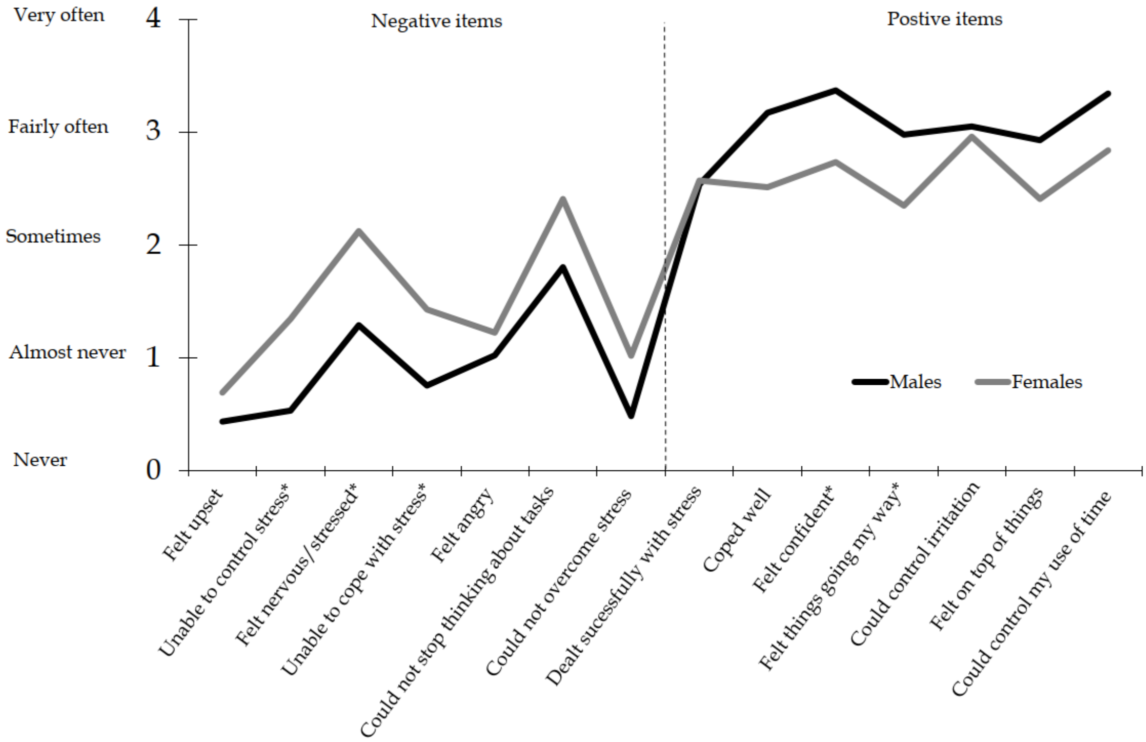

FIGURE 17 Scores on the different items of the perceived stress test during the pre-season training. *Significant difference between sex ($p < 0.05$).

5.4 Changes induced by endurance training in young athletes (Article IV)

Results presented in Article IV illustrate what one year of endurance training may exhibit on various performance-related measures of young athletes. In addition, the potential relationships between any changes observed to one another and/or to perceived levels of stress and certain blood parameters are also presented in TABLE 7.

TABLE 7 Mean (±SD) changes in performance-related variables, blood parameters and perceived stress in male and female cross-country skiers after 1-year of endurance training.

	Males, n = 5			Females, n = 7		
	Test 1	**Test 2**	**% Change**	**Test 1**	**Test 2**	**% Change**
VO_2max						
mL ·kg^{-1} ·min^{-1}**	68.4 ± 4.3	68.0 ± 2.7	-0.2 ± 7.8	54.3 ± 4.3	53.4 ± 4.3	-1.3 ± 8.1
L ·min^{-1}**	4.9 ± 0.6	5.1 ± 0.7	3.2 ± 6.7	3.4 ± 0.3	3.4 ± 0.3	0.3 ± 8.3
TTE (min)**	24.8 ± 2.2	24.9 ± 1.1	0.6 ± 5.9	20.4 ± 1.2	19.9 ± 1.1	-2.0 ±8.6
LT						
% VO_2max	85.4 ± 7.4	83.9 ± 6.8	-1.5 ± 6.9	80.8 ± 4.7	83.4 ± 5.1	3.4 ± 6.7
Time LT (min)	17.2 ± 1.0	16.8 ± 2.0	-2.4 ± 8.9	13.2 ± 1.1	13.4 ± 1.1	2.0 ± 5.2
DP						
TTE (min)*	8.5 ± 1.8	9.5 ± 1.4	13.1 ± 9.3	8.0 ± 0.9	8.6 ± 0.9	8.6 ± 7.4
HRmax (bmp)	196 ± 9	194 ± 7	-0.8 ± 1.5	197 ± 8	195 ± 7	-1.0 ± 2.0
CMJ						
Height (cm)**	36.6 ± 4.2	37.1 ± 4.9	1.3 ± 7.0	30.6 ± 4.2	29.8 ± 5.0	-2.8 ± 8.4
Blood parameter						
Ferritin (ug · L^{-1})**	68.5 ± 15.8	68.3 ± 19.9	-0.7 ± 36.4	47.9 ± 26.9	38.0 ± 17.3	-5.4 ± 72.2
Hemoglobin (g · L^{-1})**	151 ± 6	155 ± 4	1.9 ± 3.8	143 ± 7	145 ± 9	1.1 ± 4.4
Vitamin D (nmol · L^{-1})*	68.8 ± 35.9	82.2 ± 39.4	11.5 ± 19.2	81.4 ± 20.0	97.5 ± 25.1	4.5 ± 52.7
Perceived Stress						
PSS (score)	12.6 ± 6.0	19.6 ± 12.5	66.7 ± 81.0	21.4 ± 9.4	23.9 ± 11.5	12.7 ± 56.2

VO_{2max}, maximal oxygen uptake; TTE, Time to exhaustion during test; LT, lactate threshold; DP, double pole test; HRmax, maximal heart rate during the double pole test; CMJ, countermovement jump test; PSS. Perceived Stress Survey.
*Significant effect of time ($p < 0.05$) on the linear mixed model (LMM) for each sex.
**Significant difference in LMM ($p < 0.05$) between the males and females.

5.4.1 Performance tests

Ski-specific double pole test performance improved by 10 ± 8% ($p < 0.001$). Detailed analysis of individual performances revealed that 10 subjects improved, and the remaining two subjects displayed a decline of 0-2% (FIGURE 18). No significant correlations were displayed between DPP and the remaining variables.

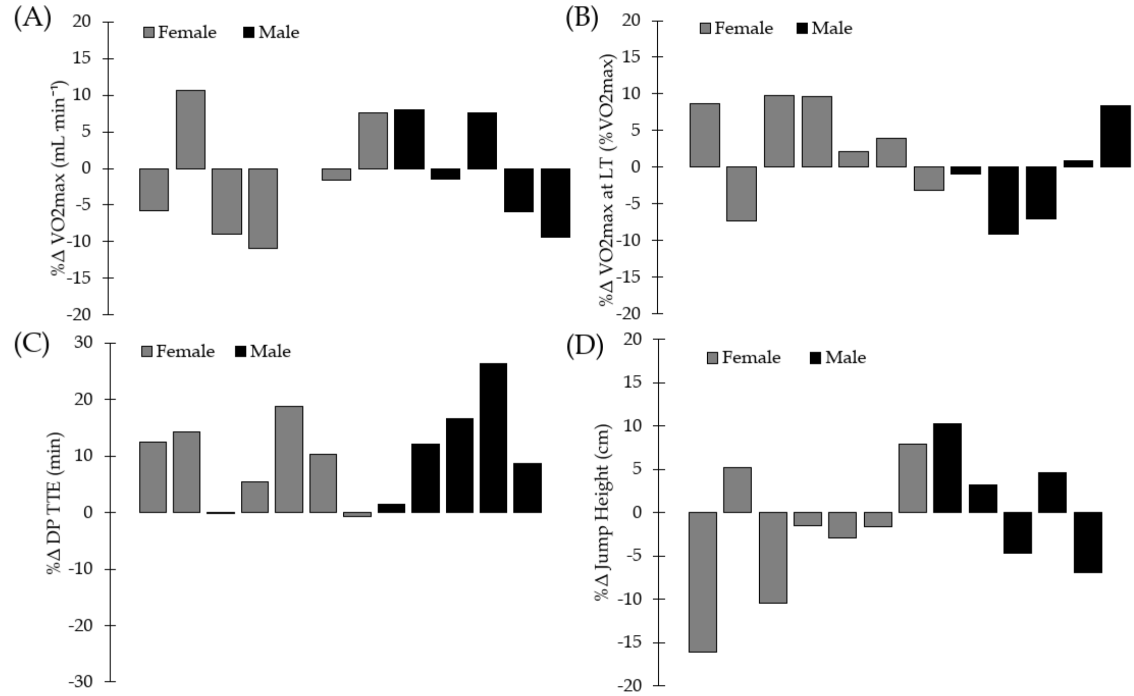

FIGURE 18 Individual changes (percentages) in (A) VO_{2max} (mL · kg^{-1} · min^{-1}), (B) percentage of VO2max at the lactate threshold (LT), (C) duration of DP performance (DP TTE) and (D) counter-movement jump (CMJ) height during the one-year of training. Modified and reproduced (Mishica et al., 2023).

In the other performance-related variables, no significant changes were present. Individual changes in VO2max showed a decrease for seven subjects, an increase for four subjects and no change for one subject (FIGURE 18). Maximal physiological values, independent of sex, during each stage of the maximal incremental treadmill tests, are presented in FIGURE 19. With regards to CMJ performance, a strong correlation was found between the changes in CMJ and absolute ($r = 0.669$, $p = 0.017$) and relative VO2max ($r = 0.598$, $p = 0.040$).

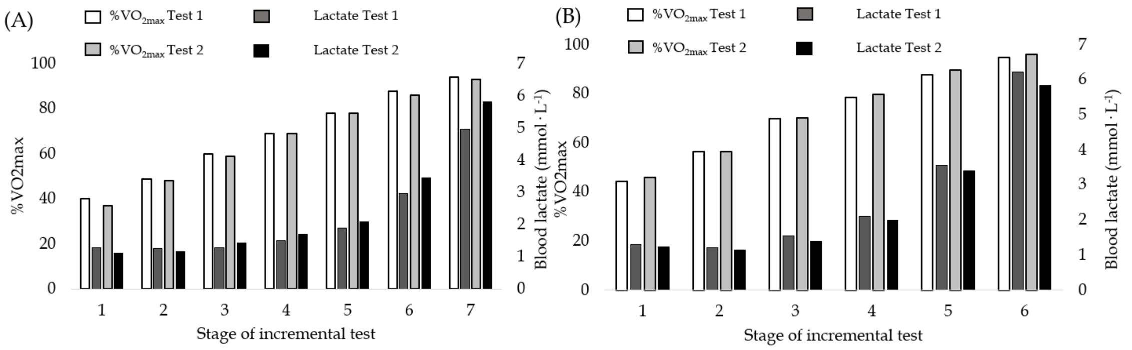

FIGURE 19 Changes in oxygen utilization and blood concentration of lactate during each individual stage of an incremental test following one year of endurance training in male (A) and female (B) cross-country skiers. Modified and reproduced (Mishica et al., 2023).

The only performance-related measures to show significant change during the one-year period was the ski-specific double pole performance. FIGURE 20 shows the development of both male and female athletes that occurred during study II.

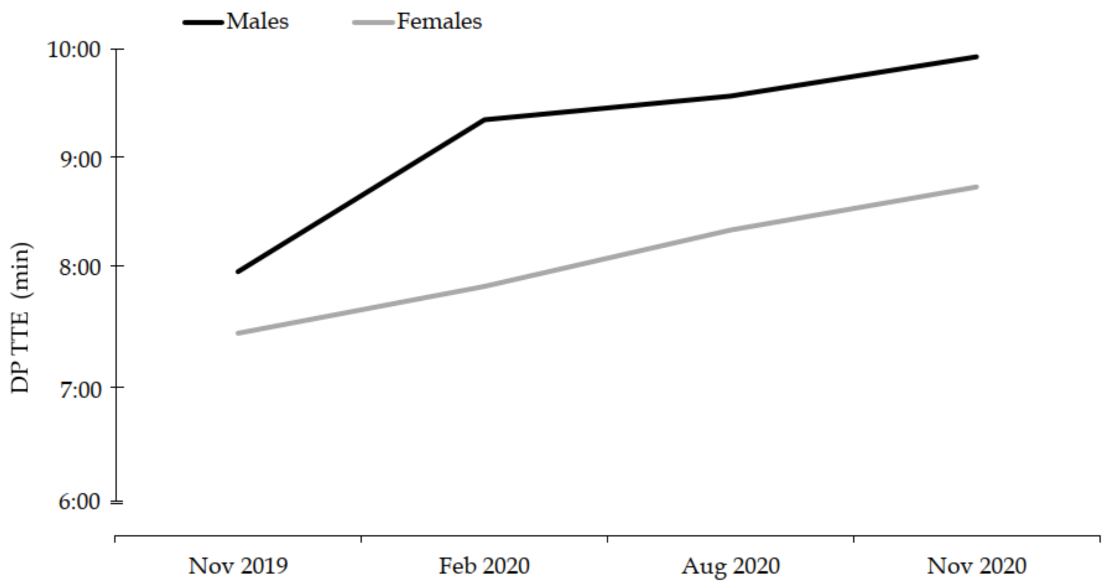

FIGURE 20 Development of ski-specific double pole tests during 1-year of endurance training.

During the longitudinal research period, feedback on performance-related jump performance tests was provided to all subjects, which consisted of individual and group average results. FIGURE 21 represents an example of jump performance (CMJ) feedback that was provided to subjects.

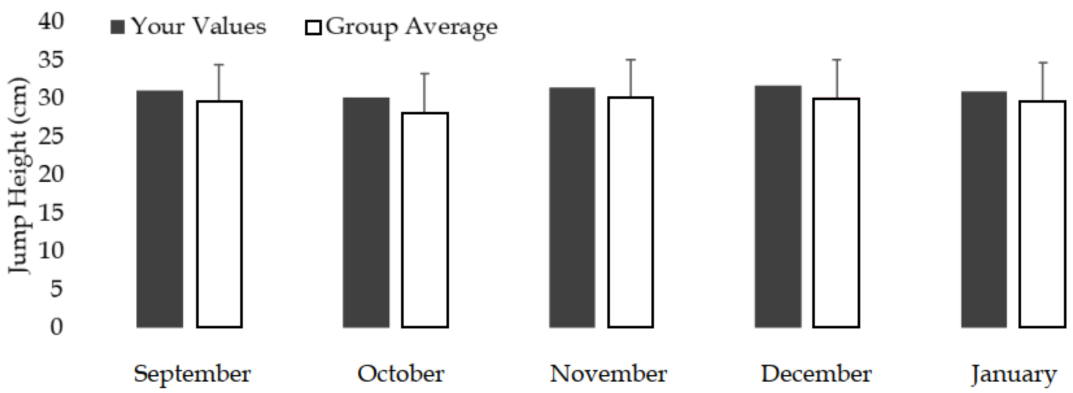

FIGURE 21 Representation of subject feedback for jump performance (CMJ) tests that was provided to present subjects with a visual representation of jump performance overtime.

5.4.2 Perceived stress and training

Levels of perceived stress showed some changes, but individual variation was high. Throughout the longitudinal period, PSS surveys were frequently monitored. FIGURE 22 reveals the combined perceived stress levels during this time specific to the month and independent of sex. On average, females measured 5.4 points higher than males on their monthly perceived level of stress.

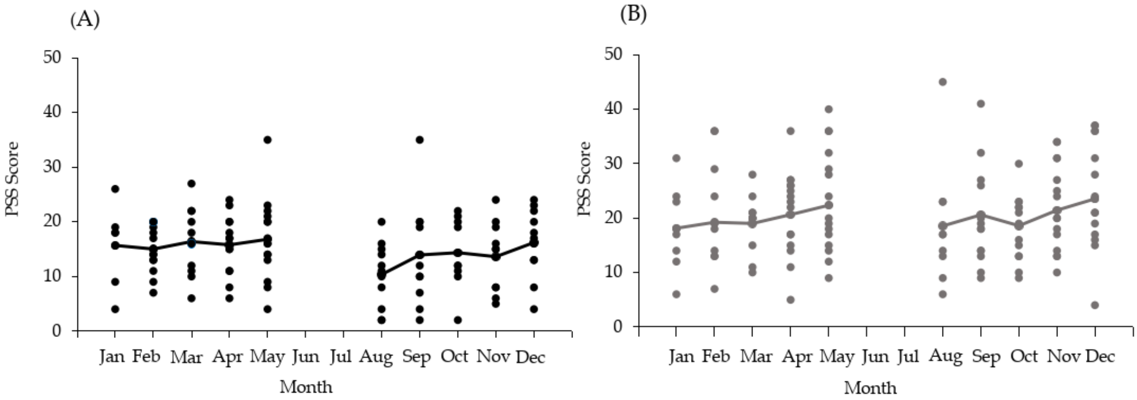

FIGURE 22 Monthly measures of perceived stress (PSS, Cohen) for males (A) and females (B) individually during the longitudinal research period.

During the 1-year of endurance training, training volume, intensity and mode of exercise were similar for both males and females. The average annual training hours for males was (587 ± 35) and (562 ± 53) for females. Annual organization of training by month distributed by intensity (LI, LT and HI) as well as a specific mode of exercise are shown in FIGURE 23.

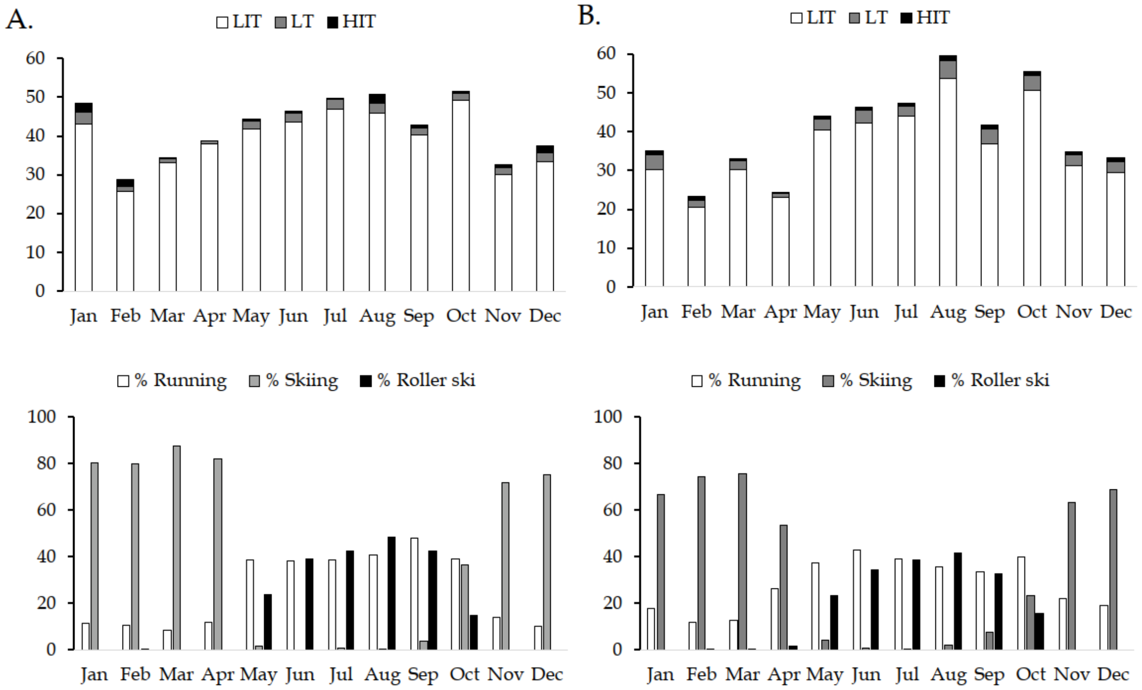

FIGURE 23 Monthly training distribution corresponding to volume of intensity (LIT, low-intensity training, LT, lactate threshold, HIT, high-intensity training) and percentage of training that occurred in the three most common training modes in both males (A) and females (B).

5.4.3 Blood values

During the one-year period, hemoglobin (Hg) nor ferritin (Fer) significantly changed, whereas blood values for vitamin D (VitD) increased in all subjects ($p = 0.042$) (TABLE 8). No significant correlations were found between blood values and the other variables observed.

TABLE 8 Average values (mean ± SD) of different hematological values followed during study II.

	November 2019	February 2020	August 2020	November 2020
Hemoglobin (g · L^{-1})				
Males	151 ± 6	151 ± 12	150 ± 5	155 ± 4
Females	143 ± 7	147 ± 7	139 ± 10	145 ± 9
Ferritin (ug · L^{-1})				
Males	68.5 ± 15.8	83.0 ± 19.4	71.2 ± 19.7	68.3 ± 39.4
Females	47.9 ± 26.9	58.7 ± 17.4	35.7 ± 23.2	38.0 ± 17.3
Vitamin D (nmol · L^{-1})				
Males	68.8 ± 35.9	62.5 ± 31.9	88.3 ± 23.2	82.2* ± 19.9
Females	81.4 ± 20.0	96.6 ± 30.9	101.7 22.2	97.5* ± 25.1

* $p \leq 0.05$

6 DISCUSSION

6.1 Relationship between heart rate variability and cortisol and the influence of training and sleep duration (Article I)

6.1.1 Nocturnal HRV and salivary cortisol

The first main finding of the thesis was an inverse relationship was found between nocturnal HRV and salivary cortisol levels during a 7-week training period. This finding indicates that cortisol levels decreased with increasing HRV indices during the 7-week training period in our young athletes. Furthermore, this relationship displayed the strongest correlation during the weeks when nocturnal HRV values were low and/or decreased and consequently, an increase in the level of salivary cortisol also occurred.

The present thesis evaluated three-day nocturnal RMSSD values for the analysis of HRV. While day-time recordings are commonly used, previous research suggests that night readings may provide increased reliability due to the exclusion of external factors that are no longer present during sleep (Buchheit et al., 2004; Nummela et al., 2010; Pichot et al., 2000). Earlier research has revealed that both daily (Carter et al., 2003; Hautala et al., 2001; Herzig et al., 2017; Hynynen et al., 2010; Kiviniemi et al., 2007) and weekly (Nummela et al., 2010; Pichot et al., 2000) HRV values appear to respond to training, and therefore, both methods are commonly used in sports. However, weekly measures may provide an improved indication of training response and/or adaptations due to the exclusion of diurnal variation that may influence daily measurements (Plews et al., 2013a). In addition, earlier research also utilizing BCG-based measures of nocturnal HRV has shown good agreement with validated values found using electrocardiographic measures (Vesterinen et al., 2020). Thus, these findings indicate that weekly values of nocturnal HRV may be a useful method to evaluate individual responses to endurance training.

Salivary levels of cortisol have shown a moderate association to perceived stress and are a frequent biomarker for the evaluation of psychological stress (Hellhammer et al., 2009). In response to exercise, salivary cortisol levels substantially increased following high-intensity exercise but displayed minimal to no changes after low and moderate exercise (VanBruggen et al., 2011). The current findings support earlier research that also found an increase in morning and afternoon levels of salivary cortisol, despite following a similar training volume and level of intensity (Iellamo et al., 2003). Thus, competition appears to influence the physiology of stress-related hormones and following salivary levels of cortisol, especially during the competition season, may provide a valuable insight on how well an individual is handling their present training and/or competition plan.

6.1.2 Associations between nocturnal HRV and other measures

Nocturnal HRV and Training

The investigated training characteristics displayed a mixed effect on nocturnal HRV values. Previous research has also revealed conflicting results with increases, decreases and no changes in HRV, all occurring when the training load was increased (Carter et al., 2003; Hautala et al., 2001; Hynynen et al., 2010; Pichot et al., 2000). It is apparent that the exact effects of endurance training are not yet well-defined (Herzig et al., 2017). In the present study, nocturnal HRV was the highest during week 4, when the training load also reached its highest value. This suggests that subjects were tolerating the present training stimulus since enhancement in vagal-related HRV indices are related to moderate amounts of training (Buchheit et al., 2004). However, results also revealed a decline in HRV (week 3–week 7) correlated to a reduction in both high-intensity training and training load. Conflicting findings show that decreases in HRV have occurred after both moderate to heavy endurance training sessions (Hynynen et al., 2010) and/or prolonged intensive training periods (Pichot et al., 2000). In the present study, the reduction in HRV occurred despite a decrease in training load and volume. Therefore, changes in HRV appear to be unrelated to training stress and may be related to the transition from training to the early competition period. Acute stress, such as an influence of an anticipatory task (e.g. competition), is previously associated with a decrease in HRV during sleep (Hall et al., 2004), supporting the idea that physiological stress levels may have increased during this time. However, in the present study, independent measures of anxiety were not evaluated and therefore, it is hard to identify if alterations in HRV were influenced by the stress of competition.

Nocturnal HRV and sleep

An additional factor that may have influenced daily changes in HRV and/or recovery is sleep (Shinar et al., 2006). Sleep, particularly in adolescents, plays a critical role in the execution of daily tasks (Brand & Kirov, 2011). Recommendations for youth (13–18 years of age) suggest that 8–10 hours of sleep are obtained each night (Paruthi et al., 2016), with athletes advised to obtain additional sleep to reduce the negative impact sleep loss may cause on human performance (Simpson et al., 2017). Furthermore, when observing female subjects, it is important to recognize that both HRV (Altini & Plews, 2021) and sleep (Baker & Lee, 2018) may be affected by the menstrual cycle. Therefore, in connection with longitudinal monitoring of HRV and sleep in females, the menstrual cycle is beneficial to consider, given that several studies report both decreased sleep quality during the premenstrual and menstrual phase (Baker & Lee, 2018) and slightly suppressed HRV (Altini & Plews, 2021; Kokts-Porietis et al., 2020) during the luteal phase of the menstrual cycle.

In the current study, sleep duration remained within the recommended hours, but sleep was not a controlled variable and therefore, individual sleeping habits, personal commitments, etc. may have influenced the overall quality and/or duration of sleep. Nonetheless, prior research with young gymnasts reported that sleep quality was unaffected during competition (Sartor et al., 2017). Thus, this may indicate that the positive relationship between sleep and HRV displayed during the competition period ($r = 0.786$) is due to an improved recovery that occurred when more sleep was present.

6.1.3 Associations between salivary cortisol and other measures

Salivary cortisol and training

Throughout the investigated period, the lowest cortisol levels were displayed when the highest training volume occurred (TABLE 5). In addition, changes in salivary cortisol (week 3 to week 5) appeared to react to the reduction in the training volume and a positive correlation ($r = 0.810$) was revealed. These findings suggest that young athletes appear to handle high training loads as long the amount of hard training is reduced is supported by earlier research that found large increases in low-intensity training (100% increase in training load) showed no changes in cortisol, despite a decrease in performance (Jürimäe et al., 2004). The current study displayed the most substantial increase in cortisol is seen during the early competition phase (week 5–week 7), indicating that changes in cortisol may be more related to the influence of the early season competitions rather than training stress. The competition season began during week 6, when 7/8 subjects participated in their first race. Stressful situations are known to affect the ANS and hormonal responses, and prior research has indicated that the stress from competition may affect the physiological regulation of hormones, such as cortisol levels (Iellamo et al., 2003). As a result, an important factor to take into consideration when interpreting cortisol results is the additional stress from racing.

Salivary cortisol and sleep

Salivary cortisol and sleep were previously evaluated and a decrease in sleep quality and/or duration were associated with an increase in both sympathetic activity and cortisol concentrations (Spiegel et al., 1999). The current analysis of training stimulus and sleep duration revealed that the investigated test weeks remained similar throughout the duration of the research period, further supporting the idea that competition stress may have increased morning cortisol levels. However, the current analysis of salivary cortisol was assessed using three-day average values, which shifts the focus to the total stress that was occurring each week rather than the stress response of one specific day or competition. Therefore, the increase in cortisol may be the result of prolonged stress, which is known to cause an increase in cortisol as well as a decrease in parasympathetic activity (McEwen, 2007). Thus, the increase in physical training during week 5, followed by the competition stress during week 6, may have led to a delayed fatiguing effect that may explain why an increase in cortisol occurred during week 7.

6.2 Assessment of nocturnal and morning measures of heart rate indices (Article II)

The main result from the evaluation of nocturnal versus morning measures of HR indices suggests that nocturnal measurements, as well as measurements in the morning with orthostatic tests, provide reliable weekly values of HR and HRV for young endurance athletes.

Differences between nocturnal versus morning values
Previously, assessment of HRV under real-life conditions revealed a lower variation in orthostatic values when compared to values during nocturnal rest, suggesting that parasympathetic withdrawal that occurs upon awakening may explain these differences (Hynynen et al., 2011). However, it is important to note that the reported decrease of HRV values during orthostatic tests was also associated to an increase in self-reported stress (Hynynen et al., 2011). Similarly, analysis of HRV values in overtrained athletes revealed a disruption in morning HRV values but not during night sleep (Hynynen et al., 2006). Therefore, in the current investigation, the fact that none of the individuals were in a stressed and/or overtrained state, may be one explanation for the lack of change in HR and the increase in HRV values that occurred during morning measurements.

Furthermore, HRV responds to countless stimuli and is influenced by not only psychological and physiological factors but also environmental surroundings (Fatisson et al., 2016). While sleeping, external stimuli is no longer present, and therefore, an enhanced reliability may exist (Buchheit et al., 2004; Nummela et al., 2010; Pichot et al., 2000). Consequently, the slight differences in nocturnal and morning values may be due to the difference in the duration and/or time of day of the two separate measurement procedures used in the present study. Nonetheless, regardless of current differences, nocturnal and morning HRV values consistently displayed a strong relationship with one another and thus, measurements collected during nocturnal and morning rest are both acceptable.

HR indices and performance-related tests
During the present study, it was hypothesized that changes in HR and HRV would be associated with changes in SRT and CMJ tests. However, the weekly training stress remained similar each week, and no significant differences occurred. Previous research, also monitoring normal in-season training, has suggested that both CMJ and SRT are reliable measures to predict changes in performance (Garrett et al., 2020), indicating that the present changes in HR and HRV were not due to alterations in training load or volume.

HR indices for athlete monitoring
The use of weekly average values appears to be a useful method for evaluating individual response to endurance training (Nummela et al., 2010), suggesting long-term monitoring may be an effective tool for young endurance athletes. Although both morning and nocturnal methods are valuable, nocturnal values may have several advantages, including an increased ability to compare results. In earlier studies, variations in body position, time of day, sleep cycle, and the use of daily versus weekly values have led to conflicting results in the direction of change in vagal-regulated HRV indices that are likely due to methodological differences (Buchheit, 2014). Application of whole night average values may help eliminate these differences and provide an increased ability for comparison between studies to occur. In addition, nocturnal measures are more easily attainable when compared to morning orthostatic measurements that are obtained using electrodes or HR straps that require added effort and hence, reduce daily athlete compliance (Plews et al., 2017). Furthermore, nocturnal measures of HR indices, obtained with a BCG-based sleep device, provide a monitoring method that is fully automatic and contact-free with no time constraints (Vesterinen et al., 2020). Therefore, athlete monitoring is able to occur under relatively free-living conditions (Waldeck & Lambert, 2003) with a balance of validity and comfort-of-use in mind. Consequently, implementation and continuous monitoring of HR indices would be more feasible, suggesting that nocturnal measures may be a more favourable option than morning values obtained from orthostatic tests.

6.3 Relationships between objective measures of training, recovery and subjective stress (Article III)

The major findings of the present study revealed a negative association between perceived stress and sleep duration, between sleep latency and jump performance, and between blood lactate levels during high-intensity running and sleep duration. In addition, the females demonstrated considerably higher levels of stress and blood lactate during submaximal running.

Perceived stress and sleep
During the current training period, subjective monthly measures of perceived stress displayed a negative relationship to sleep duration with associations

between sleep and PSS revealing that for every 1 hour increase in sleep duration, the PSS is estimated to decrease by 2.8 points (
TABLE 6). Previously, perceived stress in junior athletes has appeared to be a significant predictor of sleep quality and effective management of individual stress levels may help to prevent poor sleep (Hrozanova et al., 2019). While the current study did not focus on direct measures of sleep quality, the impact of sleep duration on competitive performance has been examined (Kirschen et al., 2018) and showed that an increase in time in bed as well as an increase in sleep duration may result in an improved athletic success (Juliff et al., 2018). During this study, athletes regularly attained the recommended 8 hours of sleep each night (Riederer, 2020), suggesting that sleep duration was sufficient throughout the 4-month testing period. However, the ideal amount of sleep may be individual and some young athletes may need additional sleep (Chaput et al., 2018). Hence, recommendation advising an adequate sleep duration may need to be made on an individual basis.

Stress-related measures and performance
A unique attribute of this study is the continuous assessment of HRV and perceived stress in combination with performance-related measures. HRV is known to react to changes in stress (Kim et al., 2018); nonetheless, we were not able to detect any meaningful associations between PSS and nocturnal HRV. Interestingly, we found that females displayed higher nocturnal HRV values which is typically linked to positive training adaptations (Plews et al., 2013b) as well as higher levels of perceived stress which is commonly related to a decline in performance (Otter et al., 2015). Possible explanation for these inconsistent findings may be due to the current methods since PSS is presenting a subjective measure that is individually assessed (Cohen et al., 1983) and HRV is an objective measure that is connected to changes in the ANS (Kim et al., 2018). Furthermore, HRV was measured nocturnally each night and weekly average values were used for analysis, while retrospective PSS values were collected monthly (e.g., 'in the past month'). Additional reduction in associations may be due to the investigated phase of preseason training when external factors such as travel, and competition pressure are significantly reduced. It is likely that athletes perceived and/or direct exposure of stress is elevated during the competition season and frequent monitoring during this time may reveal new associations that may help prevent a decline in performance. Thus, additional studies, particularly during a competitive phase that measures subjective (PSS) and objective (HRV) measures of stress, would interest both athletes and coaches.

Sleep and performance-related tests
When examining the performance-related measures of SRT, sleep duration displayed a negative relationship with blood lactate concentrations suggesting that a decline in sleep appears to influence blood lactate levels. A recent review on performance in youth showed a decrease in well-being, higher perceived ratings of training load, and a decline in performance, were related to inadequate sleep (Riederer, 2020). At controlled submaximal intensities, an increase in lactate

levels often implies there is a decline in performance (Jacobs, 1986), supporting the idea that the increase in blood lactate levels may be due to the reduction in sleep. However, it is important to acknowledge that submaximal lactate and performance-related associations are not well-established and factors such as levels of muscle glycogen, hydration status, muscle damage and ambient temperature may influence lactate levels, hence, changes in blood lactate concentrations should continue to be evaluated with caution (Jacobs, 1986; Swart & Jennings, 2004).

Another investigated measure of sleep monitored in this study was sleep-latency, which is a suitable marker to assess sleep quality with latency periods of 30 minutes or more, indicating good sleep quality in both adolescents and adults (Ohayon et al., 2017). In this study, sleep latency displayed a negative relationship with jump performance. Although large amounts of imposed sleep restrictions have shown impairment in vertical jump height (Mah et al., 2019), the present changes occurred naturally, and therefore, differences in sleep variables remained relatively small. Thus, the current results only suggest that when reductions in jump height are present, evaluation of sleep may be of importance, but more research is necessary to effectively identify associations between sleep and jump performance in young athletes.

Sex differences

Throughout the 4-month training period, several sex differences occurred. Females displayed higher stress scores each month, implying that during this preseason training period, they perceived a greater amount of perceived stress. On average, females measured 6.8 points higher than males suggesting their overall level of stress was moderate compared to the low-stress level in males (Hrozanova et al., 2019). Similar sex differences have been shown in adult endurance athletes, with females experiencing higher stress during pre-competition on several different stress-related items (Roberts et al., 2022). Research studying university students has also revealed that females consistently report more perceived stress (Graves et al., 2021) and a higher level of overall stress when compared to their male counterparts (Brougham et al., 2009).

When examining sex-specific responses, differences were the most pronounced in areas of negative stress with "feelings of nervousness/stress" in addition to "feeling unable to cope and/or control" revealing significantly higher scores for females (FIGURE 17). This provides an extension on earlier research that indicated a higher risk of anxiety and stress are commonly found in females (de Visser et al., 2010). While higher levels of perceived stress may be detrimental to performance, previous studies have suggested that females utilize coping strategies (Nqankwo & Onyishi, 2012) and social support systems (Crocker & Graham, 1995) more than males. Additionally, subjects in the current study were attending a sports academy which provides a supportive environment that may aid in the balance and ability to handle current stress loads more effectively.

6.4 One year of endurance training in young athletes (Article IV)

Detailed analysis of one year of endurance training revealed a progressive development in ski-specific DP test performance in young XC skiers without significantly altering any of the other performance-related parameters. No major differences in perceived stress levels were present, nor for blood values in Hg and Fer, although slight increases in blood levels of VitD were present.

6.4.1 Ski-specific performance tests

The improvements in DPP presented in this study indicate that during one-year of endurance training, subjects developed their upper-body performance. Earlier research has revealed that improvements in incremental DP tests appear to translate to race results suggesting they may be a good predictor for XC ski performance (Børve et al., 2017). The development of DPP presented here occurred while completing normal endurance training specific to XC ski and biathlon. Previously, 6 months of XC ski training has reported a 3% increase in DPP (Johansen et al., 2020), which is considerably less than the current improvement shown here. The present increase (+10%) is comparable to previous studies that implemented a 6-week period of high-intensity DP intervals and found 19.5% (Johansen et al., 2021) and 16% (Nilsson et al., 2004) increases in performance.

During the incremental DP tests, maximal HR values were similar to the HR values that were reached during VO_{2max} tests, indicating that the current DP protocol was appropriate and that the provided familiarization period was adequate. Therefore, it appears improvements were likely due to an increase in upper-body performance. Earlier research reported that maximal upper-body strength appears to show a substantial impact on DP roller skiing performance (Sunde et al., 2019). However, time to exhaustion change in percentage does not directly correspond to an underlying physiological capacity and/or performance time change over a given distance. Previous studies suggest that a 10% change in time till exhaustion (TTE) may represent a change of approximately 1% in physiological capacity (Hopkins et al., 2001). Furthermore, age-related differences are an additional factor to consider. When investigating time trial performance in the DP technique, differences between junior (16–18 years of age) and adult athletes were around 10%. It is evident that young skiers are still progressing, and advancements in performance are likely due to benefits obtained from additional training and the development of their neuromuscular system (Johansen et al., 2020).

Throughout the 1-year period, improvement in TTE in the DP performance test consistently increased (FIGURE 20). To our knowledge, there are no longitudinal studies following the progress of the DP technique in young XC skiers. However, following the development and monitoring of performance tests that are technique specific, such as the DP technique, may be beneficial for coaches and young athletes. This would not only provide valuable information regarding athlete development, but when a plateau in test performance appears, coaches and athletes could consider if the addition of a technique-specific training intervention and/or technique-specific training would be an appropriate training stimulus to aid in performance enhancement.

6.4.2 Maximal oxygen uptake

The effectiveness of endurance training is commonly evaluated based off changes in VO_{2max}, especially in young athletes (Ingjer, 1992; Landgraff et al., 2021). Thus, athletes aim to improve VO_{2max} values, but even with sufficient training a lack of change in VO_{2max} frequently occurs. Research with young endurance athletes has revealed that despite 3 years of consistent training, changes in VO_{2max} were insignificant (Landgraff et al., 2021). Additionally, increases in ski-specific training volume over a 6-month period also displayed minimal to no change in physiological and/or performance variables (Johansen et al., 2020) and one year of high-level training with 17-year-old male XC skiers showed no change in absolute and relative physiological parameters at the maximal level and only a slight increase for females (Zoppirolli et al., 2020). Furthermore, when investigating the influence of a training stimulus, 8 weeks of an increase in both low and high-intensity endurance training, following a period of standardized training, showed little to no effect on the development of performance or physiological indices in young skiers (Talsnes et al., 2022a). A similar study, also comparing increased training load in junior skiers, revealed that although an increase of both low and high-intensity training revealed a slight development in performance, greater changes were present in measures of VO_{2max} and VO_{2peak} in the high-intensity training group. This finding indicates that an increase in high-intensity for 8 weeks may elicit slightly greater maximal energy delivery capacities in young endurance athletes, however, differences in running and/or skate roller-skiing performance were not significant (Talsnes et al., 2022b). Hence, the lack of change observed in the present study is supported by previous work. Nonetheless, the interpretation of change must consider the effects of individual variation. Although the investigated athlete cohort was highly homogeneous, inter-individual variation was present. This is most likely due to variations in the trainability of VO_{2max}, which is highly hereditary and influenced by age, body composition and body mass (Bouchard et al., 1999). FIGURE 18 reveals individual changes that occurred during one year of endurance training for different performance-related measures, and interestingly, when looking at sex-specific results, the oxygen consumption at LT increased for most of the female subjects. However, the relative change in VO_{2max} values did not reflect a similar increase, and changes in the values at LT were not significant, and therefore, no conclusion can be drawn.

6.4.3 Power performance tests

Throughout the research period, the evaluation of power was measured with CMJ tests. Similar to the VO_{2max} values, analysis of jump height over one year revealed minimal change in both males and females. However, investigation of change between the selected tests during one-year revealed a positive relationship with changes in relative and absolute VO_{2max}. Earlier research investigating performance in 10-km running competitions (Sinnett et al., 2001) and TTE in middle-distance runners (Houmard et al., 1991) revealed a positive relationship to vertical jump height, indicating that neuromuscular performance may help to predict endurance performance. Nonetheless, additional research is needed before relationships between jump performance and endurance-related measures of performance can be made.

Although measures of neuromuscular function via the CMJ test are popular (Taylor et al., 2012) assessment of results are challenging to interpret. A recent review with endurance runners has highlighted the lack of sensitivity that jump height may have on internal training load when measured during training sessions but indicates that improved jump performance in subsequent running sessions may suggest lower fatigue and/or positive training adaptation (García-Pinillos et al., 2021). Other research, also with endurance runners, revealed that an increase in volume and intensity that was linked with a decrease in perceived recovery had no effect on the CMJ results (Nuuttila et al., 2021), further supporting the idea that a lack of sensitivity may be present. However, when comparing seasonal best and seasonal worst running performances, the week prior to season-best races were associated with significantly higher CMJ performance (Sinnett et al., 2001), and XC skiers' jump performance also displayed a positive relationship with maximal skiing speed (Stöggl et al., 2011). Therefore, taken together, the above results indicate that CMJ may be a valuable monitoring tool, but further research is needed to clarify what parameters should be used and how to best evaluate results.

6.4.4 Measures of perceived stress levels

As previously stated, no significant relationships between PSS and performance variables were present. However, cumulative results presented in FIGURE 22 display that variation between months was present. In addition, females had higher levels of perceived stress throughout all measured months of the training year. Stress appears to display a substantial impact on performance (Meeusen et al., 2013; Nuutilla et al., 2021; Saw et al., 2016) and thus, stress levels are an important variable to consider when evaluating performance results. Nonetheless, successive days of high-intensity competition in trained athletes had no change on psychological response, suggesting training and preparation does not consistently have a major influence on an individual's stress response (Väänänen & Vihko, 2005). One possible explanation for the lack of change in the present study may be the specific environment that a sports academy provides. Athletes had the support of coaches, teammates and additional support staff to aid in balancing the necessary combination of training, competition and studying (e.g. measurements were always planned around training and examination schedules and training). Thus, future studies should consider whether the subjects report receiving adequate support to handle current stress loads in order to obtain more accurate associations.

6.4.5 Measures of blood variables

Routine measures of blood are often recommended for athletes to ensure proper nutrition is met and help support athlete health and performance across a wide range of physiological systems (Pedlar et al., 2019). In order to increase the benefit and application that blood variables may provide, research following these parameters is needed. Currently, despite the fact that following various blood variables is common practice, limited studies include such measures. Therefore, the inclusion is a strength of the present study.

The current study investigated three different parameters from blood (Vit D, Hg and Fer) that are regularly monitored by endurance athletes. While the physiological processes that underline performance are supported by a variety of vitamin and minerals, evidence shows that nutritional deficiencies commonly occur in athletes (Lee et al., 2017). Previous research has indicated that iron depletion (Fer $< 12\ \mu g \cdot L^{-1}$, Hg $< 13\ ug \cdot L^{-1}$ for females and $< 30\ ug \cdot L^{-1}$ for males), is a common occurrence among athletes and is regularly associated with an inadequate energy intake (Nutrition and Athletic Performance, 2009). Additionally, VitD deficiency is also prevalent and insufficient levels of VitD are linked to low iron levels, implying that assessment of iron and VitD levels is highly beneficial (Constantini et al., 2010). Furthermore, when exposure to natural sunlight is limited, the risk for VitD deficiency increases (Powers et al., 2011). Previous research with Finnish runners revealed VitD deficiency levels in 68% of subjects in the winter months (Lehtonen-Veromaa et al., 1999), further supporting the idea that monitoring the VitD status in young XC skiers would be highly valuable.

To maintain bone health, VitD values of $> 50\ nmol \cdot L^{-1}$ (25(OH)D serum concentrations) are recommended (Ross & Institute of Medicine (U. S.), 2011). Throughout Article IV, athletes were advised to aim for values within 50–75 $nmol \cdot L^{-1}$ and no direction of supplementation was provided, but a suggestion that athletes may want values $> 75\ nmol \cdot L^{-1}$ was provided (Owens et al., 2018). Evaluation of dietary VitD intake was not performed, but 75% of the subjects reported ingesting VitD, iron and other supplements intermittently throughout the investigated training period. Analysis revealed a significant increase in 25(OH)D serum levels from test 1 (October 2019) to test 2 (October 2020) but minimal to no change in Fer and Hg values. These findings indicate that while performing one-year of endurance training analysis of Vit D, Hg and Fer revealed that young athletes were able to maintain proper nutritional status and good metabolic health.

6.5 Limitations

Research does not take place without limitations, and the studies presented here are no exception. For the investigated field of sport-practice, research findings are frequently reported as group results, and thus application of the results may not apply to all subjects. Each individual athlete represents their own unique case and therefore, findings only reveal information that may aid in understanding the mechanisms behind performance development. Furthermore, multiple factors influence individual development and knowledge and understanding this complex interaction is far from complete, with a large emphasis still placed on knowledge and training insight gathered by "experience" rather than evidence-based knowledge (Haugen, 2021). Subsequently, longitudinal research that incorporates collaboration between research and practice is needed to produce evidence-based knowledge that is applicable and relevant to practice in sport. Lastly, to provide high-quality research, a proper balance of ease-of-use and validity need to be at the forefront when planning future applicable studies in sports science. Additional limitations specific to each Article are presented below:

In Article I, the small sample size and grouping of both sexes may have influenced the results. In addition, changes in levels of psycho-physiological stress may have increased when the period of competition began, due to, e.g., the pressure to perform well. This potential change may have influenced the HRV, sleep duration and saliva cortisol levels. However, this limitation led to the addition of perceived stress measures in the upcoming studies so independent evaluation of stress could also be assessed. Finally, it is important to acknowledge that no information regarding the menstrual status and/or the use of hormonal contraceptives was collected, and the addition of this data may have provided additional insight for changes that occurred in female participants.

In Article II, lack of standardization due to the collection of HR indices occurring in individual home environments is the main concern for this investigation. It is important to recognize that the values calculated by the device may not provide the most reliable measures of HR indices since the accuracy of this device for long-term monitoring is yet to be determined. Nonetheless, the main objective was to mimic typical every-day use, and the current methods were able to provide routine measures of sleep and training.

In Article III, although the presented statistical approach was chosen to minimize the influence of missing data is still an important factor to acknowledge. Furthermore, a performance-related measure and/or race result would have been a valuable measure to include in this study to provide further details on the relationships that perceived stress may have on young athletes' competition-specific performance.

In Article IV, limitations are due to the small sample size of 12 individuals examined in this study. This frequently occurs in longitudinal research with high-level athletes, and hence, the current sample size is similar to previous research that also monitored the development and/or performance in XC skiers (Fabre et al., 2010; Ingjer, 1992; Losnegard et al., 2013; Rusko, 1992). In addition, analysis of individual training diaries was limited due to inconsistency in how subjects reported various individual training characteristics. Lastly, supplementary measures, such as VO_{2max} during DPP, ski-specific upper-body training and DP economy, should be incorporated into future studies to better understand what factors contribute to improved DPP in young athletes.

7 PRIMARY FINDINGS AND CONCLUSION

The motivation for this dissertation was to expand on the developmental understanding and aid in the identification of what practical and relevant measures in both free-living and laboratory conditions are valuable for monitoring training and/or performance in young endurance athletes. *Study I* was designed to investigate the application of nocturnal HR measures in several different contexts related to both training and development in young athletes. The *first article* displayed the associations between nocturnal HRV and salivary cortisol and their relationship to various characteristics of training/recovery. Whereas the *second article* focused on exploring the validation of nocturnal HR indices to ensure nocturnal HR and HRV measures would be a viable method for monitoring during long-term measurements. With the validation of nocturnal HR measures, in our *third article,* we explored the associations between common performance measures with subjective measures of perceived stress, to determine which measures may be the most valuable. To conclude this PhD thesis with a more comprehensive assessment, the *fourth article* investigated a one-year endurance training period in young XC skiers and biathletes to provide details on various changes in performance-related tests.

The main findings of this thesis were:

1. That nocturnal HRV appears to correlate negatively with salivary levels of cortisol in young endurance athletes. During the competition period, a decrease in HRV was associated with a decline in physical training, and sleep displayed a positive relationship with HRV, indicating that during this period, full recovery requires more time, and additional sleep may be beneficial.
2. Nocturnal values for weekly measures of HR and HRV obtained during sleep with a BCG-based sleep device are accurate and reliable. Thus, day-to-day monitoring of nocturnal HR and HRV appears to be a convenient and valid approach for long-term monitoring of young endurance athletes.
3. Increases in subjective levels of perceived stress are associated with diminished sleep duration. Based on monthly measures of perceived stress

throughout a 4-month training period, females appear to experience higher amounts of stress than males.

4. One year of ski-specific endurance training in young athletes improved ski-specific performance, but no change in maximal oxygen uptake, fractional oxygen utilization at LT, explosive power, perceived stress, or selected blood biomarkers were present. Based on these findings, analysis of one-year of endurance training in young skiers should include ski-specific assessments of performance and additional measures may not reveal improvements that may motivate and provide young athletes with valuable insight needed for their future athletic careers.

Practical Implications and Future Perspectives

Today, training in sports can be monitored in numerous ways and coaches and athletes can easily have access to valuable information both in and out of a laboratory setting. The present measures of HRV confirm that nocturnal HRV values correlate with salivary levels of cortisol and support previous findings that the application of HRV values in training may help identify more individualized profiles of training (Nummela et al., 2010). Additionally, our findings indicate that three-day average values for HRV may provide an accurate representation of young endurance athlete's status of recovery and, thus, may be useful values to monitor, especially during periods of high training and competition. Furthermore, sleep appears to be another tool that may facilitate success in young athletes. Although the current research did not examine sleep quality, our findings offer insights into the potential value improvements in sleep may provide. Additionally, when examining weekly average values for HR and HRV obtained during sleep, both accurate and reliable values were found with the BCG-based device used throughout this research. The application of BCG-based values provides an automatic and contact-free method for monitoring HR indices, providing an attainable way to continuously measure these values in free-living conditions. It is important to acknowledge that for a monitoring system to be feasible, especially for young athletes, validity and accessibility are critical factors to consider. Thus, the daily monitoring of nocturnal HR and HRV utilized in this study appears to be a valid approach for long-term monitoring of these values in athletes.

In relation to subjective stress, we found that an increase in perceived stress levels was connected to a decline in sleep duration. Sex differences also revealed that females experience higher stress levels than males suggesting that monthly measures of perceived stress levels may be valuable. Furthermore, perceived stress levels, sleep duration and sleep latency can be easily measured and do not demand any physical exertion, and therefore, the implementation of these variables to aid in athlete monitoring may be relevant for young athletes that are preparing for a competition season.

Lastly, regular endurance training in young athletes may not directly influence performance-related measures such as maximal oxygen uptake, fractional utilization of LT, or explosive power. A lack of change in the following measures does not automatically infer that young athletes are no longer developing or improving with training. During one-year of training, young XC skiers displayed significant improvements in ski-specific DPP despite no change in the additional performance-related factors.

Longitudinal studies, related to training and performance in young athletes are challenging to conduct and hence, are limited. However, this PhD work suggests that technological advancements and improved accuracy in non-invasive monitoring techniques may be suitable tools to increase long-term athlete testing and monitoring. In addition, simple subjective measures, such as perceived stress surveys, may provide support for coaches and young athletes striving to balance a high level of performance while attending school. With regards to athlete development, performance-related measures do not always follow a linear trend and assessment of junior athletes should include sport-specific tests to reveal developmental improvements that otherwise may go undetected. Therefore, future research should consider what new opportunities we can incorporate into athlete monitoring and testing over longer periods of time that are both general and sport-specific to help expand developmental understanding and performance for young endurance athletes.

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ORIGINAL PUBLICATIONS

I

RELATIONSHIPS BETWEEN HEART RATE VARIABILITY, SLEEP DURATION, CORTISOL AND PHYSICAL TRAINING IN YOUNG ATHLETES

by

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Research article

Relationships between Heart Rate Variability, Sleep Duration, Cortisol and Physical Training in Young Athletes

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Abstract
The aims of the current study were to examine the relationships between heart rate variability (HRV), salivary cortisol, sleep duration and training in young athletes. Eight athletes (16 ± 1 years) were monitored for 7 weeks during training and competition seasons. Subjects were training for endurance-based winter sports (cross-country skiing and biathlon). Training was divided into two zones (K1, easy training and K2, hard training). Heart rate and blood lactate during submaximal running tests (SRT), as well as cortisol, sleep duration and nocturnal HRV (RMSSD), were determined every other week. HRV and cortisol levels were correlated throughout the 7-week period ($r = -0.552$, $P = 0.01$), with the strongest correlation during week 7 ($r = -0.879$, $P = 0.01$). The relative changes in K1 and HRV showed a positive correlation from weeks 1-3 ($r = 0.863$, $P = 0.006$) and a negative correlation during weeks 3-5 ($r = -0.760$, $P = 0.029$). The relative change in sleep during weeks 1-3 were negatively correlated with cortisol ($r = -0.762$, $P = 0.028$) and K2 ($r = -0.762$, $P = 0.028$). In conclusion, HRV appears to reflect the recovery of young athletes during high loads of physical and/or physiological stress. Cortisol levels also reflected this recovery, but significant change required a longer period than HRV, suggesting that cortisol may be less sensitive to stress than HRV. Moreover, our results indicated that during the competition season, recovery for young endurance athletes increased in duration and additional sleep may be beneficial.

Key words: Recovery, endurance training, physiological stress, individual adaptations, submaximal tests, autonomic nervous system.

Introduction

During the past four decades, increasing emphasis has been placed on training young athletes, many of whom now train all year round (Brenner, 2016). At the same time, most investigations on responses to endurance training have involved adult subjects and considerably less is known about the trainability and development of younger individuals (Naughton et al., 2000; Murray, 2017). Moreover, in addition to the stress of daily training, the added stress of their studies affects the recovery of young athletes. Therefore, research on the training of young athletes, focused on reaching a high level of performance, is necessary for attaining maximal gains and allowing young athletes to succeed in elite sports (Brenner, 2016; Murray, 2017).

Physiological processes that occur during sleep are a fundamental aspect of an athlete's recovery and subsequent ability to train and compete at maximal capacity (Samuels, 2008; Brand and Kirov, 2011). However, both the quality and quantity of sleep by young athletes has been declining (Samuels, 2008; Copenhaver and Diamond, 2017) for a number of reasons, including training schedules, education, social events and travel plans (Copenhaven and Diamond, 2017; Simpson et al., 2017) as well as chronic and acute stress. It is known that the human response to stress is largely regulated by the autonomic nervous system (ANS) and, therefore, can be observed easily and non-invasively by measuring beat-to-beat variation in resting heart rates, also known as heart rate variability (HRV) (Electrophysiology, 1996; McEwen, 2007). Numerous studies have investigated the effects of endurance training on HRV (Pichot et al., 2000; Hautala et al., 2001; Carter et al., 2003; Kiviniemi et al., 2007) and recent studies suggest that nocturnal recordings help further evaluate an individual's accumulated training load (Pichot et al., 2000; Hynynen et al., 2010). HRV measurements during sleep provide a measurement that is independent of external factors and therefore, enhances their reliability (Pichot et al., 2000; Buchheit et al., 2004; Nummela et al., 2010). Furthermore, nocturnal HRV appears to have a dose-response relationship with increased exercise intensity causing a reduction in nocturnal HRV (Hynynen et al., 2010). Thus, sleep duration and nocturnal HRV measurements may be effective measures to monitor recovery in young athletes.

Cortisol is one of the most frequently investigated hormones as a measure of overtraining and stress. Extended periods of increased or decreased levels of cortisol have a negative impact on health and therefore, may hinder athletic performance (Duclos et al., 2007). The relationship between cortisol and exercise as well as the different methods used for measuring cortisol secretion have conflicting results (Neary et al., 2002; Duclos et al., 2007). However, several studies have shown a strong relationship between serum and salivary cortisol levels indicating that salivary cortisol is a reliable measurement method, a good biomarker for physiological stress and a non-invasive option for monitoring athletes (Neary et al., 2002; Gustafsson et al., 2008; Hellhammer et al., 2009). During a 37-week follow up, salivary cortisol increased with increased training but when training was reduced, no change was observed and no relationship was found between performances (Chatard et al., 2002). In addition, a

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repeated exercise prescription does not appear to elicit the same changes in resting levels of cortisol with previous research finding that cortisol decreased considerably between two different maximal tests (Hedelin et al., 2000). Maximal tests are a good measure of performance but require a highly intensive exertion, reducing their application to everyday training (Lamberts et al., 2011; Capostagno et al., 2016). As a result, submaximal tests are used more frequently to monitor athletes and predict performance (Lamberts et al., 2011). Submaximal treadmill tests have been compared to cycle tests in athletes training for triathlon, and findings showed that both testing modes could be used interchangeably (Basset and Boulay, 2003). Running is a common training mode for both cross-country skiers and biathletes, allowing SRT to be a valid test for monitor training in this study.

Although monitoring training load at the elite level is common practice, there is no well-defined boundary between effective and ineffective training adaptations (Gustafsson et al., 2008). Previous research has shown that the utilization of HRV measurements in sport is challenging due to inconsistent procedures making comparison of results problematic as well as time constraints reducing the overall athlete compliance, especially over a long season (Rave et al., 2018). Increases in technology have introduced monitoring options that are easily accessible and collected with minimal effort. However, these measures are often performed in home environments. Therefore, investigating "real-life" values is highly relevant and may provide future understanding that is highly applicable for athletes and coaches. Accordingly, this study was designed to characterize the relationships between HRV, salivary levels of cortisol, sleep duration, and blood lactate during submaximal running tests (SRT) in young athletes during their training and competition seasons. Our main hypothesis was that nocturnal HRV exhibits a negative relationship to salivary cortisol levels in the morning. We also hypothesized that a decrease in sleep duration alone or in combination with more intense and prolonged training reduces nocturnal HRV and elevates morning cortisol levels.

Methods

Participants

Eight well-trained young endurance athletes participated in this study. The participants were all athletes at a sports academy high school competing and training for cross-country skiing (6 subjects) or biathlon (2 subjects) year round and participating at the national level. Characteristics of the athletes are presented in Table 1. All subjects were fully informed of the study procedures and gave written consent to participate in the project. The ethics committee of the University of Jyväskylä, Finland, approved the study and the measurements were performed in accordance with the declaration of Helsinki.

Study Design

This study was performed during a 7-week period (November-December) that involved both a training (T1) and early competition (T2) training phase. ANS state was assessed with nocturnal HRV analysis, collected using a ballistiocardiographic (BCG) sleep-tracking device (Emfit QS, Jyväskylä, Finland). Additional assessment tests occurred on four separate occasions: twice during the training season (weeks 1, 3; T1) and twice during the competition season (weeks 5, 7; T2). During each test week, subjects participated in SRT and saliva samples were collected for three consecutive days; one day before SRT, the test day and one day after SRT. Athletes recorded their own individual training plans during this time and training characteristics (easy training, hard training and training load) were evaluated via electronic training diaries. Body fat percentage was measured using the bioimpedance method (InBody 720, Inbody CO., Cerritos, California, USA) in the beginning of the training period.

Table 1. Characteristics of the subjects (means ± SD).

	Women (n = 5)	Men (n = 3)
Age (yrs)	16 ± 1	16 ± 1
Body mass (kg)	58 ± 5	67 ± 5
Body fat (%)[a]	16.2 ± 8.8	14.5 ± 11.1
Training hours (y)[b]	500 ± 76	600 ± 71

[a] Assessed on the basis of bioimpedence measurements.
[b] recorded in electronic training diaries.

HRV and Sleep Analysis

Previous research has demonstrated that determination of HRV on the basis of BCG is both accurate and reliable (Shin et al., 2011; Wang et al., 2015). The Emfit QS device (EMFIT QS, Emfit OY, Jyväskylä, Finland) consists of a contactless pressure sensor (542mm x 70mm x 1.4mm) that utilizes BCG to interpret repeated movements of the human body, such as heartbeat, by representing them graphically (Pinheiro et al., 2010). Evaluation of this device during one night of sleep under real-life conditions revealed good agreement with the measurements provided by a reference device that employs electrocardiography and has been validated in laboratory studies, with only very minor differences in the mean HR and HRV values obtained (Vesterinen et al., 2020). Thus, although the reliability of this device for monitoring these parameters during sleep has yet to be established, it would appear to provide a simple and effective tool for automatic daily analysis of HRV.

Therefore, the time spent sleeping, nature of the sleep, and associated HR and HRV were monitored with an Emfit QS device for 7 weeks here. To minimize the distance to the heart and thereby maximize signal quality, this device was placed under the mattress near the chest in a manner such that the subject was unaware of its presence. The device began to record automatically at a sampling rate of 100 Hz when it sensed body weight and continued throughout the night, stopping when the subject got out of bed in the morning.

This monitoring of nocturnal HRV and HR was collected in continuous 3-minute periods and data from periods in which the signal was poor and/or disrupted was excluded. The magnitude of the HRV is expressed relative to time, utilizing the root-mean-squared difference between successive RR intervals (RMSSD, ms). For this purpose, the average RMSSD for each 3-minute period was calculated and these averages used to visualize the nocturnal HRV values graphically (Figure 1). The endpoints of the best linear fit for each night were considered to be the average RMSSD values for evening and morning sleep and the latter taken to be the HRV RMSSD value (HRV_{NOC}) during that night of sleep. Although previously the average 3-minute values for the entire night have been used to calculate this value (Vesterinen et al., 2020), the current investigation focused on an individual's current state of recovery and readiness to train, so the morning value was considered to be more relevant. For monitoring sleep patterns outside the laboratory, wrist actigraphy is the approach most widely used and best validated (Van De Water et al., 2011), but, at the same time, devices incorporated into the bed are highly convenient. These eliminate the need for attachment of electrodes or sensors to the body, providing a valuable option for longer-term monitoring of sleep at home. Such devices identify the different classes of sleep, as well as periods of wakefulness, with good accuracy (Yi et al., 2019).

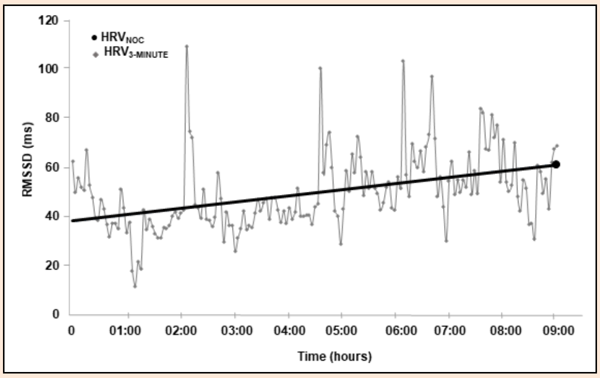

Figure 1. A representative example of the 3-minute sampling of the root mean square of successive differences between RR intervals (RMSSD) for heart rate variability (HRV) during sleep. The line shows the best fit used to calculate nocturnal HRV (HRV_{NOC}).

The sleep duration and HRV_{NOC} values analyzed here are presented automatically on the user interface of the Emfit QS device, providing easy daily access to both coaches and athletes. These values were also calculated as 3-day averages for comparison with cortisol values and the periods of SRT testing.

Cortisol analysis

Cortisol levels were analyzed from morning saliva samples. At the beginning of the testing period, all subjects were instructed on how to handle and collect the saliva samples. Collection occurred immediately after waking up before eating, drinking or brushing teeth. The passive drool-method was used for saliva collection and small cups were provided so that subjects could take 100 mL of water to wash out their mouth before collecting the sample. Subjects were asked to provide at least 3 mL of saliva for each sample as well as record the date, the time of day and how long it took to complete the procedure. Saliva samples were taken on 3 consecutive days, allowing for a sample the day before, the day of and one day after the SRT measurements occurred. Saliva samples were immediately Placed into subjects' freezers and collected every other week throughout the testing period.

Saliva samples were analyzed using the chemiluminescence method with the IMMULITE 2000 XPi Analyzer (Siemens Healthcare Diagnostics Products Ltd., Glyn Rhonwy, Llanberis, UK). The sensitivity of the saliva assay for cortisol was 5.5 nmol/l with inter-assay precision 8.2 % at 12.5 nmol/l. This method provides a noninvasive, easily repeatable and practical way to assess the cortisol response. Saliva cortisol values were analyzed in 3-day average values to coincide with the HRV values and SRT testing periods.

Training analysis

Individual training plans were followed throughout the testing period. Subjects were asked to write down all training sessions daily including the intensity, duration and exercise mode in their electronic training diaries (elogger.net, Espoo, Finland). Subjects had participated in prior maximal graded exercise tests that provided individually determined heart rate zones to guide training intensity on a daily basis with their individual heart rate monitors. Endurance training intensities were based on a 5-zone training distribution with zone 1 and 2 representing basic training (estimated: ≤ 2 mM blood lactate) and zone 3-5 representing all high intensity training. Training was analyzed according to the electronic training diaries. Easy training (zone 1 and 2) was defined as all training below aerobic threshold (K1, minutes) and hard training (zone 3-5) was defined as all training above aerobic threshold (K2). Weekly distribution of specific training modes for K1 and K2 training during the 7-week testing period are presented in Figure 2. Training load was quantified using a modified version of Lucia's simplified TRIMP system (Anta and Esteve-Lanao, 2011). Training load was calculated with the following equation:

$$\boldsymbol{TL = 1 \times K1 + 2.5 \times K2}$$

TL = training load, K1 = time training under aerobic threshold, K2 = time training at/above aerobic threshold. Training values were expressed in minutes. The training load, K1 and K2 were calculated to determine weekly values and three-day average values when STR and cortisol measurements occurred.

Submaximal Running Test

The SRT was 16-minutes in length and included 4 stages. Tests were performed on a Tunturi GO Run 50 Treadmill (Tunturi Fitness, Flevoland, Netherlands). The SRT was standardized for speed (women: 10 km/h, men: 11.7 km/h) with inclination increasing every 4 minutes, starting at 2%, then 4%, 7%, and 9%. One familiarization SRT was conducted so that all subjects were familiar with the test protocol. The SRT used in this study was designed for junior cross-country skiers and biathletes. Although we do not have validation of this protocol, it is a classic method used as a control test by athletes and coaches in Finland. Due to the homogenous group, the standardized protocol was appropriate and submaximal intensities were reached for all subjects. Heart rate (HR) was monitored with a HR-monitor (Polar V800, Polar Electro Oy, Kempele, Finland) and HR values were recorded when 15 s of each load remained. Subjects briefly stopped running and blood samples (20 μL) were taken from the fingertip every 4 minutes to determine blood lactate concentrations (Biosen C_line Lactate Analyzer. EKF Diagnostic, Magdeburg, Germany). Sample collection time (approx. 15 s) was included in the 4 minutes of upcoming stage.

Statistical Analysis

All statistical analyses were performed in the SPSS for Windows software (IBM SPSS Statistics 24 (SPSS, Inc., Chicago, IL, USA)). Since the number of subjects was small, Friedman's non-parametric test for related samples was applied to analyze changes in training, HRV and salivary cortisol levels. Post-hoc analyses were performed with the Wilcox signed rank test. Spearman's correlation coefficient was used to determine the relationship between 3-day average HRV and cortisol levels, as well as between HRV, sleep duration, cortisol levels and training characteristics. In addition, the relative changes in HRV, cortisol levels, sleep, SRT and training characteristics from week to week were also investigated using Spearman's correlation coefficient. All values of HRV, sleep duration and cortisol level utilized were 3-day averages, whereas weekly averages were employed in the case of SRT and training characteristics. All values shown are means ± SD and statistical significance defined as $p < 0.05$.

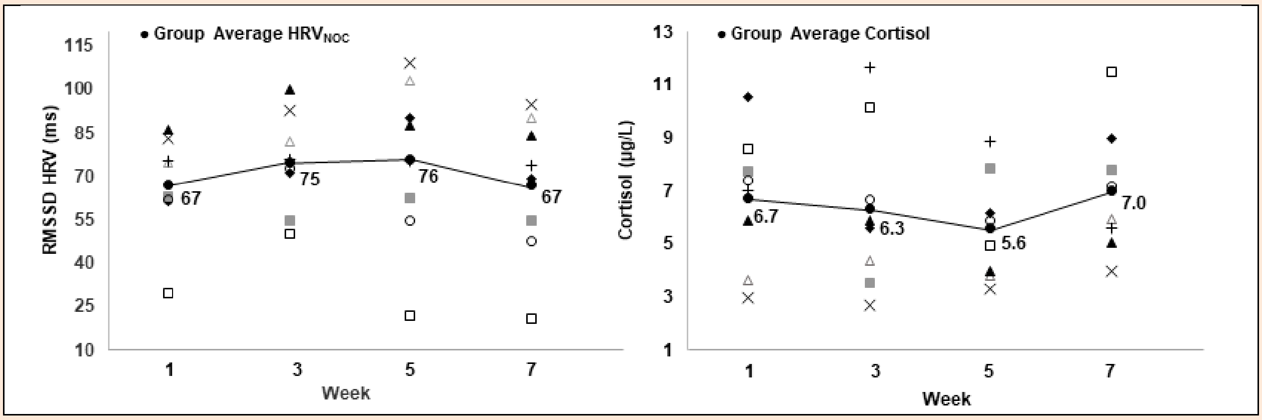

Figure 3. Average individual 3-day values for the root mean square of successive differences between RR intervals (RMSSD) and morning salivary levels of cortisol at different time-points during the 7-week study.

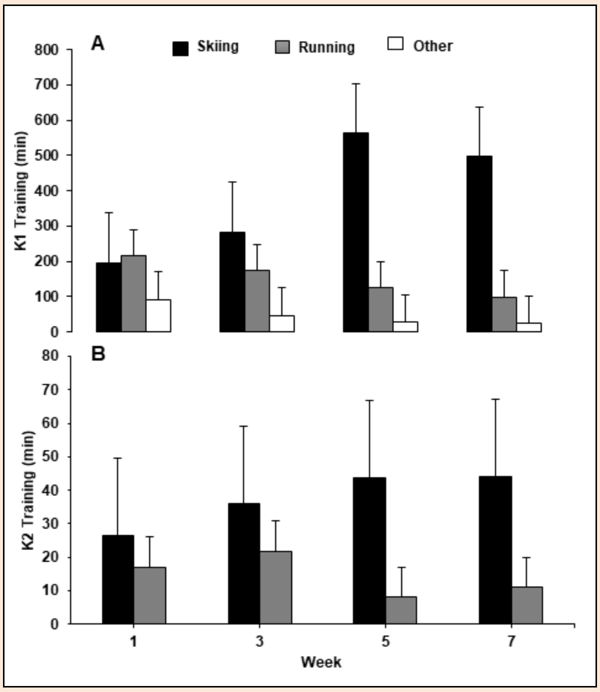

Figure 2. Graphs A and B represent the weekly distribution of exercise training modes for easy (K1) and hard (K2) training during all weeks of testing.

Results

During all weeks of testing, HRV and salivary levels of cortisol were inversely related. Although the inter-individual differences were pronounced (Figure 3), the highest HRV and lowest level of cortisol were observed during week 5.

Relationships between HRV, Cortisol levels, sleep and training

Average values for HRV, levels of cortisol, sleep and weekly training values are shown in Table 2. Weekly correlations between these parameters are shown in Table 3. Figure 4 is a scatter plot of the relationship between HRV and cortisol during all weeks of testing combined. Figure 5 shows average daily values of HRV and cortisol throughout the testing period.

As can be seen, the most pronounced correlation between HRV and morning cortisol levels was observed during week 1 and week 7 ($r = -0.833$, $p < 0.05$) (Table 3). The HRV values were significantly different between week 1 and week 3 ($p < 0.05$) and between week 5 and week 7 ($p < 0.05$). In addition, sleep and nocturnal sleep HR were both significantly different between week 1 and week 7 ($p < 0.05$) (Table 2). Differences between test weeks for cortisol and training values were not significant.

Throughout the training period (T1, weeks 1-3), a negative correlation was observed for relative changes in cortisol levels and sleep ($r = -0.762$, $p < 0.05$). The same relationship was demonstrated for K2 and sleep (week 1-3, $r = -0.762$, $p < 0.05$). During the shift from the training to the early competition season (week 3-5), cortisol displayed a positive relationship with K2 ($r = 0.810$, $p < 0.05$). When looking at HRV, a positive relationship was displayed for relative changes from week 5 to week 7 with sleep ($r = 0.786$, $p < 05$) and a negative relationship was observed for differences between weeks 1-7 ($r = -0.714$, $p < 0.05$) with sleep HR. In addition, the relative changes in K2 and HRV showed a positive correlation weeks 3-7 ($r = 0.736$, $p < 0.05$) and the same pattern was observed for relative changes in HRV and TL (week 3-7, $r = 0.810$, $p < 0.05$).

Associations between cortisol levels, training, and heart rate and levels of blood lactate during Submaximal Running Tests (SRT)

Cortisol's response to changes in SRT varied throughout the testing period. Relative differences in week 3-5 demonstrated a negative relationship to both HR ($r = -0.929$, $p < 0.01$) and blood lactate ($r = -0.857$, $p < 0.05$). However, during the end of the testing period (week 5-7), the differences between cortisol levels displayed a positive relationship with the changes in the SRT HR values ($r= 0.929$, $p < 0.01$). In addition, a negative relationship between blood lactate and cortisol was observed during week 7 ($r = -0.714$, $p < 0.05$).

Monitoring of the distribution of training revealed that volume of K2 was the highest during week 3, while K1 and training load were both greatest during week 5, i.e., at the beginning of the competition season (Table 2). Distribution of training modes for K1 and K2 throughout the testing period can be viewed in Figure 2.

Table 2. HRV, morning salivary levels of cortisol, nocturnal heart rate and sleep, amounts of hard and easy training, overall training load, and blood level of lactate and heart rate during submaximal running tests (SRT) at different time-points during the 7-week study period (means ± SD).

	T1, week 1	T1, week 3	T2, week 5	T2, week 7
RMSSD (ms)[a]	66.9 ± 17.8	75.0 ± 17.0*	75.6 ± 28.7	67.0 ± 24.8*
Cortisol (µg/L)[a]	6.7 ± 2.5	6.3 ± 3.1	5.6 ± 2.0	7.0 ± 2.4
Hard training (min)[b]	44 ± 30	64 ± 28	45 ± 17	53 ± 14*
Easy training (min)[b]	508 ± 190	555 ± 151	743 ± 251	577 ± 136
Training load[b]	616 ± 246	714 ± 209	856 ± 283	710 ± 132*
Blood lactate (mmol/L)[c]	3.8 ± 1.0	3.6 ± 0.6	3.5 ± 0.6	3.7 ± 1.1
Heart rate (bpm)[c]	183 ± 6	184 ± 8	180 ± 9	182 ± 9
Sleep (min)[a]	482 ± 29**	513 ± 36	513 ± 52	527 ± 44
Nocturnal heart rate (bpm)[a]	58 ± 5**	55 ± 4	55 ± 3	54 ± 5

[a] Average 3-day values for the nocturnal root mean square of successive differences between RR intervals (RMSSD), nocturnal heart rate, night sleep and saliva levels of cortisol. [b] Weekly values. [c] Average during the final stage of the SRT. * $p < 0.05$ compared to the previous week. ** $p < 0.05$ when compared to week 7.

Table 3. Relationships (Spearman's correlations) between HRV_{NOC} and morning salivary level of cortisol, the amounts of hard and easy training, and overall training load during the different weeks of the study.

Week	Cortisol (µg/L)	Hard training (min)	Easy training (min)	Training load
1	-0.833*	-0.287	-0.071	-0.095
2	-0.238	-0.036	-0.048	-0.132
3	-0.524	0.024	-0.238	-0.143
4	-0.833*	-0.571	-0.264	-0.143

* $p < 0.05$

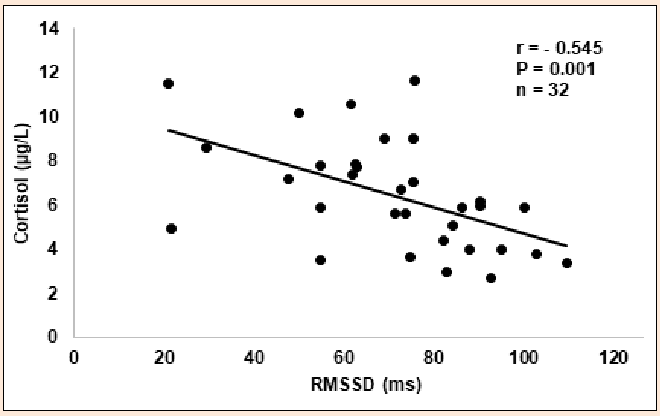

Figure 4. Graphs A and B represent the weekly distribution of exercise training modes for easy (K1) and hard (K2) training during all weeks of testing.

Discussion

The major findings of the current investigation were as follows: 1) HRV_{NOC} and salivary cortisol correlated significantly during test weeks that displayed the lowest HRV_{NOC} and highest salivary cortisol levels. 2) The decline in HRV from week 3 to week 7 was correlated with a reduced volume of intense training/training load, suggesting that this decline was not due to training stress, but rather the increase in fatigue/stress associated with the beginning of the competition period. 3) When the athletes were focused on training (weeks 1-3), the change in sleep duration was negatively correlated to both K2 and salivary cortisol levels, indicating that a reduction in the amount of sleep may be associated with elevated weekly strain. Finally, 4) although the amount of easy training and training load increased, a reduction in the volume of hard training is reflected in salivary cortisol levels with a

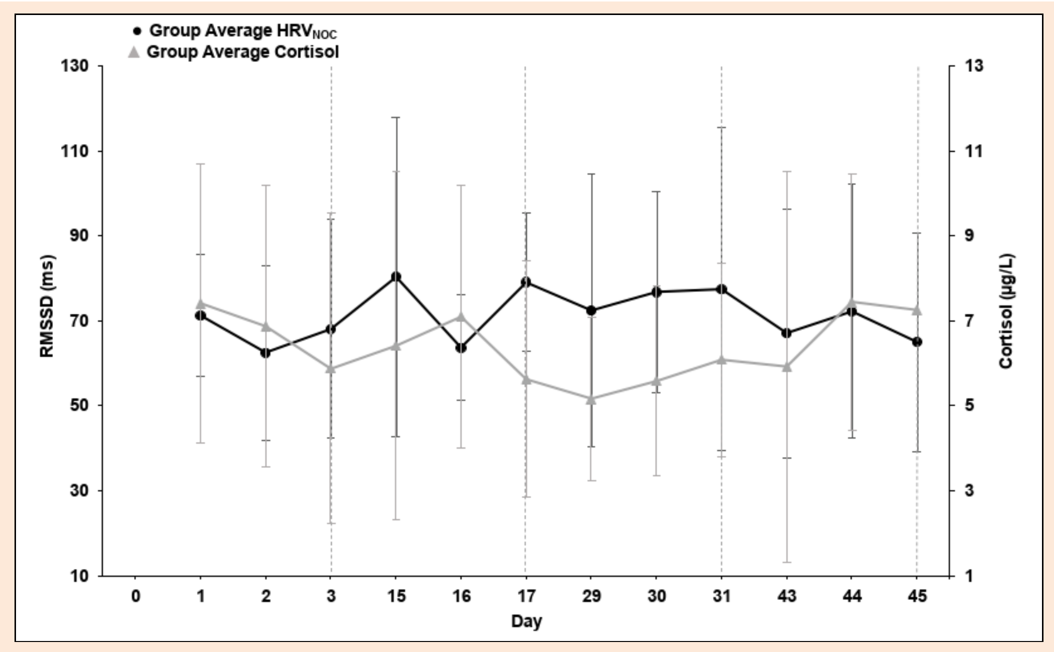

Figure 5. Daily values for the average root mean square of successive differences between RR intervals (RMSSD, ms) and morning salivary level of cortisol during the 7-week testing period.

positive relationship between changes in K2 and cortisol during weeks 3-5. Thus, young endurance athletes appear to handle large amounts of easy training when the volume of hard training is reduced.

During the 7-week study period, HRV$_{NOC}$ and cortisol displayed a negative relationship (Figure 4). When observing each test week separately, we found strong negative correlations between HRV$_{NOC}$ and cortisol during week 1 and 7 and moderate negative correlations for all remaining test weeks (Table 3). This indicates that cortisol and HRV$_{NOC}$ have a negative relationship for young endurance athletes.

The evaluation of three-day nocturnal RMSSD for the HRV analysis used in the current study is based on previous findings. Although daytime recordings are commonly used, it has been suggested that night readings enhance reliability since external factors are no longer affecting an individual during sleep (Pichot et al., 2000; Buchheit et al., 2004; Nummela et al., 2010). Earlier research has found that the time domain variable (RMSSD) has shown similar recovery times and changes to the commonly used high-frequency variables of HRV (Hautala et al., 2001; Carter et al., 2003), indicating RMSSD effectively evaluates change in autonomic regulation. Additionally, RMSSD measures have shown a high correlation to the high-frequency variability (Otzenberger et al., 1998; Esco et al., 2018) with various breathing frequencies having minimal effects on RMSSD values (Electrophysiology, 1996). Moreover, similar ballistocardiographic-based measures of nocturnal HRV have been compared to electrocardiographic measures and results showed that both HRV and HR data agreed with electrocardiography data that was also tested in real-life conditions (Vesterinen et al., 2020). These findings support the idea that HRV$_{NOC}$ may be a useful method to evaluate individual response for endurance training. Daily (Hautala et al., 2001; Carter et al., 2003; Kiviniemi et al., 2007; Hynynen et al., 2010; Herzig et al., 2017) and weekly HRV (Pichot et al., 2000; Nummela et al., 2010) values, which are both responsive to training, are commonly analyzed. However, since daily measurements can be influenced by pronounced diurnal variations, weekly averages may provide a better indication of adaptation to training and are recommended for use (Plews et al., 2013). At the same time, utilizing the combined values for nocturnal and morning HRV over several days instead of weekly values provides a better measure of rapid responses by the autonomic nervous system (Nuuttila et al., 2017). Additionally, although several studies found no sex differences in resting HRV values response to training of cross-country skiers (Hedelin et al., 2000, Schäfer et al., 2015), the averaging of several HRV values may help diminish the effect of individual confounders, such as gender, on HRV (Schäfer et al., 2015). Therefore, we analyzed the nocturnal morning RMSSD for three successive days, an approach that does not require special software and involves calculations that can be made easily, allowing its use not only in the laboratory, but in real life as well (Hynynen et al., 2010).

One important factor that may influence the daily changes in HRV values as well as the current state of recovery is sleep (Shinar et al., 2006). Sleep and overall levels of fatigue are highly interconnected, and sleep appears to have a critical role in the daily functioning during the adolescent years (Brand and Kirov, 2011). Sleep duration is a frequently and easily investigated measure for overall health and recommendations suggest that adolescents (13-18 years of age) should obtain 8-10 hours of sleep each night (Paruthi et al., 2016). In addition, athletes are advised to obtain additional sleep and ample research has reported the detrimental effects of sleep loss on human performance; demonstrating sleep is a valuable factor to observe in athletes (Fullagar et al., 2015; Simpson et al., 2017). Although monitored each night, sleep was not controlled during this study. Therefore, personal commitments (i.e. socializing, studying) and individual sleeping habits likely influenced the relationships between sleep and other investigated variables.

Previous research has discovered that even elite athletes are often unable to obtain the recommended amount of sleep (Roberts et al., 2019) with a recent review finding greater deficiencies in athletes' sleep during competition periods (O'Donnell et al., 2018). Our findings differ from this tendency, with the greatest volume of sleep occurring during the competition period (Table 2). Additionally, the sleep duration during this study consistently remained within the recommended 8-10 hours. Although the quality of sleep was not monitored, previous research with young gymnasts found the overall quality of sleep was unaffected during a competition period (Sartor et al., 2017). Therefore, the positive correlation between changes in sleep and HRV_{NOC} observed during the competition period ($r = 0.786$) indicates that individuals who obtained more sleep may have also experienced an enhanced recovery.

During this 7-week training period, training characteristics had a mixed effect on HRV_{NOC} . Previous research has found conflicting responses with increases, decreases and no changes in HRV all occurring with an increased training load (Pichot et al., 2000; Hautala et al., 2001; Carter et al., 2003; Hynynen et al., 2010). It is evident that the exact mechanisms behind the effects of endurance training on HRV are not well-defined (Herzig et al., 2017). Moderate amounts of exercise have been shown to enhance vagal-related HRV indexes (Buchheit et al., 2004). Thus, the increase in HRV_{NOC} from week 1 to week 3, when physical training increased, supports this previous finding. Moreover, HRV_{NOC} was the highest, during week 5, when training load also reached its highest values, indicating there was a good tolerance to the present training stimulus. However, we found that the changes from week 3 to week 7 showed a positive correlation with decreases in volume of hard training, training load and HRV. Previous research found a decrease in nocturnal HRV values after both moderate and heavy endurance training sessions (Hynynen et al., 2010). Additionally, a progressive decrease in HRV values were found following a 3-week period of intensive training (Pichot et al., 2000). In the present study, the volume of hard training and training load were reduced, indicating that the decrease in HRV_{NOC} was not associated to current training induced stress. Since the period of training analyzed in this study occurred during the initial phase of competition, all subjects also had the common goal of preparing for the early stage of their competition season. Therefore, as a group, weekly training followed similar and expected training programs with an intentional increase in ski-specific training throughout the study to reduce training stress as competitions approached. Similar to physical stress (physical work, fatigue, dietary stress), physiological stress (emotional, anxiety, cognitive), such as anticipatory stress, may have increased during the competition phase due to race-induced pressure and mental preparations that occur prior to competition. An independent measure of anxiety was not included in this study; as a result, although it appears, it is hard to identify if the alterations in HRV_{NOC} and cortisol were induced by competition stress. Nevertheless, previous research has shown that acute stress, due to an anticipatory task, had an impact on HRV during sleep and was associated with a decreased parasympathetic modulation, and therefore, resulted in lower HRV values (Hall et al., 2004), further supporting our current finding.

Salivary cortisol levels are frequently used as a biomarker of psychological stress and have shown a moderate association to perceived stress (Hellhammer et al., 2009). Previous literature has investigated salivary cortisol level's response to exercise and found cortisol only significantly increased after high-intensity exercise with no changes occurring after low and moderate exercise (VanBruggen et al., 2011). Additionally, when investigating cortisol levels in over-trained and control athletes no significant differences between groups were found (Hynynen et al., 2006). Furthermore, both baseline and response to training cortisol levels are influenced by genetics (Feitosa et al., 2002) so individual variation has an added effect on cortisol values. In the present study, changes during week 3 to week 5 showed that salivary cortisol appeared to respond to the reduced volume of hard training by demonstrating a positive correlation ($r = 0.810$). The fact that week 5 included the highest amount of physical training and lowest salivary cortisol levels suggests that young athletes appear to handle large volumes of easy training and high training loads as long as the amount of hard training is reduced (Table 3). This current finding supports previous research that found, an increase of low-intensity training, equivalent to about 100% increase in training load, showed no changes in cortisol, although decreased performance occurred (Jürimäe et al., 2004). Our study displayed the greatest increase in cortisol from week 5 to week 7, during the early competition period, suggesting changes in salivary cortisol may be more related to the early season race schedule rather than amount of hard training. During week 6, the competition season began with 7/8 subjects participating in their first race. Therefore, when interpreting cortisol results, the stress from racing is an important factor to consider. Previous research has found an increase in morning and afternoon salivary cortisol levels during a competition day, regardless of similar training volume and intensity, indicating competition may alter the physiology of stress-related hormones (Iellamo et al., 2003).

In addition, a decrease in sleep quality and duration has been associated with raised cortisol concentrations as well as an increase in activity of the sympathetic nervous system (Spiegel et al., 1999). In the present study, the training stimulus and sleep duration remained similar each week; therefore, our findings support the idea that competition stress may have increased morning cortisol levels. However, the cortisol values presented in our study were not collected on race day, so it is hard to know if a competition-induced stress was still present. The analysis of 3-day average salivary cortisol levels used in the present study shifts the focus to the total stress that was occurring each week rather than the stress response of an individual competition. When the subjects were focused on training (week 1 to week 3), changes in sleep duration revealed a negative relationship with both K2 and cortisol, proposing a decrease in sleep may be associated to an increased amount of weekly strain. This agrees with findings that found high intensity training negatively affected both subjective sleep parameters and recovery-related ratings (Kölling et al., 2016).

Furthermore, in endurance sports, the parasympathetic form of overtraining syndrome often dominates (Lehmann et al., 1993). Therefore, the increase in physical training during week 5, followed by the competition stress during week 6, may have resulted in a delayed fatiguing affect that was displayed during week 7. Pro-longed stress causes an increase in cortisol as well a decrease parasympathetic activity (McEwen, 2007) which may explain why an increase in cortisol was found as well as a decrease in HRV during week 7. Additionally, an anti-inflammatory process occurs due to training as well as muscle damage. Therefore, the elevation of cortisol may be associated to the greater training volume during week 5 or a result of a maximal race effort causing added stress and increased stimulation of glycogen re-synthesis (Kirwan et al., 1998).

In the present study, performance/recovery status was followed with SRT. As illustrated in Figure 2, running is a common exercise mode in both hard and easy training; therefore, a SRT was applicable for monitoring fatigue with this group of subjects. The easily repeatable design (based on speed and inclination) of this testing protocol provides a test that can be conducted in various training environments, such as at training camps or after long travels, to help athletes and coaches determine current levels of fatigue. Although the lack of individualized exercise intensities may reduce the reliability of the test, the repeated design provides valuable heart rate and lactate data at standardized exercise intensities during the 7-week period and significant changes in this data would indicate that levels of fatigue should be further investigated. Previous literature supports the application of submaximal tests for monitoring and predicting performance (Lamberts et al., 2004), but details the importance of implementing multiple variables so adequate insight of individual status is applied when interpreting results (Capostagno et al., 2016). As a result, we investigated the relationships between SRT heart rate, SRT blood lactate, morning salivary cortisol, HRV_{NOC}, and physical training. During controlled submaximal intensities, HR has shown to remain constant with the lowest variation occurring at 90% HRmax values (Lamberts et al., 2004). In the current study, changes of SRT heart rate (around 90% VO_2max) between week 5 and 7 displayed a strong relationship with changes in cortisol ($r = 0.929$). Cortisol demonstrated an additional relationship between week 3 and 7 with a negative correlation to changes in SRT heart rate ($r = -0.929$) and blood lactate ($r = -0.857$). Common assumptions about changes in HR at submaximal intensities suggest that an increase in aerobic fitness is linked to decreases in HR, while increases in HR are associated with a decline in fitness, dehydration or overtraining (Lamberts et al., 2004). Earlier research additionally suggests reduced submaximal HR is only a sign of effective endurance training when no decline in maximal performance is present (Hedelin et al., 2000). Therefore, without maximal HR values it is hard to evaluate the relationship between SRT HR and resting cortisol values, which also have mixed results. In addition, in order to detect significant changes in SRT, it is recommended that the HR values are approximately 7 bpm different at 90% HRmax workload (Lamberts et al., 2004) and therefore, fluctuations during the present study were too small to interpret any training induced changes.

Since variation in response to training stress is an apparent difference, it is logical to assume that monitoring variables, such as HRV, that also have an individualized response to training stress would be beneficial for optimizing performance. Research has investigated the response to endurance training and numerous factors have helped explain these differences such as, genotype, training background, gender, age, training load, etc (Carter et al., 2003; Buchheit et al., 2004; Nummela et al., 2010). In addition, large differences were observed despite prescribing the same amount of volume and modifying intensity training individually (Nummela et al., 2010). During this study, although physical training was not standardized, comparable training occurred due to group training and competition schedules. Present findings showed similar weekly trends for both HRV and cortisol but individual differences were high, agreeing with previous findings (Figure 3). Due to this high intra-individuality, previous research has investigated and implemented HRV-guided training into endurance training programs. HRV-guided training resulted in a lower frequency of high-intensity exercises and therefore, a decreased training load (Kiviniemi et al., 2007). When the timing and amount of high intensity exercise is adjusted, a slight change in the training periodization occurs. A large training focus for young endurance athletes is building their aerobic capacity and an improved endurance comes from accumulated years of effective training. As a result, further research should follow the long-term effects on HRV and endurance training before implementing a HRV-guided approach to training in young athletes.

Limitations

Limitations of this study may have occurred due to the small sample size and the grouping of both genders, as well as the decreased standardization due to the collections of nocturnal HRV, sleep duration and morning cortisol values occurring at home. There may be various factors such as poor or disrupted sleep that are not associated to training but still affect morning cortisol and HRV values. Additionally, the assessment of physical training and training load came from self-accessed training diaries; therefore, they were solely based on subjective estimations of the training-induced stress that was occurring during this period. Finally, our subjects' level of psycho-physiological stress may have risen when the period of competition began, due, e.g., to the pressure to perform well. This potential change was not evaluated independently and may have influenced the HRV, sleep duration and saliva cortisol levels. However, autonomic stress reactions do not differ between the source of stress, and most likely, the greatest influence is the overall stress/recovery balance.

Practical applications

Young athletes are in a developmental period and therefore, may display a greater sensitivity to stress (McEwen, 2007). Application of HRV values to monitor training could identify better-individualized training profiles (Nummela et al., 2010). This would be valuable information for coaches and athletes and when no access to laboratory settings is required, it can be used daily as well as at training camps where training load increases. Thus, our findings suggest three-day average HRV values may provide an accurate representation of young athletes' current recovery status of the autonomic nervous system and would be a beneficial value to follow during the high stress training and competition periods.

In addition, it appears sleep is another tool that could be utilized to further facilitate success in young athletes. Although we did not examine sleep quality or the circadian rhythm of sleep in detail, our findings on sleep duration provide insights of potential value to many athletes. Our findings provide support for previous suggestions that improved awareness of the negative consequences of sub-optimal sleep, in particular between races during the season of competition, can help athletes optimize their training (Simpson et al., 2017). During the period of competition, the weekly training load becomes slightly more constant in order to maximize preparation for important competitions. As a result, daily stress often increases and, therefore, young endurance athletes may benefit from additional sleep during this period.

Conclusion

In conclusion, the present study shows that nocturnal HRV appears to correlate negatively with salivary levels of cortisol in young endurance athletes. Coaches and athletes should be aware that as the training season ended, the decline in physical training correlated to the decrease in HRV, suggesting more time is needed to reach full recovery once the competition season has begun. This indication that full recovery from competition requires more time is supported further by the positive relationship between sleep duration and HRV during the competition season which implies additional sleep may also be beneficial for performance. In addition, changes in cortisol during the competition season suggest that an increased stress occurs but whether this stress is specific to competition is still unknown. Future research should be designed to determine which specific variables best reflect recovery and should be utilized to monitor this aspect of training. Additional focus should also be placed on determining what variables and changes are most closely associated with improved performance, so that further characterization of these patterns and variables can help young athletes improve their performance.

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Key points

- Nocturnal HRV appears to correlate with salivary levels of cortisol in young endurance athletes.
- Recovery during the competition season, despite a decrease in physical training, may require additional time.
- Throughout the race season, young athletes may benefit from increased sleep.

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II

EVALUATION OF NOCTURNAL VS. MORNING MEASURES OF HEART RATE INDICES IN YOUNG ATHLETES

by

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RESEARCH ARTICLE

Evaluation of nocturnal vs. morning measures of heart rate indices in young athletes

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Data Availability Statement: Ethical restrictions were placed upon this data due to the sensitive subject information and possible identifying information it may contain. In the informed consent form that was approved by the Research Ethical Committee we have stated that all data is confidential and it will not be given to third parties. Our data includes sensitive health data (such as HR indices, sleep values, etc.) and since all the subjects participating in this study were adolescents it is possible that they can be identified even after anonymization of this data, therefore,

Abstract

Purpose

The purpose of this study was to compare heart rate (HR) and heart rate variability in young endurance athletes during nocturnal sleep and in the morning; and to assess whether changes in these values are associated with changes in submaximal running (SRT) and counter-movement jump (CMJ) performance.

Methods

During a three-week period of similar training, eleven athletes (16 ± 1 years) determined daily HR and heart rate variability (RMSSD) during sleep utilizing a ballistocardiographic device (Emfit QS), as well as in the morning with a HR monitor (Polar V800). Aerobic fitness and power production were assessed employing SRT and CMJ test.

Results

Comparison of the average values for week 1 and week 3 revealed no significant differences with respect to nocturnal RMSSD (6.8%, $P = 0.344$), morning RMSSD (13.4%, $P = 0.151$), morning HR (-3.9 bpm, $P = 0.063$), SRT HR (-0.7 bpm, $P = 0.447$), SRT blood lactate (4.9%, $P = 0.781$), CMJ (-4.2%, $P = 0.122$) or training volume (16%, $P = 0.499$). There was a strong correlation between morning and nocturnal HRs during week 1 ($r = 0.800$, $P = 0.003$) and week 3 ($r = 0.815$, $P = 0.002$), as well as between morning and nocturnal RMSSD values (for week 1, $r = 0.895$, $P<0.001$ and week 3, $r = 0.878$, $P = 0.001$).

Conclusion

This study concluded that HR and RMSSD obtained during nocturnal sleep and in the morning did not differ significantly. In addition, weekly changes in training and performance were small indicating that fitness was similar throughout the 3-week period of observation. Consequently, daily measurement of HR indices during nocturnal sleep provide a potential tool for long-term monitoring of young endurance athletes.

our data cannot be shared publicly. Only the authors and an institutional point of contact have access to the data and the corresponding author Christina Mishica has the coding key. Possible requests for limited data should be sent either to christina.m.mishica@jyu.fi, to professor vesa.linnamo@jyu.fi or to Secretary of the University of Jyväskylä Ethical Committee at secretary-ethicomm@jyu.fi.

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Introduction

Over the past several decades, the scientific approach to finding a balance between endurance training and recovery has continued to grow [1], resulting in the application of various tools and methods to monitor athlete recovery [2]. Since the outcome of an endurance competition is often influenced by relatively small differences in performance, tests of performance, such as submaximal running (SRT) and counter-movement jump (CMJ) tests, are commonly utilized for monitoring athletes' current physical condition [3]. For instance, during a two-week period with an increasing training load, the submaximal lactate levels observed during a SRT declined, with a subsequent return to normal following two weeks of recovery [4], demonstrating that physiological changes are reflected rapidly in the results of a SRT. In addition, decreases in the submaximal heart rate (HR) and/or increases in heart rate variability (HRV), measured using the root-mean-squared difference between successive RR intervals (RMSSD), for young soccer players as their training season progressed most likely reflect improvements in performance [5], indicating that these values might be of use for monitoring young athletes.

Recovery from endurance training involves a multitude of physiological responses, with the cardiovascular system playing a key role in this context [6, 7]. This process is regulated by the autonomic nervous system (ANS) and the time required for autonomic recovery is an indicator of cardiovascular homeostasis [7, 8]. Autonomic cardiovascular function can be assessed non-invasively by measuring HRV, i.e., the beat-to-beat variation in heart rate [9, 10] and advances in technology have provided affordable and reliable means for monitoring HRV and its response to daily stress [10]. Consequently, use of this approach, both for practical and research purposes, has become increasingly common [11].

HRV is regulated by the sympathetic and parasympathetic activity of ANS, and two common methods for measuring HRV are time and frequency domain [12]. RMSSD measures are a time domain method where sympathetic activity decreases the time between heartbeats while increases are due to parasympathetic activity [10]. During rest, the ANS favors parasympathetic activity [7], therefore an easy and practical way to monitor HRV is to follow resting RMSSD values [13]. There is considerable variation in procedures concerning what HRV methods are the best compromise between quality and accuracy of recordings as well as ease of use for athletes [13]. Sport technology devices are not specifically designed for research application, but validation of the recorded data is critical. Developments in technology with heart rate monitor devices have shown to produce recordings of RR intervals consistent to electrocardiograph (ECG) recordings and HRV parameters resulting from these recordings are comparable for performing orthostatic tests in healthy subjects [10]. Furthermore, mixed results in previous research indicate that HRV alone may not provide a comprehensive view of an athletes' overall wellbeing [2]. However, when used in combination with performance tests, and/or training data and questionnaires these values appear to be some of the most valuable variables to monitor [2].

Therefore, the purpose of this study was to access the ability of nocturnal HR and RMSSD values determined by ballistocardiography (BCG) to predict the respective validated morning values recorded with a heart rate monitor during orthostatic tests [10]. The aim was to compare HR and RMSSD values to provide results that can reflect the agreement between the two methods with additional SRT and CMJ tests to follow changes in physical performance during the 3-week training period. It was hypothesized that nocturnal and morning weekly HR and RMSSD values would show agreement, but morning orthostatic values would have a slightly lower RMSSD and slightly higher HR than nocturnal values due to additional thoughts and disturbances that may affect autonomic regulation when awake [14]. Secondly, it was hypothesized that changes in RMSSD and HR would be related to fatigue and therefore, associated to performance changes in SRT and CMJ tests. Finally, if weekly agreement between nocturnal and morning HRV tests was present, the goal was to determine if nocturnal measures are a valid method for monitoring young endurance athletes.

Methods

Participants and design

Eleven well-trained young endurance athletes participated in this study. All participants were students at a sports academy high school competing and training for cross-country skiing (10 subjects) and biathlon (1 subject) year-round. Characteristics of the athletes are presented in Table 1. All were fully informed of the experimental procedures and provided written consent from their legal guardians before taking part. The ethics committee of the University of Jyväskylä, Finland, approved the study and measurements were performed in accordance with the declaration of Helsinki.

This study occurred during a 3-week period of normal endurance training in preparation for the upcoming racing season (early November). Preceding participation, practice tests were provided so subjects were familiarized with the CMJ and SRT protocols. Running and jump exercises were already incorporated into the tested individuals' normal training so a preparatory training period was unnecessary. The SRT and CMJ tests were performed during week 1 and week 3 of the study. During night sleep, ANS state was assessed with nocturnal HRV and HR analysis, collected using a ballistiocardiographic (BCG) sleep-tracking device (Emfit QS, Emfit Oy, Jyväskylä, Finland). In addition, morning values for HRV and HR were also evaluated with orthostatic tests performed using a Polar V800 heart rate monitor (Polar Electro Oy, Kempele, Finland) and H10 heart sensor (Polar Electro Oy, Kempele, Finland). Morning and nocturnal values for HR and HRV were measured daily throughout the study. At the beginning of the study, body fat percentage and weight were measured using the bioimpedance method (InBody 720, Inbody CO., Cerritos, California, USA). Fig 1 illustrates the study design and when each measurement occurred.

HRV and HR analysis

Nocturnal HR and HRV were monitored using a contactless sleep-tracking device. This device employs BCG to numerically and graphically depict repeated movements [15], such as heartbeat, with a contactless pressure (542 mm x 70 mm x 1.4 mm) and then presents the values numerically. Under free-living conditions, evaluation of this device revealed good agreement to a laboratory validated reference device utilizing electrocardiography, with only minor differences in mean HR and HRV values [16]. Therefore, although the accuracy of this device for continuous monitoring of nocturnal HRV and HR is yet to be determined, it seems to be an effective tool and convenient method for automatic analysis of HR and HRV.

Table 1. Characteristics of the subjects (means ± SD).

	Women (n = 4)	Men (n = 7)
Age (yrs)	16 ± 1	16 ± 1
Height (m)	1.67 ± 0.09	1.79 ± 0.02
Body mass (kg)	61 ± 10	68 ± 5
Body fat (%)[a]	17.6 ± 1.5	7.9 ± 2.3
BMI ($kg.m^{-2}$)	21.6 ± 1.3	21.1 ± 1.3

[a] Assessed on the basis of bioimpedance measurements.

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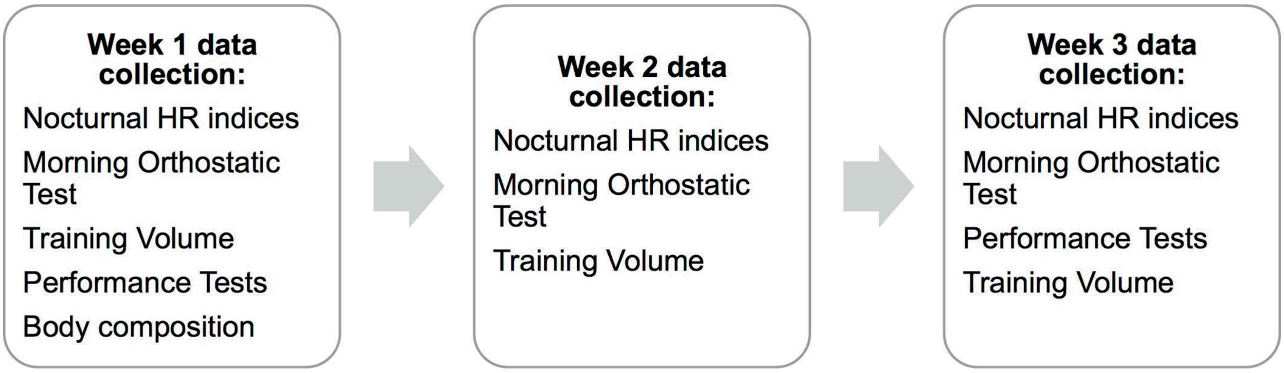

Fig 1. Flow chart of study design.

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Subjects were instructed to place the device under the mattress near the chest area to minimize the distance to the heart and thus, maximize the signal quality. The subjects were unable to detect the device's presence, but the device sensed when body weight was present and automatically began the recording process. The recording continued at a sampling rate of 100 Hz, stopping when the subject left the bed in the morning. Nocturnal HR and HRV data were collected using continuous 3-minute periods, and disrupted and/or poor signals were excluded from the data. The average HR values for each 3-minute period were calculated and these averages were used to determine whole night daily averages. Nocturnal HRV was interpreted relative to time, by utilizing RMSSD. To determine several different nocturnal RMSSD values during each night, average RMSSD for each 3-minute period was calculated and used to produce a graphical representation of nocturnal RMSSD values. The endpoints of the best linear fit for each night were chosen to represent the average RMSSD values for evening ($RMSSD_{pm}$) and morning ($RMSSD_{am}$) sleep and the whole night average ($RMSSD_{NOC}$) was considered to be the mean of both values. Although previously the average 3-minute values for the entire night have been used for analysis [16], the current investigation focused on using values that are automatically presented on the user interface of the sleep device. Values presented there were obtained from approximately 8–10 hours of sleep each night.

Morning orthostatic tests were performed using a Polar V800 heart rate monitor and H10 heart sensor. This sensor has previously shown acceptable levels of agreement with a 12-lead electrocardiogram system for recording RMSSD [17] and the Polar V800 monitor is a previous validated method for detection of RR intervals during an orthostatic test [10]. All subjects were instructed to keep the heart rate monitor and electrode strap next to their bedside and to conduct the orthostatic test upon waking up each morning. This assured minimal physical activity occurred. No attempts were made to control the breathing frequency, and subjects were asked to remain relaxed and repeat the test using the same routine each morning. Instructions were provided in the orthostatic test feature of the Polar V800 watch with the test beginning at the click of a button. Subjects were directed to remain in a supine position for 3 minutes before a beeping occurred from their watch signifying that the subject needed to stand up. The standing portion of the test was also 3 minutes in length with a beep indicating the test was complete. Orthostatic tests measures HR and HRV in R-R intervals using a sampling frequency of 1000 Hz with a reference window of 60–120 seconds in each position. Collected data was analyzed with polar flow (www.flow.polar.com). Test results provide average values for HR and HRV in both the supine and standing position as well as peak HR when standing. Minimal compliance for HRV and HR analysis was set at 3 days each week, therefore, if a subject did not have 3 days in one of the test weeks they were omitted from the study.

Submaximal running test and counter movement jumps

Counter movement jump (CMJ) performance tests [18] were evaluated using a force plate (HUR FP8, HUR Oy, Kokkola, Finland). Subjects were instructed to keep their hands fixed to their hips, feet shoulder-width apart and to bend their knees to a 90-degree angle when jumping as high as possible. A total of three jumps were performed with about 1 minute of recovery between jumps. Jumping height was analyzed from the force impulse. The analysis was completed using coach tech system (Vuokatti Sports Technology Unit, University of Jyväskylä, Finland) [19]. The highest jump was used for the current measure of performance.

SRT tests were conducted to evaluate aerobic fitness. The SRT performed during this study was designed to elicit heart rates (HR) at approximately 90% of maximal HR so significant changes in HR variation were measurable [20]. The SRT included 4 stages and was 16-minutes in length. The test was performed on a Tunturi GO Run 50 Treadmill (Tunturi Fitness, Flevoland, Netherlands) and speed was standardized (women: 10.0 km/h, men: 11.7 km/h) with inclination increasing every 4 minutes, starting at 2%, then 4%, 7%, and 9%. The subjects' HR was continuously monitored with a Polar HR-monitor and when 15 seconds of each load remained HR values were recorded. Every 4 minutes the subjects briefly stop running and blood samples (20 μL) were taken from the fingertip to determine blood lactate concentrations (Biosen C_line Lactate Analyzer, EKF Diagnostic, Magdeburg, Germany). Sample collection time (approx. 15 s) was included in the 4 minutes of the upcoming stage. Due to the highly homogenous nature of the group, the utilized protocol was appropriate and submaximal intensities were reached for all subjects. Although we do not have validation of this specific protocol, it is a familiar and standardized protocol that is commonly used as a control test by junior cross-country skiers and biathletes in Finland. Performance test measures occurred during week 1 and week 3 of the study period and measurements were required to be completed by all subjects each test week.

Statistical analysis

Descriptive statistics were calculated for all variables and all values are reported as means ± SD with the 95% confidence interval (CI). Sample distribution was tested using the Shapiro-Wilk test for nocturnal and morning HR indices (i.e., HR and RMSSD) as well as performance test variables. Normal distribution was present for all weekly variables as well as the difference between weeks for nocturnal and morning HR indices. A within-subject approach was applied by utilizing paired sample t-tests to examine the differences between nocturnal versus morning measurements and the differences between the different test weeks. To evaluate the extent of agreement between morning and nocturnal values Pearson's correlation coefficient (r) was calculated. To assess the difference between measurements made in the morning or during the night, the coefficient of variance and 95% CI for HR (HRCV) and RMSSD were determined each week for all HRV parameters (HRVCV).

The extent of correlation among the morning and nocturnal measures for HR and three different nocturnal HRV measures (AM, PM and Nocturnal) was tested using Intra-class correlation coefficient (ICC) or reliability coefficient, a measure of the reliability of measurements. ICC estimates and their 95% confidence intervals were calculated by a single-rating, consistency-agreement, 2-way mixed-effects model [21]. Values less than 0.5 suggest poor reliability with values between 0.5 and 0.75 suggesting moderate reliability and values between 0.75 and 0.9 indicative of good reliability [21].

Table 2. Comparison of heart rate variability, heart rate, sleep, performance tests and training during the study period.

	Week 1		Week 3			
	mean ± SD	95% CI	mean ± SD	95% CI	% diff	P
Nocturnal						
$RMSSD^{AM}$ (ms)	75 ± 26[a]	57.7, 92.7	77 ± 17[a]	65.6, 88.7	8.0	0.654
$RMSSD^{PM}$ (ms)	60 ± 20[a]	46.7, 73.2	63 ± 21[a]	49.5, 77.2	6.6	0.306
$RMSSD^{NOC}$ (ms)	68 ± 22[a]	52.6, 82.4	70 ± 17[a]	58.9, 81.5	6.8	0.344
Heart rate (bpm)	57 ± 5	53.0, 60.0	54 ± 5	50.2, 56.9	-5.2	0.002*
Sleep (hours)	8.2 ± 0.4	8.0, 8.5	8.6 ± 0.6	8.2, 9.0	3.8	0.095
Morning Orthostatic						
$RMSSD^{Rest}$ (ms)	104 ± 39[a]	77.8, 130.2	115 ± 41[a]	87.1, 142.4	13.4	0.151
$RMSSD^{Stand}$ (ms)	26 ± 8.6	18.3, 33.5	29 ± 9	19.9, 37.3	14.5	0.361
Heart rate (bpm)	57 ± 6	53.3, 61.3	55 ± 5	51.3, 58.5	-3.9	0.063
Performance Test						
Heart rate (bpm)	181 ± 6.9	176.3, 185.0	180 ± 8.2	174.0, 185.0	-0.2	0.447
Heart rate range (bpm)	28 ± 5.8	24.4, 32.2	30 ± 5.4	26.1, 33.3	1.3	0.181
Blood lactate (mmol/l)	3.6 ± 0.9	3.0, 4.1	3.6 ± 1.0	3.0, 4.3	0.4	0.781
Counter movement jump (cm)	34.1 ± 6.3	29.9, 38.4	32.5 ± 5.3	28.9, 36.0	-1.2	0.122
Training						
Volume (min/week)	514 ± 141	396.1, 632.4	563 ± 156	432.7, 693.0	16	0.499

* $p < 0.01$.

[a] Significant difference between nocturnal and morning $RMSSD_{Rest}$

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Results

Table 2 shows weekly means and standard deviations of all measured variables. Paired samples t-tests revealed no significant differences between week 1 and week 3 for $RMSSD_{NOC}$ (mean difference 6.8%, P = 0.344), morning RMSSD (mean difference 13.4%, P = 0.151), morning HR (mean difference -3.9%, P = 0.063), SRT HR (mean difference -0.7%, P = 0.447), SRT blood lactate (mean difference 4.9%, P = 0.781), CMJ (mean difference -4.2%, P = 0.122) or training volume (mean difference 16%, P = 0.499). Individual and group values for HR and RMSSD during morning and sleep are presented in Table 3. HR_{CV} and $RMSSD_{CV}$ values are presented in Table 4 with HR presenting similar values in both morning and nocturnal measurements. Furthermore, no significant differences were found in HR between the measurements obtained during night sleep (Emfit QS) vs. morning orthostatic tests (Polar V800). During both test weeks, significant differences were found for RMSSD values between devices (P<0.008) and very high correlations were observed for $RMSSD_{NOC}$ during week 1 (r = 0 .895, P<0.001) and week 3 (r = 0.878, P<0.001). Table 5 presents the IIC values of the measured HR indices. Moderate to good reliability was found for all HRV measures with nocturnal sleep and morning HR values showing the best agreement. Fig 2 displays the relationships for HR and RMSSD between nocturnal and morning measurements during the whole test period.

Discussion

The main results from the current study were that HR and RMSSD values obtained with a BCG device under real-life conditions during sleep were in good agreement to morning values derived from orthostatic tests (Fig 2), providing support for our hypothesis that measurements in the morning with orthostatic tests as well as nocturnal measurements give reliable weekly values for the HR and RMSSD of young endurance athletes.

Table 3. Descriptive statistics and coefficient of variance of individual and group weekly means ± SD for heart rate and RMSSD values during nocturnal sleep and morning tests.

	Average Heart Rate						Average RMSSD					
	Morning			Sleep			Morning			Sleep		
Subject	W1	W3	CV*	W1	W3	CV*	W1	W3	CV*	W1	W3	CV*
1	50	54	8.16	49	51	6.23	98	83	26.46	60	56	10.46
2	63	60	3.14	61	58	3.92	45	64	8.26	42	52	15.56
3	62	65	7.10	62	61	3.27	74	60	41.65	48	50	10.85
4	69	59	5.27	60	56	4.00	54	67	36.87	44	53	13.95
5	55	52	4.66	57	52	1.63	94	95	15.69	56	65	8.00
6	58	56	9.39	53	49	5.33	116	129	22.37	73	74	7.45
7	56	51	3.83	54	53	3.22	135	162	17.70	64	67	7.02
8	48	45	4.28	50	44	5.11	169	151	9.25	100	93	9.94
9	58	54	5.86	60	55	4.25	88	147	26.22	70	84	20.54
10	59	56	4.50	64	60	2.95	120	144	14.54	74	84	4.71
11	52	52	4.18	52	51	5.34	153	159	12.15	112	95	11.67
Mean	**57**	**55**	**5.49**	**57**	**54**	**4.11**	**104**	**115**	**20.01**	**68**	**70**	**11**
SD	**6**	**5**	**1.96**	**5**	**5**	**1.33**	**39**	**41**	**22.17**	**17**	**11**	**4.46**

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At the same time, our hypothesis that resting RMSSD and HR would be slightly lower and higher, respectively, than the corresponding nocturnal values turned out to be incorrect, since the former value obtained with orthostatic tests in the morning was higher and HR very similar at both time-points. Previous studies on the HRV of individuals with high levels of stress under real-life conditions revealed a lower variation in the values obtained with orthostatic tests after awakening, but not during nocturnal rest, suggesting that parasympathetic withdrawal occurs upon awakening [22]. In the case of our own investigation, in which none of the participants was in a stressed or over-trained state, the HR was similar at both time-points and the differences observed are likely to be due to the two different measurement procedures.

Previous research has challenged the evaluation of HR and RMSSD to monitor athletes, suggesting that the high day-to-day variation and variability of these values limits their usefulness because small and moderate changes often occur inside differences that are normally expected [23]. However, findings show that when comparing weekly vs. single day HRV values, the analysis of weekly HRV values provided a more meaningful assessment of ANS response in endurance athletes [24]. Additionally, longitudinal studies following RMSSD

Table 4. Coefficient of variance (CV) during nocturnal and morning heart rate measures.

	Week 1		Week 3	
	CV (%)	95% CI	CV (%)	95% CI
Nocturnal				
$RMSSD^{AM}$ (ms)	16.20	10.99, 21.42	13.02	9.33, 16.71
$RMSSD^{PM}$ (ms)	21.13	16.73, 25.46	21.37	15.43, 27.30
$RMSSD^{NOC}$ (ms)	12.35	8.08, 16.63	9.49	7.33, 11.66
Heart rate (bpm)	4.17	2.88, 5.45	4.06	2.59, 5.53
Morning				
$RMSSD^{Rest}$ (ms)	22.60	11.51, 33.68	19.43	9.43, 29.44
$RMSSD^{Stand}$ (ms)	38.11	22.19, 54.02	39.96	22.00, 57.93
Heart rate (bpm)	5.78	3.57, 8.00	5.19	4.00, 6.39

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Table 5. Intra-class correlation coefficient of morning versus nocturnal heart rate measures.

	Week 1			Week 3		
	ICC	95% CI	P	ICC	95% CI	P
Nocturnal						
$RMSSD^{AM}$ (ms)	0.813	0.45, 0.95	0.001	0.534	0.06, 0.85	0.037
$RMSSD^{PM}$ (ms)	0.691	0.20–0.91	0.006	0.650	0.12, 0.89	0.011
$RMSSD^{NOC}$ (ms)	0.769	0.35, 0.93	0.002	0.617	0.06, 0.88	0.016
Heart rate (bpm)	0.793	0.40–0.94	0.016	0.810	0.44, 0.95	0.001

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support this idea suggesting that weekly and rolling averages appear to have a more meaningful assessment of change in cardiac autonomic balance compared to isolated daily values [24]. As a result, using weekly mean values obtained from daily HRV recordings may improve the diagnostic utility of using HRV indices [24, 25]. Hence, the present study measured the daily HR and RMSSD values but utilized the weekly average values for further analysis.

Heart rate variability corresponds to countless stimulus and is influenced by physiological, psychological and environmental factors [26]. As a result, observable differences in nocturnal vs. morning RMSSD measures were present (Table 2). During sleep, individuals are no longer affected by external stimuli and therefore, enhanced reliability may exist for recordings that occur during sleep [14, 27, 28]. Contrary to the hypothesis, the current study showed significantly higher variation and higher RMSSD values during morning tests. In addition, slight differences in reliability between morning and nocturnal values was also present (Table 5). This may be the result of the difference in duration and/or time of day that were measured. Previous research has suggested that combining sleep periods into a single segment introduces a noise that reduces the detection of changes in HRV in over-trained athletes [29], which may also explain the differences in the present study. In addition, cardiac autonomic regulation was disrupted in HRV values, obtained after awakening, of over-trained athletes but not during night sleep [30]. However, this study did not investigate over-trained athletes and despite slight differences in weekly mean values, the nocturnal and morning RMSSD values showed strong relationships during both weeks. This finding suggests that when following weekly average values, measurements taken during nocturnal and morning rest are both acceptable. Although sleep values may be affected by movement and sleep stages, the use of weekly mean values has shown an increased ability to detect changes in performance [18], suggesting long-term weekly monitoring may be an effective tool for young athletes. Moreover, an additional advantage to the average values obtained during night sleep may be an increased ability to compare results. In previous research, the use of variation in body posture, time of day, sleep cycle and daily-

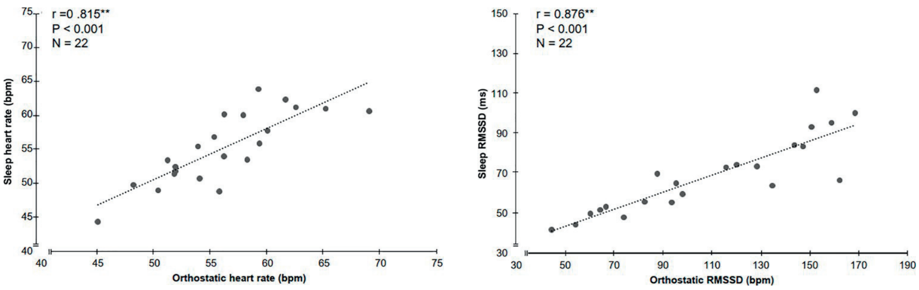

Fig 2. The relationship between nocturnal (sleep) and morning measurements for HR and RMSSD during the whole test period.

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vs.-weekly averages has presented disagreement in the direction of change in vagal-related HRV indices, which may be a result of methodological differences [2]. The use of whole night average values may help eliminate these differences and allow for comparison between studies.

Although coaches and athletes have followed HR for decades, current research has placed greater emphasis on HRV, considering it a more sensitive tool [2]. Research monitoring changes in HR during sleep found daily HR variation of about 8 bpm [31]. However, when observing the fatigue status of endurance athletes over a 4 year-period, the HR variation observed during morning supine tests was reduced, with average daily values being 6 bpm higher in fatigue vs. non-fatigued states [32]. In addition, when investigating weekly values for HRV and HR, both measures were able to indicate non-functional overreaching with minimal differences, supporting the idea that weekly HR values are useful [18]. A valuable addition in this present study is the inclusion of HR during two different periods of rest. The HR values showed the highest level of agreement among morning and nocturnal values (Table 5), suggesting that the nocturnal HR values may be a valuable way to monitor resting HR. Previous findings found significant increases in nocturnal HR during a short period of intensified training and no changes in morning HR, suggesting that an improved accuracy may exist for nocturnal HR values [4]. In the current study, similar findings were observed with a significant decrease between the week 1 and week 3 nocturnal HR values, whereas the morning HR values displayed a decrease but at a slightly reduced level and without significance (Table 3). Similar to HRV, these differences may be due to external influences that are decreased during sleep and therefore, nocturnal HR values may provide a more observable relationship between HR and training status [31].

We hypothesized that changes in HR and RMSSD would be associated to changes in SRT and CMJ tests. However, during the present study, training remained similar and no significant differences were present in SRT or CMJ, suggesting that the above changes are not due to changes in training load or volume (Table 3). Previous literature has suggested unloaded jumps are a common and useful way to monitor fatigue [4]. Research measuring weekly CMJ in distance runners found that jump performances the week before the season best competition were significantly higher than jump performance before the season worst completion [33]. In the present study, minimal changes between jump and performances tests, both as a group (Table 3) and on an individual level (Fig 3), during the test weeks illustrates that similar training was conducted during each test week and no significant differences were expected. However, a previous study with young athletes following a normal training protocol found submaximal HR values were associated with changes in performance variables over the entire season [5].

Additionally, during normal in-season training, good reliability was shown in both CMJ and SRT with a slight indication that SRT may be a more sensitive monitoring tool to predict performance changes [34]. Therefore, these findings imply that although no changes were found in our study, CMJ and SRT are acceptable tests to monitor young endurance athletes.

Our main concern in connection with our current investigation may be the lack of standardization of the measurement procedure, due to the collection of HR indices in individual home environments. Furthermore, although previous research has shown that resting HRV values do not differ between males and females [35, 36], the small sample size and grouping of gender are an additional limitation that should be noted. In addition, values calculated by the device may not provide the most reliable measures of HR indices since the accuracy of this device for continuous monitoring is yet to be determined. Since the extent of training stress also appeared to remain relatively constant, variation in the HRV was expected to be minimal.

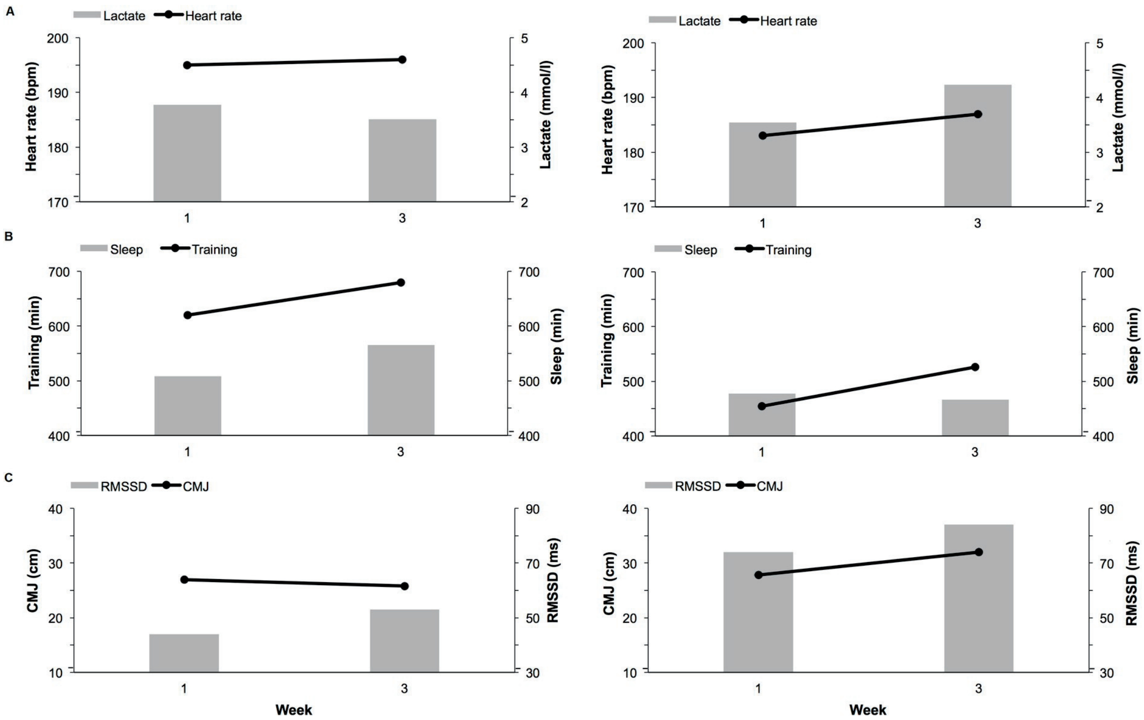

Fig 3. Comparisons of performance tests, nocturnal measures, and training results of two subjects during the study period. (A) Submaximal running test blood lactate and heart rate values during week 1 and week 3 for two different subjects. (B) Average values for night sleep and training during week 1 and week 3 for two different subjects. (C) Counter movement jump height and nocturnal RMSSD values during week 1 and week 3 for two different subjects.

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However, since our objective was to mimic typical every-day use, the subjects were instructed to maintain their routine patterns of sleeping and training.

Conclusions

The present findings indicate that the accuracy and reliability of weekly average values for HR and RMSSD obtained during sleep employing a BCG-based device is acceptable. Thus, day-to-day monitoring of nocturnal HR and RMSSD appears to be a convenient and valid approach for long-term monitoring of young endurance athletes.

Practical application

The application of BCG-based values used in the present study, provides facile monitoring of HR and RMSSD with a fully automatic and contact-free analysis without added time constraints [16]. Based on the data from this study, the nocturnal HR and nocturnal RMSSD values obtained appear to be a reliable way for coaches and athletes to monitor weekly average values. Therefore, long-term measurements are more attainable compared to morning measurements collected using electrodes or HR straps that require additional effort and thus, reduce daily athlete compliance [17]. It is important to note, that a practical monitoring system, especially for young athletes, should occur under relatively free-living conditions [31] with a balance between validity and ease-of-use in mind. Furthermore, the implementation of results is likely more effective when utilizing continuous measures that help identify individual variations rather than a less frequently collected value of slightly more power [2].

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Data curation: Christina Mishica.

Formal analysis: Christina Mishica.

Funding acquisition: Vesa Linnamo.

Project administration: Vesa Linnamo.

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Writing – original draft: Christina Mishica.

Writing – review & editing: Christina Mishica, Heikki Kyröläinen, Esa Hynynen, Ari Nummela, Hans-Christer Holmberg, Vesa Linnamo.

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III

ASSOCIATIONS BETWEEN OBJECTIVE MEASURES OF PERFORMANCE-RELATED CHARACTERISTICS AND PERCEIVED STRESS IN YOUNG CROSS-COUNTRY SKIERS DURING PRE-SEASON TRAINING

by

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IV

PERFORMANCE-RELATED PHYSIOLOGICAL CHANGES INDUCED BY ONE YEAR OF ENDURANCE TRAINING IN YOUNG ATHELES

by

Mishica, C., Kyröläinen, H., Valtonen, M., Holmberg, H.-C., & Linnamo, V.
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Performance-related physiological changes induced by one year of endurance training in young athletes

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Introduction: Although maximal oxygen uptake (VO_2max) is generally recognized as the single best indicator of aerobic fitness in youth, interpretation of this parameter and the extent to which it can be improved by training remain controversial, as does the relative importance of VO_2max for performance in comparison to other factors such as power production. Here, we examined the influence of endurance training on the VO_2max, muscle power and sports-related performance of cross-country skiers attending a school specializing in sports, as well as potential relationships between any changes observed to one another and/or to perceived stress scale (Cohen) and certain blood parameters.
Methods: On two separate occasions, prior to the competition season and separated by one year of endurance training, the 12 participants (5 males, 7 females, 17 ± 1 years) carried out tests for VO_2max on a treadmill, explosive power utilizing countermovement jumps (CMJ) and ski-specific maximal double pole performance (DPP) employing roller skis on a treadmill. Blood levels of ferritin (Fer), vitamin D (VitD) and hemoglobin (Hg) were monitored, and stress assessed with a questionnaire.
Results: DPP improved by $10 \pm 8\%$ ($P < 0.001$), but no other significant changes were observed. There were no significant correlations between the changes in DPP and any other variable.
Discussion: Whereas one year of endurance training improved the cross-country ski-specific performance of young athletes significantly, the increase in their maximal oxygen uptake was minimal. Since DPP was not correlated with VO_2max, jumping power or the levels of certain blood parameters, the improvement observed probably reflected better upper-body performance.

KEYWORDS

VO_2max (maximal oxygen uptake), cross-country skiing, adolescent & youth, sport-specific, double poling performance

Introduction

Maximal oxygen uptake (VO_2max) has been suggested to be the best single measure of performance (1). Thus, it has been studied for more than a century, yet the interpretation and trainability of VO_2max is still a topic of controversy, especially in adolescents (2). Previous literature, concentrating on longitudinal development in young cross-country (XC) skiers, reveals mixed results. Research on athletes between 15 and 20 years of age, indicates that VO_2max continued to increase, and high-level skiers continued to improve absolute VO_2max

values even after 20 years of age (3). Conversely, when adjusted for body weight maximal physiological parameters displayed minimal to no change in skiers from 17 to 18 years of age (4) with additional findings suggesting that males reach a VO_2max plateau around 19-years of age (5). In junior XC skiers, the effect of endurance training is often evaluated on the basis of changes in VO_2max (5, 6), although many other factors appear to be involved as well.

Previous research on juniors (18 years of age) found that roller-skiing performance in both diagonal-stride and double poling technique were accurate predictors for both male and females and numerous studies have shown that upper body power plays a major role in XC ski performance (7–10). With regards to upper-body muscular endurance training, as little as 6 weeks in well-trained adult skiers, showed improved double pole performance (7). These findings indicate that upper-body endurance and power are important determinants of XC performance, as well as that one year of ski-specific training should improve these parameters. Therefore, performance-related variables, such as the results of incremental double poling tests, would be of value to assess in connection with the development of young XC skiers.

Although it is now clear that the aerobic capacity of young athletes can be improved by training (2), the most appropriate method for monitoring and optimizing this training remains to be determined. Therefore, a variety of psychological, physiological, performance-related and biochemical measures are utilized to assess the effects of training (11–13). Psychological aspects are often evaluated on the basis of self-reported measures of perceived stress or mood, whereas physiological parameters are often quantified on the basis of performance, e.g., in submaximal or maximal exercise tests (12). Furthermore, biochemical and hematological measures help optimize an individualized balance between training and recovery, as well as reveal any potential nutritional deficiencies, which are common among athletes (14). Many athletes monitor their progress with a combination of these various markers (13), but it remains unknown which of them provide(s) the most validated and useful indicator(s) of performance (14), especially for young athletes.

The current investigation examined the potential influence of one year of endurance training on the results of performance-related tests and on other parameters commonly utilized to monitor the training of young XC skiers, in the present case students at a high school specializing in sports. Particular emphasis was placed on determining VO_2max, fractional oxygen utilization at the timepoint at which the lactate threshold was reached, muscular power and double poling performance. Furthermore, we assessed potential relationships between these different performance-related measures, as well as between these and perceived stress and/or hematological parameters.

Materials and methods

Subjects

Twelve well-trained young endurance athletes who trained and competed in XC ski (9 subjects) and biathlon (3 subjects) year-round participated in this study. All subjects were attending a sports high school and had a minimum of 3 years of competition experience at the national level and can be classified as tier 4/tier 3 athletes according to McKay et al. 2022 (15). Baseline characteristics and training background are shown in **Table 1** and details on gender specific training modes as well as training intensity distribution during the 12-month research period are shown in **Figure 1**. Subjects were fully informed of all the experimental procedures and provided written consent to participate in the study. The ethics committee of the University of Jyväskylä, Finland, approved the study, and measurements were performed in accordance with the declaration of Helsinki.

Study protocol

The study was carried out during a one-year period that began and ended in November before the winter competition season began. Subjects were instructed to train according to their own individual training plans and report their training *via* electronic training diaries. In order to evaluate changes in physiological, psychological and performance-related variables, subjects were tested on two different occasions with one year time between tests. Subjects were administered the perceived stress survey (PSS) followed by assessment of explosive power with countermovement jumps (CMJ) and then a VO_2max test to measure maximal oxygen consumption. Additional assessment included a ski-specific double pole test (DPT) on a roller ski treadmill and the monitoring of three commonly followed biomarkers (Fer, Vit D and Hg) from blood. Testing procedures were the same for all testing sessions, and subjects were instructed to do light training for 24 h prior to maximal performance tests. Jump exercises were already incorporated into the tested individuals' normal training so that subjects previously performed maximal tests a minimum of 2 times. Thus, a preparatory training period was unnecessary. Due to the longitudinal design of this study, measurement weeks were carefully considered with coaches to avoid weeks that included training camps, or exams and tests occurred during a 2-week period.

Maximal oxygen uptake (VO_2max)

Subjects completed a continuous, incremental, maximal test by walking or running with poles on a large motor-driven treadmill (Telineyhtymä, Kotka, Finland). The test started at an inclination of 2.8° with a speed of 5.5 $km \cdot h^{-1}$ for men at an inclination of

TABLE 1 Characteristics of subjects (means ± SD).

	Males (*N* = 5)	Females (*N* = 7)
Height (cm)	179.6 ± 5.4	169.2 ± 7.6
Body mass (kg)	72.4 ± 9.7	63.2 ± 6.3
VO_2max (ml/kg/min)	68.4 ± 4.3	54.3 ± 4.3
Yearly training (hrs)	587 ± 35	562 ± 53

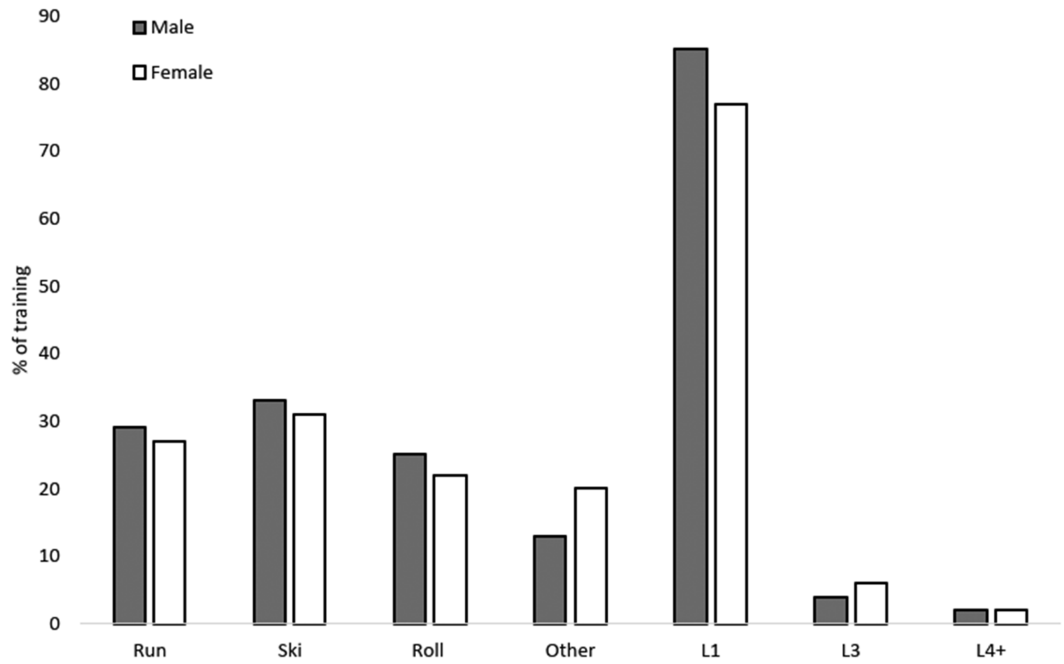

FIGURE 1
The modes of training and distribution of training intensity (TID) by young male and female cross-country skiers (endurance athletes) during a one-year period.

3.5° and speed of 5.0 km·h^{-1} for women. The workload increased every three minutes so that the predicted oxygen uptake calculated using the equation by Balke & Ware (16) increased by 6 ml·kg^{-1}·min^{-1} in every stage. All subjects were familiar with this testing protocol and the test was considered maximal when the subject demonstrated clear signs of maximal effort such as, unsteady gait and/or an inability to continue, despite strong verbal encouragement. To ensure safety was met, participants wore a harness that was attached to a rope which hung from a frame in the ceiling above the treadmill.

Before each test, volume, and gas calibration of the ergospirometer was performed and resting values of heart rate and blood lactate were recorded. Throughout the test, breathing gases were measured using a mixed chamber system (Medikro 919 Ergospirometer, Medikro Oy, Kuopio, Finland). VO_2 and respiratory exchange ratio (RER) from the last 60 s of each stage, and the highest 60 s average (VO_2max) were recorded. Time to exhaustion (TTEVO2) was defined as the total number of minutes the subject walk/ran on the treadmill during the maximal test. Heart rate was continuously monitored using a Polar H10 heart rate belt (Polar Electro Oy, Kempele, Finland) and the average heart rate from the last 60 s of each stage was recorded. During the last 10 s of each stage, fingertip blood lactate samples were collected into capillary tubes (20 µl) and placed in a 1-mL hemolyzing solution. Once the test was complete samples were immediately analyzed using Biosen C-line analyzer (EKF diagnostics, Barleben, Germany).

Lactate threshold (LT) was defined as the warm-up lactate value (i.e., measured after the first stage) + 1.5 mmol·L^{-1}. This method is in accordance with and recommended (17) by previous research (18, 19). For further analysis the percentage of oxygen utilization (VO_2) at the defined LT was calculated based off the highest VO_2 value (VO_2max) that occurred during the maximal test.

Explosive power (CMJ)

Explosive power was evaluated using CMJ (20). Prior to arrival, subjects completed a 15-minute warm-up running at a self-selected submaximal speed. Subjects were then instructed to jump as high as possible while keeping their hands fixed to their hips, feet shoulder-width apart and bending their knees to a 90-degree angle. A total of three jumps were completed with about 1-minute of recovery between jumps. Jumps were performed on a force plate (HUR FP8, HUR Oy, Kokkola, Finland), and jump height was calculated from impulse (21) using Coachtech system (Vuokatti Sports Technology Unit, University of Jyväskylä, Finland). The highest jump was recorded as the current measure of performance. CMJ are a common (13) and valid test for measuring fatigue and explosive power (20).

Ski-specific performance (DPP)

Ski-specific performance was evaluated using maximal double pole performance tests. Tests were performed on a roller ski treadmill (Rodby Innovation AB, Vänge, Sweden) with Marwe

800 XC roller skis (Marwe Oy, Hyvinkää, Finland) equipped with prolink bindings (Salomon Group, Annecy, France) and standard 6C6 wheels (Marwe Oy, Hyvinkää, Finland). Subjects were instructed to bring their own individual classic ski boots and poles. A customized tip specific for treadmill roller skiing was then placed onto the subject's ski poles. Following an individual self-selected warm-up outside the laboratory, subjects performed a 10-minute warm-up on the roller ski treadmill at a workload equal to the first stage of the test. Towards the end of the warmup, each subject performed two 12–15 s sprints at a workload equal to the fourth through sixth stage of the test. This ensured the subject was ready and verified that the poles and additional equipment was working adequately.

After the warm-up, athletes performed an incremental treadmill test in the double pole technique at an inclination of 2° with men starting at 13 $km \cdot h^{-1}$ and women starting at 10 $km \cdot h^{-1}$. Throughout the test, inclination was constant, and speed increased by one $km \cdot h^{-1}$ every minute until volitional exhaustion. Time to exhaustion (TTEDP) and heart rate (during each stage) was recorded. All subjects were familiar with this testing protocol and verbal encouragement was used to help individuals obtain their best effort. To ensure safety was met, participants wore a harness that was attached to a rope which hung from a frame in the ceiling above the treadmill.

Perceived stress scale (PSS)

Levels of perceived stress were assessed using the 14-item Cohen Perceived Stress Scale (PSS). The PSS is a reliable and validated psychological tool that was developed to evaluate stress from the psychological perspective. It consists of seven negative and seven positive items with the negative element intended to assess the lack of control and the positive element focused on the individual's ability to cope with existing stressors (14). A five-point Likert-type scale, ranging from 0, "Never" to 4, "Very Often" was used to rate each item. Possible scores ranged from 0 to 56 with higher scores indicating higher levels of perceived stress. Previously, the PSS has shown significant differences between healthy and overtrained athletes (22).

Subjects filled out the PSS upon their arrival to the testing laboratory. Surveys were conducted prior to CMJ and VO_2max tests to ensure subjects were well rested and able to properly answer all questions without any external influence.

Training analysis

Individual training plans were followed throughout the testing period. All participants recorded training data in electronic training diaries (elogger.net, Espoo, Finland). The subjects were familiar with recording training electronically and had previously reported training in this manner for at least one year prior to this research. Daily training and competition were recorded throughout the study period and training was analyzed according to the electronic training diaries. Physiological monitoring of training occurred on a daily basis with individual HR monitors (additional monitoring of blood lactate occurred on occasion) and subjects used individual training zones that were calculated from maximal exercise tests to help control and guide the intensity of their training. Due to the longitudinal design of this study, variations in how training intensity was reported were present and therefore, training intensity distribution was separated into three different training intensities: low intensity training (LI, blood lactate <2.0 mmol/L), lactate threshold training (LTT, blood lactate <4.0 mmol/L) and high intensity training (HI, blood lactate <4.0 mmol/L). In addition, the three most frequent training modes were reported including: running, skiing and roller skiing (**Figure 1**).

Blood variables

Morning fasted blood samples were obtained from the antecubital vein for the analyses of hemoglobin, ferritin, and serum total 25-hydroxyvitamin D, e.g., serum 25(OH)D. Blood for the hemoglobin analysis was drawn into EDTA tubes (Greiner-Bio-One GmbH, Kremsmünster, Austria) and immediately further analyzed with Sysmex XP300 analyzer (SysmexCo., Kobe, Japan). For serum ferritin, the blood was drawn into Vacuette gel serum tubes (Greiner-Bio-One GmbH, Kremsmünster, Austria) and centrifuged for 10 min with 3,600 rpm to collect serum, which was then frozen to −20°C for further analysis. The samples were analyzed with Siemens Immulite 2000 XPI analyzer (Siemens Healthcare Lianberis, United Kingdom), where the serum ferritin was determined by using immunometric chemiluminescence method. The sensitivity of the assay for ferritin was 0.4 μ/L and the precision (CV%) for the assay was 4.6%. The measurements of serum 25(OH)D were performed using electrochemiluminescence immunoassays (ECLIA).

Statistical analysis

Data are expressed as mean ± SD and were examined for the assumption of normal distribution before analysis using a Shapiro–Wilk test. To determine the effects of one-year of endurance training, we used a linear mixed model (LMM) for each of investigated variables. The analysis of a LMM has been widely used in longitudinal data when repeated measures of the same subjects are taken over the study period. This allows the assessment of within-subject changes over time as well as between-subject differences. The mixed model included gender (males or female), test (test 1 or test 2), and interaction terms between treatment and time as fixed effects and subject as random effects. The magnitude of differences between tests were expressed as standardized mean differences (Cohen's d effect size, ES) with the equation (M2-M1)/SDpooled. Due to gender differences, descriptive analysis was used to evaluate male and female results separately. To determine the associations between variables Pearson's correlation coefficients were used. Due to the small sample size, only whole group correlations for relative

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change between tests were analyzed. All statistical analysis were performed using SPSS version 26 (IBM SPSS Statistics 26, IBM GmbH, Munich, Germany). Statistical significance was set as $P < 0.05$.

Results

Performance tests

Performance in the Double Poling Test (TTEDP) improved by 10 ± 8% ($P < 0.001$). Analysis of individual performances revealed that 10 of the young XC skiers improved, whereas two demonstrated a decline of 0%–1% (**Figure 2**). No significant correlations between DPP performance and any other variables were observed.

There was no significant change in of the other performance-related variables monitored. With respect to absolute VO_2max, 7 of the athletes exhibited a decrease, 4 an increase and one no change (**Figure 2**). **Table 2** documents the maximal physiological values in connection with each test and **Figure 3** these values during each individual stage of the maximal incremental treadmill test, in both cases for both the men and women. A strong correlation was found between the changes in CMJ performance and absolute ($r = 0.669$, $P < 0.05$) and relative VO_2max ($r = .598$, $P < 0.05$).

Perceived stress levels and training

As shown in **Table 2**, levels of perceived stress increased throughout the study period but individual variation was high. During the one-year period monitored, 85 ± 3% (458 ± 58 h) of the training by the men and 77 ± 10% (455 ± 80 h) by the women was LI. The distribution of HI training was similar for the men (2 ± 2%, 12 ± 9 h) and women (also 2 ± 1%, 10 ± 5 h), as was also the case for LTT training (4 ± 1%, 21 ± 10 h, and 6 ± 2%, 37 ± 14 h respectively). In addition, skiing was the mode of training utilized most (for the men, 33 ± 13%, and the women, 31 ± 7%) (**Figure 1**).

Blood values

Neither Hg nor Fer significantly changed during the one-year training period, whereas the blood level of vitamin D increased in both the men and women ($P = 0.042$, **Table 2**).

Discussion

The major finding here was that one year of training by young XC skiers improved their performance in ski-specific DP tests without altering any other significant performance-related parameter. In addition, although there was a slight increase in

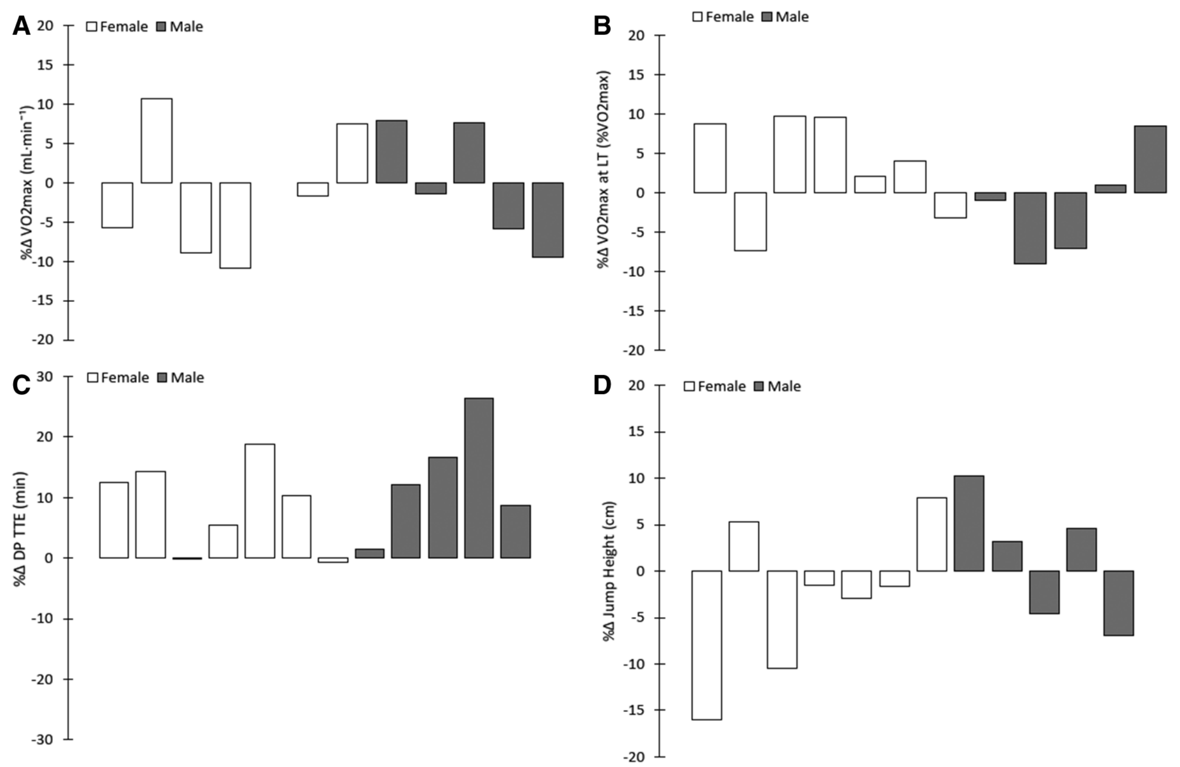

FIGURE 2
Individual changes (percentages) in (A) VO2max (ml/kg/min), (B) percentage of VO_2max at the lactate threshold (LT), (C) duration of DP performance (DP TTE) and (D) counter-movement jump (CMJ) performance during the one-year of training.

TABLE 2 Changes in performance-related variables, blood parameters and perceived stress in male and female cross-country skiers after 1-year of endurance training.

	Males, $n = 5$				Females, $n = 7$			
	Test 1	Test 2	% Change	Cohen's D	Test 1	Test 2	% Change	Cohen's D
VO_2max								
ml·kg^{-1}·min^{-1}**	68.4 ± 4.3	68.0 ± 2.7	−0.2 ± 7.8	0.11	54.3 ± 4.3	53.4 ± 4.3	−1.3 ± 8.1	0.11
L·min^{-1}**	4.9 ± 0.6	5.1 ± 0.7	3.2 ± 6.7	0.24	3.4 ± 0.3	3.4 ± 0.3	0.3 ± 8.3	0.24
TTE (min)**	24.8 ± 2.2	24.9 ± 1.1	0.6 ± 5.9	0.03	20.4 ± 1.2	19.9 ± 1.1	−2.0 ± 8.6	0.03
LT								
% VO_2max	85.4 ± 7.4	83.9 ± 6.8	−1.5 ± 6.9	0.21	80.8 ± 4.7	83.4 ± 5.1	3.4 ± 6.7	0.21
Time LT (min)	17.2 ± 1.0	16.8 ± 2.0	−2.4 ± 8.9	0.25	13.2 ± 1.1	13.4 ± 1.1	2.0 ± 5.2	0.18
DP								
TTE (min)*	8.5 ± 1.8	9.5 ± 1.4	13.1 ± 9.3	0.61	8.0 ± 0.9	8.6 ± 0.9	8.6 ± 7.4	0.61
HRmax	195.8 ± 8.5	194.2 ± 7.2	−0.8 ± 1.5	0.21	197.3 ± 8.4	195.1 ± 6.7	−1.0 ± 2.0	0.20
CMJ								
Height (cm)**	36.6 ± 4.2	37.1 ± 4.9	1.3 ± 7.0	0.10	30.6 ± 4.2	29.8 ± 5.0	−2.8 ± 8.4	0.10
Blood parameter								
Ferritin**	68.5 ± 15.8	68.3 ± 19.9	−0.7 ± 36.4	0.75	47.9 ± 26.9	38.0 ± 17.3	−5.4 ± 72.2	0.75
Hemoglobin (g/L)**	150.8 ± 5.5	155.2 ± 4.2	1.9 ± 3.8	7.1	143.3 ± 7.3	144.9 ± 9.1	1.1 ± 4.4	7.21
Vitamin D (nmol/L)*	68.8 ± 35.9	82.2 ± 39.4	11.5 ± 19.2	0.72	81.4 ± 20.0	97.5 ± 25.1	4.5 ± 52.7	0.72
Perceived stress								
PSS (score)	12.6 ± 6.0	19.6 ± 12.5	66.7 ± 81.0	0.72	21.4 ± 9.4	23.9 ± 11.5	12.7 ± 56.2	0.72

Values are means and standard deviation (mean ± SD).
VO_2max, maximal oxygen uptake; TTE, Time to exhaustion during test; LT, lactate threshold; DP, double pole test; HRmax, maximal heart rate during the double pole test; CMJ, countermovement jump test; PSS. Perceived Stress Survey.
*Significant effect of time ($P < 0.05$) on the linear mixed model (LMM) for each sex.
**Significant difference in LMM ($P < 0.05$) between the males and females.

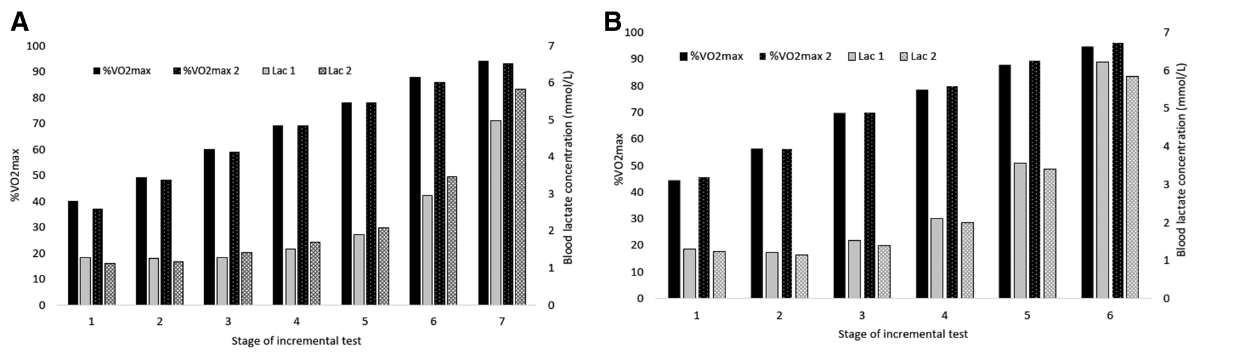

FIGURE 3
Changes in oxygen utilization and blood concentration of lactate during each individual stage of an incremental test following one year of endurance training in male (A) and female (B) cross-country skiers.

blood levels of vitamin D, the blood levels of Hg and Fer did not change. Nor was there any difference in perceived stress. Sex difference in physiological parameters was as expected, but no sex differences in the development of the different parameters were found over the one-year training period. A positive association was found between explosive power (CMJ) and VO_2max (both absolute and relative).

Performance-related variables

The increase in DP performance observed in this study indicates that during this one-year period subjects developed their upper-body performance. Previous research suggests that incremental DP tests appear to be a good predictor of XC skiers' performance (8) suggesting these improvements likely translated to competition results. It is interesting to note that the present improvement in the DP performance occurred without implementing a DP specific training intervention. This 10% improvement in TTEDP is considerably higher than previous research that reported a 3% increase in DP performance during 6 months of training (23). The current increase is comparable to previous studies that included a 6-week period of high intensity DP intervals and found 19.5% (9) and 16% (24) increases in performance. In the present study, anaerobic capacity during DP performance was not measured but maximal heart rate values during double pole tests were comparable to values obtained during VO_2max tests (**Table 2**) suggesting that subjects were able to easily reach maximal levels in the DP technique. Due to the young age of the subjects, one may suggest that familiarization of treadmill skiing may contribute to the increase in performance. However, subjects were provided with 3–4 familiarization tests (each around 30 min) before the first test with an additional 2 sessions before the second test. Therefore, we considered that the skiers were well familiarized with treadmill DP technique and improvement is likely due to increased upper-body performance. This supports previous findings that reported maximal upper body strength having a substantial impact on DP roller skiing performance in both males and females (10). It should be noted, however, that time to exhaustion change in % cannot be directly compared to the underlying physiological capacity, or performance time change over a given distance. Previous studies have demonstrated that a 10% change in TTE might correspond to a change of approximately 1% in physiological capacity (25). In addition, the age of the current subjects is a factor to consider when interpreting results. Age-related differences (−10%) were found in time trial DP performance between 16 and 18-year-old vs. adult athletes suggesting that skiers at this age are still progressing, and their performance may benefit from additional training and/or further development of their neuromuscular system (23). Thus, the improvement in DP performance in the present study may be due to an additional training/development that occurred during the one-year study period.

Although athletes aim to improve VO_2max values, a lack of change in VO_2max despite adequate training commonly occurs. Earlier studies, also focused on young endurance athletes, showed no significant changes in VO_2max during 3 years of endurance training (~7 h week) (26) and no change in physiological and/or performance variables despite an increase in ski-specific training and volume for a period of 6 months (23). Additionally, one year of high-level training for 17-year-old male XC skiers showed minimal to no difference in absolute and relative physiological parameters at the maximal level and only a slight increase for females (4), further supporting the lack of change observed in the present study. However, it is important to note that the existing lack of differences was also influenced by high individual variation. The inter-individual variation in VO_2max variables within our athlete cohort is most likely due to differences in trainability of VO_2max, which is highly hereditary with age, sex, body composition and body mass (27). **Figure 2** shows the individual changes in performance-related measures that were found between tests and interestingly, when looking at gender specific results, the oxygen consumption at LT increased for most of the female subjects. Previous research has shown an increase in oxygen consumption at similar thresholds in young male athletes but no change in females (4). However, in the present study this change was not significant and therefore, no conclusion can be drawn.

In terms of explosive power performance, CMJ results remained similar between tests in both males and females. However, when investigating the change between tests, a positive relationship was found between changes in jump height and changes in relative and absolute VO_2max. Previous research has shown that 10-km run time (28) and TTE in middle-distance runners (29) was associated to vertical jump height suggesting that muscle power (neuromuscular performance) may be an important determinant of endurance abilities. This finding suggests the improvement in jump height may be reflected in maximal oxygen uptake, however, changes in jump performance in relation to VO_2max have not been previously investigated in young skiers, therefore, the associations in the present study should be interpreted with caution.

A strength of the present study is the inclusion the of perceived stress and blood values variables. It is common practice for young athletes to monitor and follow blood values and therefore, improving knowledge and application of how to better interpret these values is important for coaches and athletes. For example, iron depletion (ferritin <12 µg/L, hemoglobin <13 µg/L for females and <30 µg/L for males), a prevalent nutrient deficiency among athletes, is frequently associated to an inadequate energy intake (30). Furthermore, young athletes have previously displayed a high rate of vitamin D insufficiency that further increased in the presence of iron depletion, suggesting that periodic screening of iron and vitamin D levels is important for young athletes (31). Few studies, assessing long-term changes in development and performance include practical applications and any indication of how these measures may aid in performance would be of high value to young athletes working to reach success on the elite stage.

As previously stated, no significant associations or changes were found between PSS and performance variables in the present study. This agrees with previous research following junior elite athletes of a similar age that showed minimal change during a 12-week intervention that was aimed at reducing PSS (32). The specific population of young elite athletes may be one possible explanation of this result since sport high schools often provide several resources and competent coaches/trainers that help balance training, competition, and school schedules by organizing the weekly training with an individual's total stress in mind (e.g., reduced training during examination weeks). Moreover, prior research has revealed that highly trained athletes are well prepared for competition with psychological response and functional strength of legs remaining unchanged after two consecutive days of high intensity competition (33). Contrary to the present findings, improved perceived recovery and stress contributed to an improved performance during one year of endurance training of female cyclists (31). These differences may be due to the reduced number of PSS measures, proposing that levels of perceived stress may need an increase in frequency to better reflect changes in performance. Considerable research has shown that stress influences performance and stress levels are important factors to consider when interpreting performance results (11, 12, 34, 35). Consequently, further research following measures of individual perceptions of stress alongside performance would be beneficial to help determine what level of perceived stress is tolerable for young athletes.

In this study, three blood parameters (Vit D, Hg and Fer) that are frequently followed by endurance athletes were analyzed. Although a variety of vitamin and minerals support the physiological processes that underline performance, evidence shows that nutritional deficiencies commonly occur in athletes, particularly for vitamin D and iron (14). In addition, living in a place where exposure to natural sunlight is limited (e.g., Finland during the winter months) amplifies the risk for vitamin D deficiency (36). Research following young Finnish runners reported vitamin D deficiency in approximately 68% of the subjects during the winter months (37) further demonstrating the importance of monitoring vitamin D status in young XC skiers. Recommended values of vitamin D based on bone health suggest 25(OH)D concentrations reach values >50 $nmol \cdot L^{-1}$ (38). In the present study vitamin D status was only monitored and no direction of supplementation was given but reference values were provided with 50–75 $nmol \cdot L^{-1}$ as a recommended range and a suggestion that athletes may want to be at levels <75 $nmol \cdot L^{-1}$ (39). In the present study, a significant increase occurred in 25(OH)D serum levels from test 1 (October 2019) to test 2 (October 2020). Although, detailed assessment of dietary vitamin D intake was not conducted, 75% of the participants reported taking vitamin D, iron, and other supplements irregularly throughout the current one-year training period. With regards to iron status, Fer and Hg both stayed at adequate levels with minimal to no change during the one-year period suggesting nutritional deficiencies or decreases in performance due to insufficient iron levels were not present. According to the findings from the present study, it appears that young XC skiers were able to maintain good metabolic and adequate nutritional profiles and vitamin D and/or iron status did not influence their performance.

As this study only had 12 subjects participating, there are some limitations due to the small sample size. This is a common issue in longitudinal research with high level athletes and this sample size is comparable to previous research following development and performance in XC skiers (5, 6, 8, 40). In addition, due to inconsistency in individual training diaries this study was unable to report strength training and training analysis was limited to three different intensities (LI, LTT and HI) and three different training modes. Furthermore, additional variables not measured in the current study, such as VO_2max during double pole performance, ski-specific upper-body training and double pole economy should be included in future models to better understand what factors contribute to improved DP performance in young athletes.

Conclusion

This study showed that young XC skiers improved ski-specific DP performance during one-year of sports high school, even though no change in maximal oxygen uptake, fractional oxygen utilization at LT, explosive power, perceived stress or selected blood biomarkers and endurance occurred. Although the improved DP performance did not directly influence the aerobic energy system (VO_2max), we can predict that race performance was likely influenced by this significant change. Since the initial blood values were within the recommended range, a lack of change indicates that subjects maintained adequate dietary levels of vitamin D and iron during this one-year period. In addition, minimal changes in jump performance suggests that there were no major alterations in lower body capacity. Hence, the improved performance can, therefore, be explained by an improved upper-body performance. In addition, it appears analysis of one-year of endurance training in young skiers may require tests that include a ski-specific component and that other assessments of performance may not uncover valuable improvements that may motivate and provide young athletes with valuable insight needed for their future athletic careers.

Data availability statement

The datasets presented in this article are not readily available because ethical restrictions were placed upon this data due to the sensitive subject information and possible identifying information it may contain. In the informed consent form that was approved by the Research Ethical Committee we have stated that all data is confidential, and it will not be given to third parties. Our data includes sensitive health data and since all the subjects participating in this study were adolescents it is possible that they can be identified even after anonymization of this data, therefore, our data cannot be shared publicly. Requests to access the datasets should be directed to christina.m.mishica@jyu.fi, or to professor vesa.linnamo@jyu.fi, or to Secretary of the University of Jyväskylä Ethical Committee at secretaryethicomm@jyu.fi.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the University of Jyväskylä, Finland. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

CM conducted the measurements, analyzed the data and prepared the manuscript. CM, VL, and HK helped in the planning of the work. VL and HK helped with interpretation of the data for the work. CM, VL, HK, MV, and H-CH edited and gave final approval of the work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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