Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Neuromedicine and Movement Science

Nils Ola Kvam

Physiological and hormonal responses to a two-week training-overload period in endurance athletes: a pilot study

Master's thesis in Exercise physiology
Supervisor: Rune Kjøsen Talsnes
Co-supervisor: Dionne Noordhof
May 2024

NTNU
Norwegian University of
Science and Technology

Nils Ola Kvam

Physiological and hormonal responses to a two-week training-overload period in endurance athletes: a pilot study

Master's thesis in Exercise physiology
Supervisor: Rune Kjøsen Talsnes
Co-supervisor: Dionne Noordhof
May 2024

Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Neuromedicine and Movement Science

NTNU
Norwegian University of
Science and Technology

Table of contents

Infographic

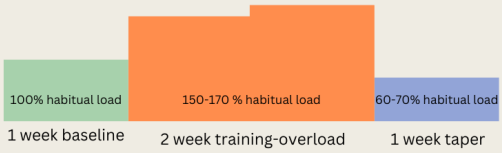
The influence of training overload on physiological and hormonal responses

Background
Early detection of imbalances between training load and recovery is crucial to prevent non-functional overreaching and overtraining syndrome

Aim
Investigate the influence of a 2-week training-overload period followed by a 1-week taper period on physiological and hormonal stress responses in endurance athletes.

Participants
- Eleven trained endurance athletes
- Distance runners, orienteers, and triathletes
- 7 males and 4 females
- Age = 25.5 ± 2.9 years
- $\dot{V}O_2max$ = 61.8 ± 7.1 mL kg^{-1} min^{-1}

Protocol
- Three training periods, 1 month total
- Laboratory testing following each period:
  - Maximal and submaximal workloads
  - Blood sampling at rest and immediately after maximal exercise



Results

Overload:	Taper:
Maximal workload: • No significant change for time to exhaustion or VO_2peak • Reduced heart rate and lactate response	• Normalization of heart rate and lactate response
Submaximal workloads: • Reduced heart rate and lactate response • No change in rating of perceived exertion	• No normalization of heart rate and lactate response
Stress hormones • Blunted ACTH/cortisol ratio response to exercise • No significant change for resting values	• Normalization of ACTH/cortisol ratio response to exercise

Conclusion
- Heart rate and lactate responses to maximal exercise are good indicators for recognizing training overload .
- Blunted heart rate and lactate response to submaximal exercise need to be put in context of other markers of fatigue and training period.
- Caution should be shown when using heart rate and lactate to control training intensity in a fatigued state. Training load can be higher than perceived and exacerbate the state of fatigue.

Acknowledgments

First and foremost, I would like to express my gratitude to my main supervisor Rune Kjøsen Talsnes for guiding me throughout the year and letting me set my mark on the research project. Secondly, I would like to thank my co-supervisor Dionne Noordhof for her help and expertise. My sincere gratitude goes to Olympiatoppen Midt-Norge and Senter for Toppidrettskorskning for allowing me to be part of their environment and inspiring me to believe that it is possible to impact theory and practice in fields I care deeply about. Moreover, I would like to express my deepest gratitude to the athlete participants for giving their time and effort to undertake a demanding training program. Finally, I would like to thank my fellow students and friends for making this process more rewarding and enjoyable.

Abstract

Introduction: Early detection of imbalances between training load and recovery (training-overload) is crucial for load management to prevent maladaptation in endurance sports. Periods of training-overload have been associated with changes in physiological and hormonal stress responses. However, a limited number of studies have investigated physiological responses and hormonal stress responses together. Therefore, this study aimed to investigate the influence of a 2-week training-overload period followed by a 1-week taper period on physiological and hormonal stress responses in endurance athletes.

Methods: Eleven trained endurance athletes (men, n = 7 and women, n = 4) completed 1-week baseline training, 2-week training-overload (50-70% increase in training load), and 1-week taper (40% reduction in training load from baseline). Immediately after each training period, physiological responses were assessed during submaximal steady-state stages and a maximal incremental time to exhaustion test while treadmill-running. Blood samples were collected at rest and immediately after the maximal exercise test for the assessment of cortisol, adrenocorticotropic hormone (ACTH), human growth hormone (hGH), and prolactin.

Results: Heart rate (HR) and blood lactate (bLa) values were reduced in response to both the submaximal and maximal exercise following training-overload ($p<.05$). HR and bLa responses to maximal exercise normalized to baseline levels after taper while submaximal HR and bLa responses did not. There was no significant influence of the training periods on any of the stress hormones. However, the ACTH/cortisol ratio response to exercise was blunted following training-overload ($p<.05$) and normalized to baseline levels after the taper period.

Conclusions: A 2-week training-overload period in endurance athletes can be recognized by blunted HR, bLa, and ACTH/cortisol ratio responses to maximal exercise. Although, physiological responses to submaximal exercise are less clear, they can still provide valuable information. These responses might therefore serve as early indicators in the context of load management to prevent maladaptation and fatigue in endurance athletes. However, these responses should be put in the context of other training-overload markers and the current training period.

Sammendrag

Introduksjon: Tidlig oppdagelse av ubalanse mellom treningsbelastning og restitusjon (treningsoverbelastning) blant utholdenhetsutøvere er avgjørende for å kunne iverksette tiltak for å kunne håndtere belastningen og med dette sikre prestasjonsutviklingen og forhindre uønskede tilstander. Treningsoverbelastning er assosiert med endringer i fysiologiske og hormonelle stressresponser, men få studier har undersøkt fysiologiske responser sammen med hormonelle stressresponser tidligere. Denne studien hadde derfor som mål å undersøke effekten på fysiologiske og hormonelle stressresponser hos utholdenhetsutøvere av en 2-ukers trenings-overbelastningsperiode etterfulgt av en 1-ukes nedtrappingsperiode.

Metode: Elleve utholdenhetsutøvere (menn, n = 7, og kvinner, n = 4) gjennomførte en uke med normal treningsbelastning, to uker med økt treningsbelastning (50-70% økning i treningsbelastning) og 1 uke med redusert treningsbelastning (40% reduksjon i treningsbelastning fra normal treningsbelastning). Fysiologiske responser ble testet med løping på tredemølle i en laktatprofil og i en maksimal trinnvis test til utmattelse. Blodprøver ble tatt i hvile og umiddelbart etter testen til utmattelse for analyse av kortisol, adrenokortikotropt hormon (ACTH), veksthormon (hGH) og prolaktin.

Resultat: Hjertefrekvens (HR) og blodlaktat (bLa) respons til både maksimalt og submaksimalt arbeid ble redusert etter treningsoverbelastning ($p<.05$). HR og bLa responser på maksimalt arbeid ble etter perioden med redusert belastning normalisert til nivået for perioden med normal treningsbelastning, derimot ble HR og bLa responsene til submaksimalt arbeid ikke normalisert tilbake til nivået for perioden med normal treningsbelastning. Det var ingen signifikant effekt av treningsperiodene på noen av stresshormonene, likevel ble ACTH/kortisol-ratioresponsen til maksimalt arbeid redusert etter treningsoverbelastning ($p<.05$) og normalisert til nivået for perioden med normal treningsbelastning etter perioden med redusert treningsbelastning.

Konklusjon: En 2-ukers periode med treningsoverbelastning hos utholdenhetsutøvere kan gjenkjennes ved reduserte HR, bLa og ACTH/kortisol-ratio -responser ved maksimalt arbeid. Effekten på submakaimale fysioloigske responser er derimot mer uklar, med også submaksimale responser kan gi verdifull informasjon. Disse responsene kan derfor fungere som tidlige

indikatorer i forbindelse med belastningsstyring for å forebygge overtrening hos utholdenhetsutøvere, men bør sees i sammenheng med andre markører for treningsoverbelastning og den aktuelle treningsperioden.

Abbreviations

Abbreviation	Definition
ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
bLa	Blood lactate
EDTA	Ethylenediaminetetraacetic acid
ES	Effect size
FOR	Functional overreaching
hGH	Human growth hormone/somatotropin
HIT	High intensity training
HPA axis	Hypothalamus-pituitary-adrenal axis
HPS axis	Hypothalamus-pituitary-somatotropic axis
HPP axis	Hypothalamus-pituitary-prolactin axis
HR	Heart rate
HRR	Heart rate recovery
LIT	Low intensity training
Min	Minutes
MIT	Moderate intensity training
NFOR	Non-functional overreaching
OTS	Overtraining syndrome
RED-S	Relative energy deficiency in sports
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
SHBG	Sex hormone-binding globulin
T3	Triiodothyronine
TRIMP	Training impulse
TTE	Time to exhaustion
VO_2 peak	Peak oxygen uptake

Introduction

Success in endurance sports requires a balance between training load and recovery to be able to adapt to training and maximize performance. If this balance is skewed towards load over time (training-overload), the result may be fatigue, blunted training adaption, and maladaptation (1–3). Prolonged excessive overload is detrimental to performance development and can pose health risks for the athlete (1–3). However, short periods of training-overload may be beneficial and/or necessary to provide sufficient physiological stimuli for adaption (4–7), although short-term term training-overload may have detrimental effects and increase the risk of injury and illness (1–3).

The outcome of training-overload in endurance sports can be categorized as a state of acute fatigue if the performance level is maintained, or a state of overreaching if the performance level is reduced from baseline following overload (1). Moreover, if the athlete improves performance after a subsequent recovery period, the state can be retrospectively categorized as functional overreaching (FOR), or as non-functional overreaching (NFOR) if performance levels are not restored (8). Prolonged training-overload may result in the more severe overtraining syndrome (OTS), which may take months to years to recover from (1). Furthermore, relative energy deficiency in sports (RED-S), has overlapping symptoms with OTS, but is caused by chronically low energy availability and needs to be excluded for an OTS diagnosis (9). At the same time, differentiation between overreaching, OTS, and RED-S is complex, and to some degree arbitrary.

Multiple factors, among them under-fuelling, illness, psychosocial and cognitive stress can impair recovery capacity (1,9–12), and impaired recovery capacity can induce fatigue and overreaching with training loads that are sustainable with normal conditions and recovery capacity. This impairment of recovery capacity may happen without the athlete’s knowledge and result in unplanned periods of training-overload or exacerbating planned training-overload periods. Recognizing when an athlete is in a state of training-overload contrary to the plan can be difficult but is crucial for adjusting training loads appropriately and preventing progression along the spectrum from acute fatigue to OTS.

Monitoring of training load and responses to load can be a valuable tool to recognize a state of training-overload in athletes. Heart rate (HR), perceived rating of exertion (RPE), and blood lactate (bLa) responses to submaximal and

maximal exercise tests are commonly used in sports practice to evaluate athletes training status. Complementing these types of tests with additional specific tests may increase the interpretation of normally collected data and therefore aid more athletes in recognizing their risk for developing severe states such as NFOR and OTS at an early stage. Furthermore, a better understanding of changes in physiological responses to different training loads can aid athletes in making better decisions in their daily load management.

Exercise acts as a stressor on the body and different physiological systems need to respond to be able to both maintain homeostasis during exercise as well as recover after exercise (12). Therefore, monitoring of athletes' training status with exercise tests can be used to evaluate the athlete's ability to respond to stress. Furthermore, the ability to respond to specific stressors is dependent on the functional capacity of different physiological systems and the regulation of these systems. High cumulative loads and impaired or insufficient recovery have been proposed to impact the regulatory mechanisms of different endocrine systems, potentially resulting in maladaptation of endocrine regulatory mechanisms, which are suggested to partly explain the long recovery time from OTS (1,12,13).

Physiological responses

In the context of training monitoring, previous training-overload studies have seen decreased HR responses during exercise, and athletes classified as overreached have been shown to have a greater decrease in peak HR at maximal aerobic workloads compared to athletes classified as acutely fatigued (3,4,6,14,15). Moreover, training-overload studies have found decreased HR responses at replicated submaximal workloads, although smaller decreases than at maximal aerobic workloads (4,6,7,15). In addition, reduced bLa responses similar to HR have been seen after training-overload to both maximal (3,4,7,14,16) and submaximal workloads (4,16–19). Additionally, heart rate recovery (HRR) following exercise in connection with training-overload periods has been seen to increase, and athletes classified as overreached have been shown to have a greater HRR increase compared to athletes classified as acutely fatigued (3,14,20). Furthermore, RPE response to submaximal exercise following a training-overload period has been seen by most studies to increase (17,20–22). However, others have shown no change in RPE following a training-overload period (14,23). Moreover, the bLa/RPE ratio at submaximal exercise has been seen to

decrease following training-overload (4,17,22), which would be expected with higher RPE. Interestingly, Bosquet et al. showed that most of the decrease in the bLa/RPE ratio came from a reduction in bLa (17).

Hormonal responses

Dysfunction in the hypothalamus-pituitary-adrenal axis (HPA axis) is a cause of adrenal insufficiency and has been proposed as the main underlying mechanism of OTS. Moreover, meaningful differences between overreached, overtrained, and healthy athletes have also been seen in the hypothalamus-pituitary-somatotropic axis (HPS axis) and the hypothalamus-pituitary-prolactin axis (HPP axis) (1,9,24). Previous training-overload studies with endurance athletes have shown different influences of training-overload on HPA, HPS, and HPP axis hormones. The influence of training-overload on basal stress hormones in the research literature has so far been inconclusive. Some training-overload studies has found no significant change in basal cortisol (14,25–30), adrenocorticotropic hormone (ACTH) (27,30), prolactin (25,27,28), and human growth hormone (hGH) (14,27,30) following training-overload. On the contrary, other training-overload studies have seen decreases in basal cortisol (28), ACTH (26,31), prolactin (28), and hGH (28) and further other training-overload studies have seen increases in basal cortisol after training-overload (31,32).

Furthermore, different influences of training-overload have been seen on stress hormone responses to exercise tests. Lehmann et al. have shown blunted hGH, cortisol and prolactin response to exercise after training-overload (28). Moreover, Meeusen et al. have shown a blunted ACTH-response to exercise after training-overload, but only after the second bout of a two-bout exercise test (27). However, Meeusen et al. could not show a significant change in either cortisol, prolactin, or hGH following training-overload (27). The two-bout exercise test used by Meeusen et al. (27) has been proposed to diagnose and differentiate between NFOR and OTS (8,33) and might be better suited to detect a state of training-overload than a one-bout test. Nonetheless, a two-bout exercise test is difficult to implement in practice, especially for early detection of risk for developing NFOR and OTS, as two maximal exercise tests are more than most top athletes are willing to do on an everyday basis. In contrast to Meeusen et al., Rietjens et al. showed small increases in ACTH and hGH response to exercise after training-overload, although these differences were not statistically significant (30).

Furthermore, could neither other studies show significant differences in exercise response after training-overload for cortisol (29,31) or ACTH (31). Nevertheless, all the above-mentioned training-overload studies investigating the stress hormone responses to exercise have had few participants and the study by Svendsen et al. with the most participants, had only a one-week training-overload period.

Modulation of the sympathovagal balance has been proposed as a possible mechanism for the impact of training-overload on HR, as either increased parasympathetic tone or decreased sympathetic tone skews the sympathovagal balance towards parasympathetic tone (6). Additionally, blunted HR responses during high-intensity exercise after training-overload in a functionally overreached group have been seen together with blunted blood catecholamine responses, whereas the same relationship was not seen in an acutely fatigued group (7). The same authors proposed adrenal insufficiency as a possible mechanism behind the training-overload effect on both HR and catecholamine response in severely affected athletes (7). Adrenal insufficiency is also associated with hypoglycemia (34) and as lactate production through glycolysis is dependent on glucose availability, adrenal insufficiency may therefore reduce bLa responses to exercise. Furthermore, ACTH, cortisol, hGH and prolactin response to insulin tolerance tests in athletes diagnosed with OTS compared to a control group of asymptotic athletes has been shown to be blunted (13,24). Moreover, the blunted HPA axis response in OTS athletes has been proposed to be caused by dysfunction in the hypothalamus or the pituitary gland as the adrenals reacted normally to ACTH stimulation tests (13). This may indicate that the proposed relationship between adrenal insufficiency and blunted HR response to high intensity exercise is caused by dysfunction at a higher level than the adrenals. No previous study has combined assessment of both ACTH and cortisol response to maximal exercise with assessment of HR and bLa responses to both maximal and submaximal exercise. Supplementing maximal and submaximal exercise testing with assesment of stress hormone response to exercise can therefore give novel information about the proposed relationship between adrenal insufficiency and blunted HR and bLa response. Furthermore, this novel combination of stress hormone responses and physiological responses may give valuable insight into the interpretation of physiological responses in everyday training monitoring.

Early detection of excessive risk for developing along the continuum from acute fatigue to OTS is integral to maintaining athletes' health and performance. Several

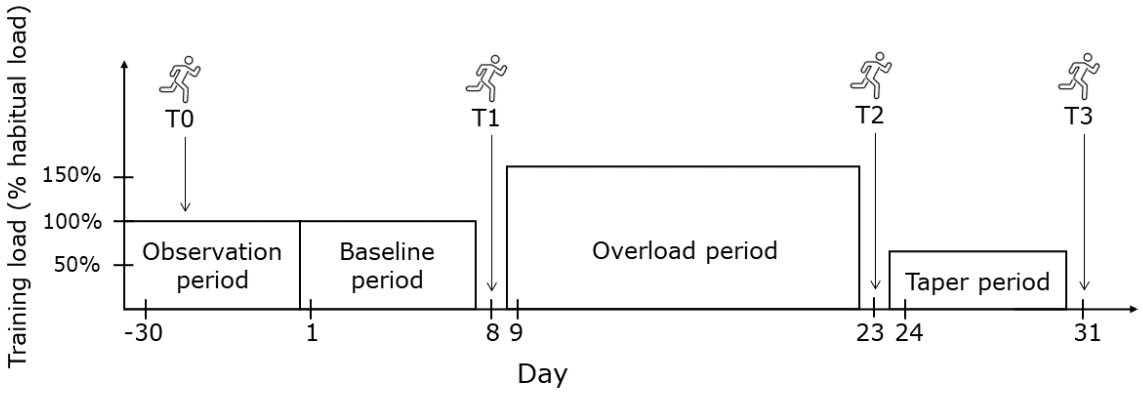
indicators for early detection of athletes in a state of fatigue and overreaching have previously been proposed although a more holistic understanding of the interaction between both physiological responses to exercise and hormonal responses are required. Therefore, the overall aim of this study was to investigate the influence of a 2-week training-overload period followed by a 1-week taper period on physiological and hormonal stress responses in endurance athletes.

Methods

Overall design

Eleven endurance athletes (men, n = 7 and women, n = 4) completed a 4-week training program consisting of a 1-week baseline period, followed by a 2-week training-overload period and a 1-week taper period. The participants attended the laboratory on four separate occasions for comprehensive physiological and hormonal testing: once prior to the baseline period for a familiarization visit (T0), and the day after each training period (T1, T2, and T3, after baseline, training-overload, and the taper period, respectively). A schematic representation of the whole study period is presented in Figure 1. The data collection was a pilot study of a larger research project that has the aim of investigating sex differences in physiological and hormonal responses, as well as RED-S indicators to training-overload in endurance athletes.

Figure 1: Schematic representation of the whole study period, 150-170% habitual load in overload, 60% habitual load in taper.



Participants

Endurance athletes (runners, triathletes, and orienteers) were recruited from local sports teams. Participant characteristics are presented in Table 1. Included participants had to be between 18 and 35 years of age, have over 5 endurance training sessions per week, and over 2 years of experience in an endurance sport.

Participants were excluded if they had current injuries or illnesses that prevented participation in regular or alternative exercise training. Both men and women were screened for indicators of RED-S before the experimental trial. Participants with high RED-S risk were excluded from study participation according to criteria suggested by Mountjoy et al. (35); at least one of the following indicators: primary amenorrhea, secondary amenorrhea, history of one high-risk (36) or two low-risk bone stress injuries within the last two years or over 6 months of training absence due to bone stress injuries in the previous 2 years, an elevated score above 2.30 for females and above 1.68 for males in the Eating Disorder Examination Questionnaire (EDE-Q global) (37). All female participants were taking hormonal contraception. All participants received both comprehensive verbal and written information about the study design, protocols, and measurements, and all participants gave their written informed consent. The study was approved by the Norwegian regional ethics committee, application number 656529.

Table 1. Participant characteristics and anthropometric measurements at baseline. n = 11.

Baseline values	Age	Bodymass	Bodyheight	Maximal HR	Oxygen uptake	Weekly training volume
Units	Years	kg	cm	beats min^{-1}	mL kg^{-1} min^{-1}	hours
Mean ± SD	25.5 ± 2.9	72.5 ± 6.9	177 ± 7	192 ± 9	61.8 ± 7.1	7.8 ± 3.1

Values are means ± SD. HR, heart rate; VO_2 max, maximal oxygen uptake.

Training load monitoing

Participants recorded their daily training, sleep duration, subjective sleep quality, resting HR, and readiness scores using an online training diary (38). In addition, a perceived recovery and well-being questionnaire was collected on each test day (39). Training data was collected from the point of their familiarization test to the end of the taper period, for participants with a brief time between the familiarization test and the start of the baseline period were training data also retrospectively extracted from other training logging tools. A three-zone model distinguishing between low intensity exercise (LIT), moderate intensity exercise (MIT), and high intensity exercise (HIT) as previously described by Solli et al. (40), was used to describe endurance training intensity. The training intensities corresponded to zones 1 and 2 (LIT), 3 (MIT), and 4 and 5 (HIT) in the five-zone

model used in the online training diary (38). Session and weekly endurance training load was calculated using a training impulse (TRIMP) model as previously described by Foster et al. (41).

Training load modulation

The average weekly TRIMP scores for the weeks before the baseline period were used to determine the baseline training load. There was a 50-70% increase in prescribed weekly endurance training load in the training-overload period compared to the baseline period, where participants with lower absolute training loads had a higher relative increase than participants with higher absolute training loads. The prescribed training load in the taper period was 40% reduced compared to the baseline period. The changes in training load were achieved by increasing endurance volume while maintaining the same intensity distribution and movement form distribution in every period. Sprint, strength, and other types of training were kept at a similar level during all three periods. There was no training in addition to the laboratory testing on each test day. Training load was adjusted together with the athletes to make sure the training load was feasible and that the athletes approached the same state of training-overload.

Test standardization

The participants were instructed to prepare for each test as they normally would do before a competition, including sleeping well and eating enough. Training on the last day before each test day was restricted to under 2 hours of low intensity exercise. Participants met in the laboratory in a fasted state between 07:00 – 09:30 for T1 and this time was replicated for T2 and T3. After a blood sample, the participants were provided a pre-exercise carbohydrate-rich meal (bread, jam, butter, orange juice, banana) corresponding to 1.5-2 g carbohydrates per kg body mass. The actual content of the meal at T1 was written down and replicated in the subsequent tests. The participants used a simple food diary to record their nutritional intake the last two days before each test and were instructed to replicate this before the subsequent tests. Additionally, the participants were instructed to increase their energy intake during the training-overload period to reduce the risk of low energy availability. Macronutrient contents from the food

diaries were analyzed by the same researcher for each participant using an online service developed by the Norwegian Directorate of Health (42).

Protocols

In the familiarization test, the participants received extensive information about the study, and the EDE-Q translated into Norwegian (37), together with instructions for recording training data, and completed the full laboratory test protocol for familiarization. The complete protocol for T1, T2, and T3 are presented in Table 2. Protocols for Stroop color test, spirometry, and countermovement jump will be presented by others.

Table 2. Order and time schedule for the full laboratory test protocol.

Procedure	Start time	Duration	Comment
Rest	00:00	10 min	
1. Blood sample	00:10	10 min	
Standarized breakfast	00:20	10 min	
Rest and questionaire	00:30	60 min	
Stroop color test	01:30	5 min	
Antropmetric measures	01:35	5 min	
1. Spirometry	01:40	5 min	
Warm-up	01:45	10 min	75 min after standarized breakfast
1. Counter movements jump	01:55	5 min	
Submaximal incremental test	02:00	20 min	Varied duration between participants
Rest	02:20	5 min	
Maximal incremental test to exhaustion	02:25	6 min	Varied duration between participants
2. Blood sample	02:31	8 min	Blood extraction 2 min after maximal test
2. Spirometry	02:39	4 min	
Submaximal running stage	02:43	5 min	
2. Counter movements jump	02:48	5 min	
Total	02:53	173 min	

Warm-up

10 min treadmill running at 1.5% elevation at 8 km/h for women, and at 10 km/h for men. Respiratory variables were collected for the last four minutes, and HR was measured continuously through all exercise protocols, and noted 30 seconds, before the end of every submaximal stage. The participants were asked to rate their RPE by the Borg scale immediately after completion of the warm-up (43). A

blood sample (20 µL) for bLa was taken from the fingertips while the participants rested standing, and HR was noted after one minute of rest for HRR.

Incremental submaximal profile

The participants ran 5-minute stages at 1.5% elevation, with 1-minute rest in between each stage. Men started at 12km/h, women at 10km/h. The speed was increased by 2 km/h for the second stage, whereas for the third and later stages, the speed increase was individualized between, 1, 1.5, and 2 km/h increments based on performance level during the familiarization test. All participants performed between 3 and 4 stages. Respiratory variables were recorded from minute two until the end of each stage. RPE, HR, HRR, and bLa were collected in the same way as in the warm-up stage.

Maximal test to exhaustion

The participants performed an incremental running test to exhaustion 5 min after the end of the lactate profile. Men started at 12 km/h, and women at 10 km/h, elevation was 5.5% for both sexes. The speed of the treadmill increased after confirmation from the participant by 1 km/h each minute. The participants had to wear a safety harness and they were blinded by time and speed updates. The treadmill was stopped if the participants signaled to stop, after 1 minute without any new confirmation for speed increase or if they were unable to keep up with the treadmill. HR and respiratory variables were recorded during the whole test. VO_2peak was determined as the average of the two highest 30 s VO_2 averages. The participants were asked for RPE immediately after test cessation, however HRR was noted, and blood lactate was sampled two minutes after test cessation.

Submaximal stage post maximal test to exhaustion

The second last lactate profile stage was repeated with the same protocol and measurements 12 minutes after cessation of the maximal exercise test to exhaustion.

Blood sampling

Venous blood samples were taken from an antecubital vein under sterile conditions two times for each visit. The first sample was performed in a fasted state after 10 minutes of rest and the second sample was taken immediately after the incremental test to exhaustion. For the first sample, blood was collected in three 5 mL serum vacutainers, (Greiner Bio-One, Vacuette Tube 5 mL CAT Serum Sep Clot Activator, Kremsmünster, Austria) for the assessment of serum hGH, cortisol, prolactin, LDL and total cholesterol, free and total testosterone, albumin, sex hormone-binding globulin (SBHG), free triiodothyronine (T3), estradiol and progesterone, and in one 4 mL EDTA vacutainer, (Greiner Bio-One, Vacuette Tube 4 mL K2E K2EDTA, Kremsmünster, Austria) used for the assessment of plasma ACTH. For the second sample, blood was collected in one 5 mL serum vacutainer for the assessment of serum hGH, cortisol and prolactin and in one 4 mL EDTA vacutainer for the assessment of plasma ACTH. Plasma and serum were centrifuged (Kubota, model 4200, Osaka, Japan) at 2200 g for 10 minutes at room temperature. Plasma was centrifuged immediately after collection and serum was stored for 30 minutes at room temperature before centrifuging. Both plasma and serum were allocated to 2 mL cryogenic vials (Avantor, VWR Cryovial®, Radnor, Pennsylvania, USA) after centrifuging and frozen. All samples were stored in a -18°C freezer at the Olympic sports center in Granåsen, Trondheim before transport with a car and dry ice to St Olavs Hospital, Trondheim within a month for deep freeze storage at -80°C and analysis at St Olavs Hospital, Trondheim. Analysis method for each hormone is presented in Appendix 1.

Equipment

Body mass was measured using a medical weight (Seca, model 708, GmbH, Hamburg Germany) and height was measured using a stadiometer (Holtain Ltd., Crymych, United Kingdom). Respiratory variables were recorded on an open-circuit indirect calorimetry (Jaeger, Vyntus CPX, Wuppertal, Germany) which was connected to a breathing tube and a mouthpiece. The respiratory flow transducer was calibrated each test day against a 2 L min-1 automatic pump attached to the Vyntus CPX. Oxygen and carbon dioxide were calibrated using a fixed gas mixture (15% ± 0.04 O2, and 5% ± 0.1 CO2). bLa was assessed in a glucose/lactate hemolyzing solution and analyzed using Bioson C-line (EKF

Diagnostics, Biosen, Cardiff, United Kingdom). HR was measured with a Garmin HRM-Pro strap (Garmin Ltd., Olathe, Kansas, USA) connected to a Garmin Forerunner 920 XT (Garmin Ltd., Olathe, Kansas, USA). All running tests were performed on a 5 x 3-m motor-driven treadmill (Forcelink B.V., Culemborg, Netherlands).

Data analysis

Max HR was determined to be the highest peak HR of the four test days. HRR was determined to be the difference between HR during and the measuring point after each stage and both HR and HRR will from here be expressed as relative values of max HR. For submaximal workload analysis, the warm-up was categorized as stage 1, while the first stage, the second last, and the last stage of the submaximal profile were categorized as stage 2, stage 3, and stage 4, respectively.

Statistical analysis

Data was evaluated for normality by Shapiro-Wilk tests and visual inspection of qq-plots. Homoscedasticity was assessed by the Levene test. Missing data was handled by listwise deletion. A significance level of $p < 0.05$ is used throughout this thesis. The effect of the training periods on variables with normal distributions was assessed by one-way repeated measures analysis of variance (ANOVA) and the Friedmann test was used for variables with non-normal distributions. Pairwise comparisons were done with Tukey's post hoc analysis and effect sizes (ES) expressed by paired Hedges g for normally distributed variables and the Nemenyi test was used for pairwise comparisons and paired Wilcox Q for ES for not normally distributed data. All statistical analysis was performed using R Statistical Software (version 4.4.0; R Foundation for Statistical Computing, Vienna, Austria) with package: rstatix: Pipe-Friendly Framework for Basic Statistical Tests, R package version 0.7.2.

Results

The weekly average training loads and volumes at each intensity zone are presented in Table 3. The participants increased their training load (TRIMP) by an average of 62.5% in the training-overload period compared to the baseline period and decreased their training load by an average of 36% in the taper period compared to the baseline period.

Table 3. Weekly training load and volume throughout the study period.

	Training week			
Variable	Baseline	Overload week 1	Overload week 2	Taper
TRIMP, week^{-1}	507 ± 179	757 ± 302	878 ± 314	312 ± 102
sRPE load AU week^{-1}	2220 ± 755	3540 ± 1650	4129 ± 1898	1497 ± 655
Total training volume, hours week^{-1}	7.5 ± 2.2	11.2 ± 3.5	12.5 ± 4.1	5.0 ± 1.7
LIT volume, hours week^{-1}	5.4 ± 1.7	8.1 ± 3.5	9.4 ± 4.3	3.7 ± 1.9
MIT volume, hours week^{-1}	0.9 ± 0.6	1.3 ± 1.0	1.5 ± 1.1	0.4 ± 0.4
HIT volume, hours week^{-1}	0.5 ± 0.4	0.7 ± 0.6	0.7 ± 0.6	0.3 ± 0.2

Values are means ± SD. TRIMP, training impulse (three-zone model); RPE, rating of perceived exertion; sRPE, session RPE training load; a.u, arbitrary units; LIT, low intensity training; MIT, moderate intensity training; HIT, high intensity training.

There was no change in average body mass throughout the study period, and there were no significant differences in macro-nutrient and energy intake between the different training periods (Table 4).

Table 4. Values for body mass at each testing day, energy and macronutrient intake measured the last two days of each training period.

	Training period			
Variable	Baseline	Overload	Taper	p
Body mass, kg	72.5 ± 6.9	72.4 ± 6.7	72.4 ± 6.6	0.999
Energy intake, kJ kg^{-1} day^{-1}	167 ± 52	188 ± 84	174 ± 87	0.801
Carbohydrate intake, g kg^{-1} day^{-1}	5.1 ± 1.6	5.7 ± 3.1	5.4 ± 3.7	0.529
Protein intake, g kg^{-1} day^{-1}	1.6 ± 0.6	1.9 ± 0.8	1.7 ± 0.7	0.673
Fat intake, g kg^{-1} day^{-1}	1.2 ± 0.4	1.6 ± 0.6	1.3 ± 0.5	0.151

Values are means ± SD.

Physiological responses

Physiological responses to maximal exercise

Data from the maximal exercise test to exhaustion are presented in Table 5. There was a significant influence of the different training periods on relative HR, bLa, RPE, and bLa/RPE ratio response to the maximal exercise test to exhaustion. Only HR response was significantly decreased following training-overload. However, there was an strong indication of decreased bLa and bLa/RPE ratio after overload and indication of increased time to exhaustion (TTE) following taper compared to baseline by large ES. Furthermore, pairwise comparisons showed a significant increase in HR, bLa, and bLa/RPE ratio response following taper compared to training-overload, sufficient to normalize these responses back to or past baseline levels.

Table 5. Values from maximal tests to exhaustion after baseline, overload and taper period.

	Training period				Effect size		
Variable	Baseline	Overload	Taper	p	T1-T2	T1-T3	T2-T3
HR peak, %max	99.2 ± 1.0	97.2 ± 1.6**	99.1 ± 1.1‡	0.001	1.142	0.091	-1.812
HRR, %max	40.9 ± 6.6	42.6 ± 6.4	39.6 ± 4.7	0.515	-0.260	0.388	0.744
TTE, s	347 ± 58	339 ± 62	374 ± 50	0.326	0.228	-1.185	-1.644
Avg speed last min, km/h	16.1 ± 1.5	16 ± 1.5	16.6 ± 1.5	0.606	0.163	-1.396	-1.568
VO_2 peak, mL min^{-1}	4457 ± 589	4414 ± 525	4533 ± 596	0.885	0.260	-0.770	-0.849
Ventilation peak, L min^{-1}	149 ± 18	146 ± 16	151 ± 16	0.797	0.176	-0.136	-0.680
RER peak	1.10 ± 0.03	1.08 ± 0.05	1.11 ± 0.05	0.161	0.420	-0.409	-2.273
bLa, mmol L^{-1}	9.9 ± 1.6	8.3 ± 2.0	11.0 ± 2.1‡	0.010	1.012	-0.592	-2.440
RPE, 6-20 a.u^{a}	19 (19.0-19.5)	19 (18.5-19.0)	19 (19.0-19.5)	0.047	0.206	-0.132	-0.392
bLa/RPE ratio, a.u	0.52 ± 0.09	0.44 ± 0.11	0.57 ± 0.11‡	0.020	1.016	-0.591	-2.378

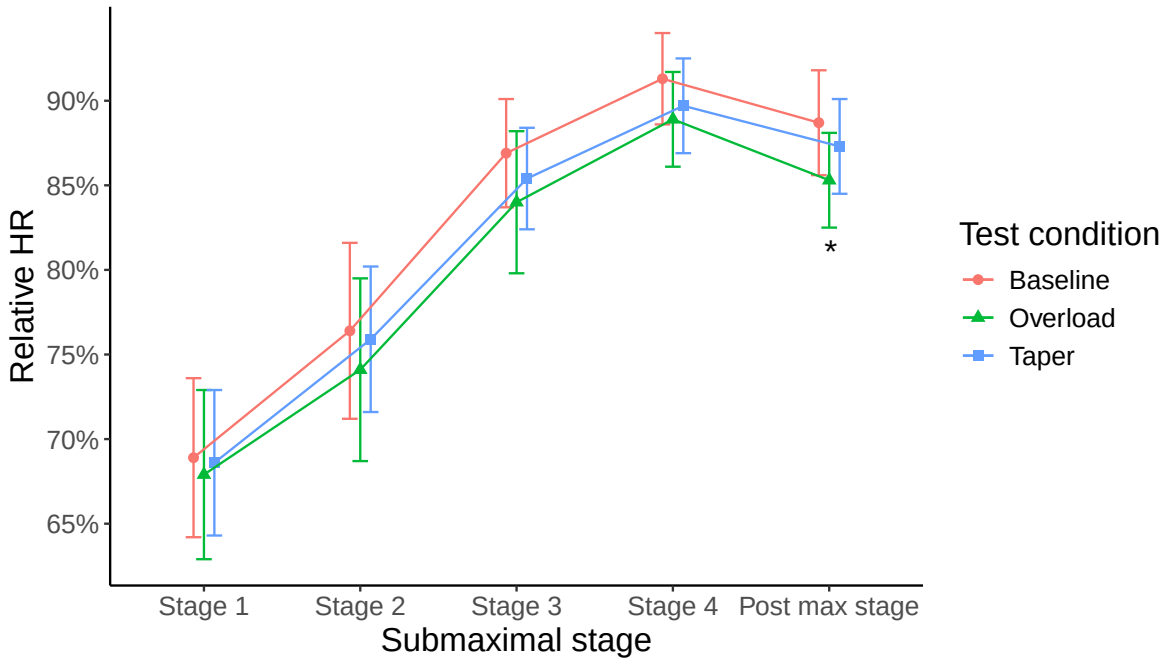
Values are means ± SD. HR, heart rate; HRR, heart rate recovery; TTE, time to exhaustion; VO_2, oxygen uptake; bLa, blood lactate; RER, respiratory exchange ratio; RPE, rating of perceived exertion; a.u, arbitrary units. **Significantly different from baseline ($p < 0.01$) ‡Significantly different from overload ($p < 0.01$) ^{a}Not normally distributed: Median (1-3 quartile).

Physiological responses to submaximal exercise

HR response

HR responses to the submaximal workloads across the different training periods are presented in Figure 2. Only relative HR during the post-max stage was significantly decreased compared to baseline, with a 3.8% reduction, p = 0.025, ES = 1.162. However,there were tendencies based on ES of lower HR following after training-overload compared to baseline for stage 2, stage 3, and stage 4 with, p = 0.534 and ES = 0.766, p = 0.170 and ES = 1.087, and p = 0.117 and ES = 1.262, respectively. There was no significant change in relative HR response to any submaximal workloads after taper compared to baseline. However, indications based on ES of reductions in relative HR between taper and baseline were evident with ES = 0.474, ES = 0.573, and ES = 0.476 for stage 3, stage 4, and the post max stage, respectively.

Figure 2: HR response to different submaximal workloads by training period.

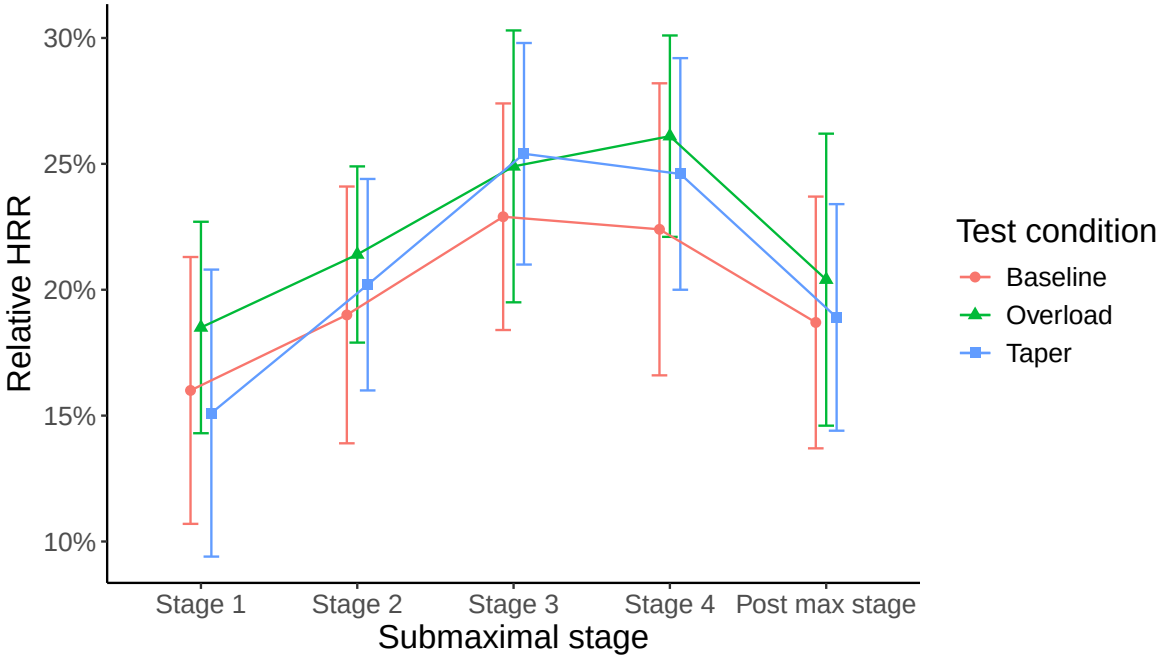


HR, heart rate. * Significantly different from baseline (p < 0.05)

HRR response

HRR response to the submaximal workloads across the different training periods is presented in Figure 3. There was no significant influence of training overload on HRR response to submaximal workloads, regardless of intensity. There are still indications based on ES of increased HRR following the training-overload period, ES = -0.756, ES = -0.608, ES = -0.329, and ES - 0.672, for stage 1 - stage 4, respectively and increased HRR after taper compared to baseline for stage 3 and stage 4 with ES = -0.496 and ES = -0.662, respectively.

Figure 3: HRR response to different submaximal workloads by training period.

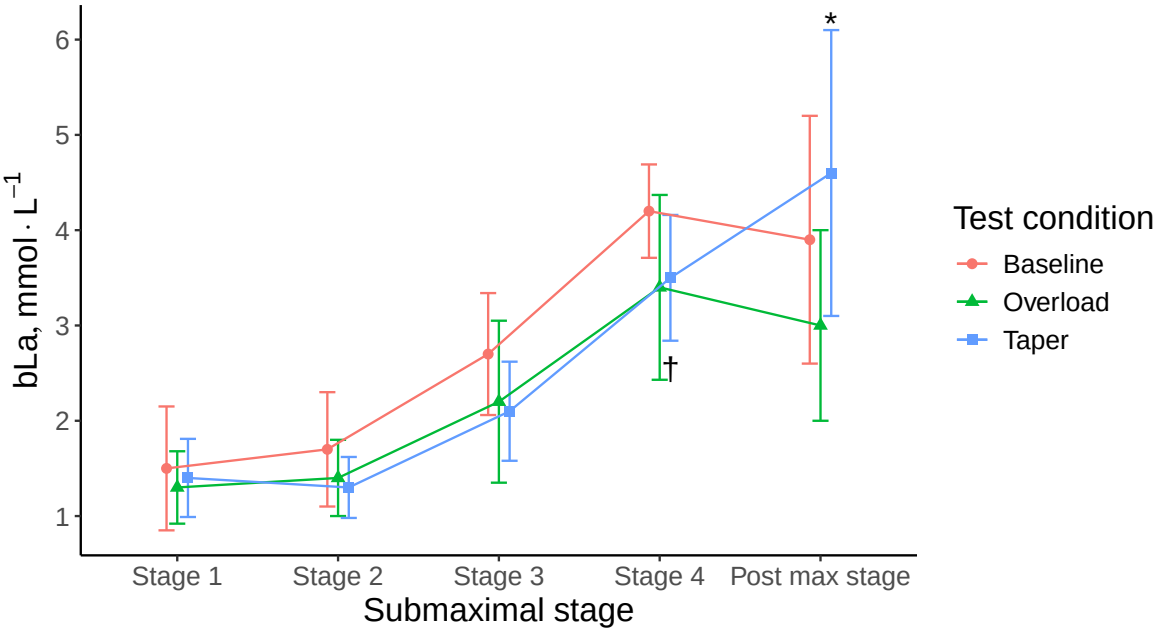


HRR, heart rate recovery.

bLa response

bLA response to the submaximal workloads across the different training periods is presented in Figure 4. There was a significant effect of the training periods on bLa for stage 4. In addition, there were indications for an effect on bLa for stage 2 and the post-max stage with p = 0.078 and p = 0.060, respectively. Pairwise comparisons showed that bLa was reduced compared to baseline after overload at stage 4 and that bLa was increased at the post-max stage after taper compared to overload. There are also indications based on ES of reduction in bLa after taper compared to baseline at stage 2 and stage 4, with ES = 0.536 and p = 0.083, and ES = 0.942 and p = 0.068, respectively.

Figure 4: bLa response to different submaximal workloads by training period.

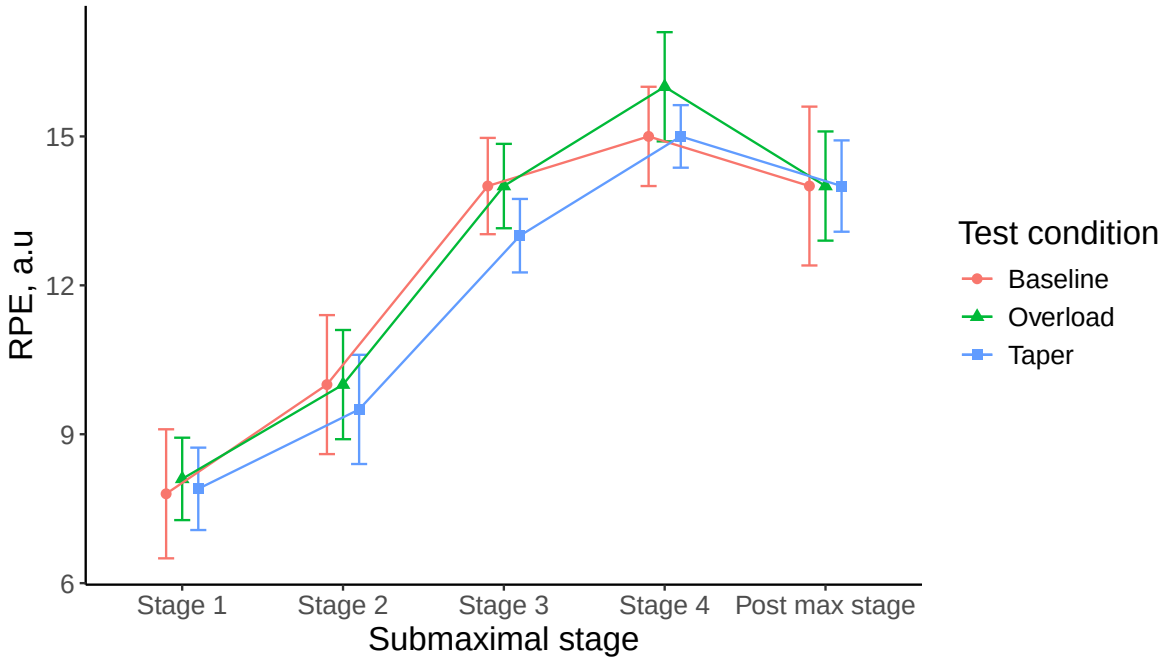


bLa, blood lactate; * Significantly different from overload (p < 0.05),
† Significantly different from baseline (p < 0.05)

RPE response

RPE responses to the submaximal workloads across the different training periods are presented in Figure 5. There was a significant effect of the training periods on RPE at stage 3, however, pairwise comparisons were not significant. The largest pairwise difference was between taper and baseline for stage 2, with ES = -0.322 and p = 0.166.

Figure 5: RPE response to different submaximal workloads by training period.

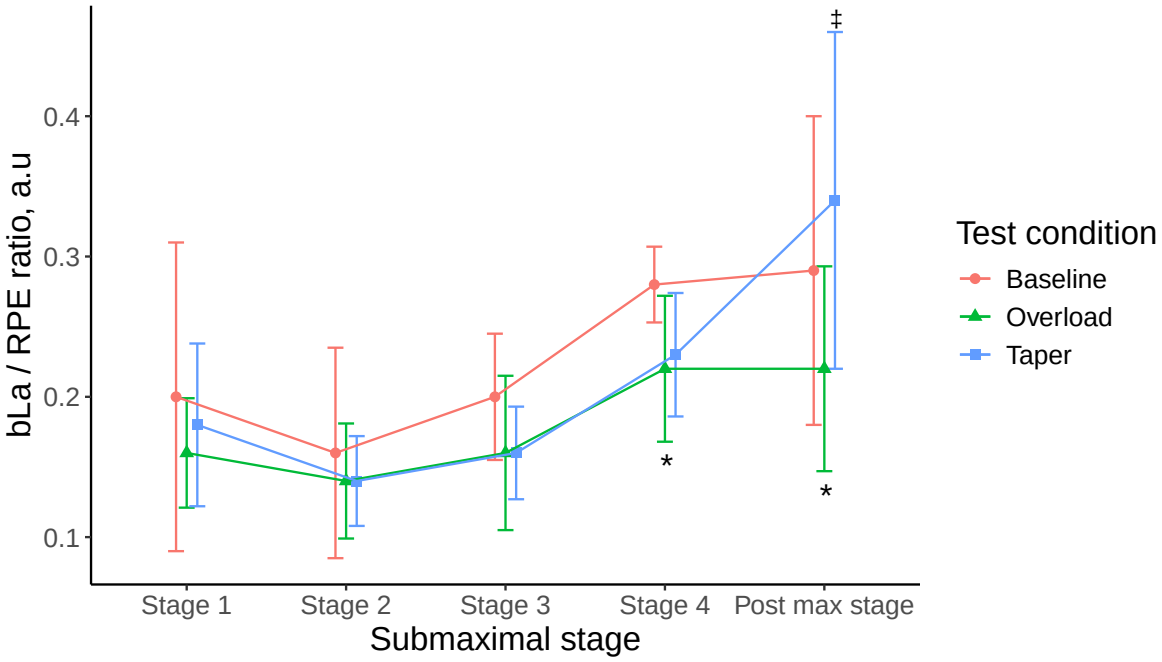


RPE, rating of perceived exertion; a.u, arbitrary units.

bLA/RPE ratio response response

There was a significant effect of the training periods on the bLa/RPE ratio at stage 4 and the post-max stage. bLa/RPE response to the submaximal workloads by training period is presented in Figure 6. Pairwise comparisons showed that the bLa/RPE ratio was decreased compared to baseline after overload at stage 4 and the post-max stage and that the bLa/RPE ratio was increased at the post-max stage following taper compared to training-overload.

Figure 6: bLa/RPE ratio response to different submaximal workloads by training period.



bLa, blood lactate; RPE, rating of perceived exertion; a.u, arbitrary units. * Significantly differe

Hormonal responses

Hormonal responses both at rest and to the maximal exercise test across the different training periods are presented in Table 6. There was a significant effect of the training periods only for ACTH/cortisol ratio and only for post-exercise and delta between rest and exercise values. There was a decrease in ACTH/cortisol ratio for the delta between rest and exercise following training-overload and an increase for both exercise values and the delta between rest and exercise following taper compared to training-overload. There was no significant change in free T3, LDL-cholesterol, or total cholesterol by the training periods.

Table 6. Values for the blood hormones measured at rest and after maximal exercise, after each training period.

	Training period				Effect size		
Hormone / condition	Baseline	Overload	Taper	p	T1-T2	T1-T3	T2-T3
Cortisol, nmol L^{-1} [a]							
Rest	622 ± 195	654 ± 220	697 ± 235	0.763	-0.993	-0.993	-0.324
Exercise	553 ± 174	527 ± 80	491 ± 162	0.662	0.540	0.540	0.262
Δ Rest-Exercise	-68.6 ± 211	-126 ± 241	-206 ± 242	0.462	1.148	1.148	0.777
ACTH, pmol L^{-1} [b]							
Rest	9.2 ± 6.0	8.2 ± 5.1	9.8 ± 7.3	0.881	-0.127	-0.127	-0.313
Exercise	24.7 ± 11.0	21.2 ± 12.0	28.6 ± 7.4	0.376	-0.460	-0.460	-0.816
Δ Rest-Exercise[c]	13.8 (7.4-22.9)	13.6 (6.8-19.0)	22.2 (17.5-24.8)	0.197	0.248	-0.248	-0.594
ACTH/cortisol,$\times 10^5$ [a]							
Rest[c]	1.3 (0.8-1.7)	1.1 (1.0-1.3)	1.0 (0.6-2.0)	0.687	0.248	0.248	0.099
Exercise[c]	3.9 (3.1-6.4)	3.8 (2.1-4.6)	6.0 (3.8-8.2)[‡]	0.011	0.743	-0.743	-0.891
Δ Rest-Exercise[c]	2.6 (2.2-5.0)	2.7 (1.3-3.5)[*]	5.0 (3.0-6.7)[†]	0.010	0.693	-0.693	-0.891
hGH, μg L^{-1} [b]							
Rest[c]	0.5 (0.2-1.6)	1.1 (0.2-3.2)	0.6 (0.3-1.4)	0.690	-0.103	0.262	0.013
Exercise	14.6 ± 9.0	12.5 ± 6.3	11.2 ± 6.5	0.610	0.412	0.412	0.265
Δ Rest-Exercise	12.2 ± 11.7	10.7 ± 6.2	9.5 ± 6.4	0.789	0.271	0.271	0.178
Prolactin, mIU L^{-1} [b]							
Rest	252 ± 46	294 ± 127	273 ± 92	0.604	-0.265	-0.265	0.262
Exercise[c]	318 (255-352)	280 (246-345)	296 (272-403)	0.773	0.257	0.257	-0.415
Δ Rest-Exercise	60 ± 75	4 ± 121	49 ± 108	0.484	0.099	0.099	-0.447

Values are means ± SD. ACTH, adrenocorticotropic hormone; hGH, human growth hormone. [a]n = 8, [b]n = 9, [c]Not normally distributed: Median (1-3 quartile). [‡]Significantly different from overload ($p < 0.01$) [*]Significantly different from baseline ($p < 0.05$) [†]Significantly different from overload ($p < 0.05$)

Discussion

The purpose of the present study was to investigate the influence of a 2-week training-overload period followed by a 1-week taper period on physiological and hormonal stress responses in endurance athletes. The main findings were that physiological responses such as HR, RPE, bLa to maximal exercise were reduced following training overload but normalized after the taper period. The same physiological responses to submaximal exercise were also reduced following training overload but did not demonstrate the same normalization following the taper period. Moreover, the ACTH/cortisol ratio response to maximal exercise was blunted following training overload and normalized following taper whereas no other changes in hormonal stress responses to training overload were evident.

Physiological responses

The reduction in HR response during the maximal exercise test to exhaustion after training-overload was accompanied by a reduction in bLa response, which aligns with previous literature in the field (3,4,6,7,14–16). This reduction in HR and bLa responses could come from a decreased relative workload. However, there was no large decrease in either RPE, TTE, or VO_2peak during the maximal exercise test, and the bLa/RPE ratio response was decreased. Therefore, the blunted HR and bLa responses after training-overload are likely an indication of a fatigue state. The small differences in TTE and VO_2peak between training-overload and baseline may suggest that performance is not a good indicator for recognizing a state of fatigue at an early stage in endurance athletes. Still, the incremental maximal exercise test to exhaustion used in the present study might be too short to have sufficient sensitivity for capturing performance decrements at an early stage. Therefore, other performance tests with a longer duration and a self-paced approach might be better suited to capture performance changes following training-overload at an early stage.

The reduced bLa response to maximal exercise following training-overload could potentially be attributed to depleted glycogen stores, given that we did not assess glycogen content. Energy and carbohydrate intake on the two last days before each test were slightly higher after training-overload than baseline, although this increase may not be sufficient to compensate for the increased energy expenditure from the increased training load. However, the training load the last

day before each test was standardized, decreasing the difference in energy expenditure the day before testing between the training periods. In addition, there were no changes in markers for low energy availability, like body mass, cholesterol, and free T3 values, indicating that the small increase in energy and carbohydrate intake might have been sufficient. The blunted bLa response after training-overload may still be caused by depleted glycogen storages if glycogenesis is impaired by the state of training-overload even with sufficient energy availability. Furthermore, HR and bLa response to maximal exercise, normalized and increased, respectively, following taper compared to baseline. A large and a moderate effect size for TTE and VO_2peak, respectively after taper compared to baseline, indicate that the participants may have had a training or tapering effect. The normalization of HR, increased bLa, and indicated increases in TTE and VO_2peak suggest that most of the participants had recovered well after the taper period.

HR responses at the different submaximal workloads tended to decrease after the training-overload period with decreases comparable to those previously reported in other training-overload studies (7,15). However, other studies have shown even greater decreases in HR at submaximal workloads (4,6), which for the study by Coutts et al. might be explained by a longer duration and a higher relative increase in the training-overload period (4). The effect size of the decrease in HR from baseline to training-overload increased by increasing exercise intensity. Impaired ability to increase HR, which is most noticeable at high exercise intensities with fatigue, could explain the problems overtrained athletes experience when training at such intensities. Furthermore, there was no significant difference in HRR at either submaximal or maximal exercise between the training periods. Nevertheless, there was an indication of increased HRR after training-overload as there were moderate ES for several submaximal stages, which is a finding consistent with previous literature in the field (3,14,20).

The blunted HR response to both maximal and submaximal exercise and the indication of increased HRR response to submaximal exercise after training-overload indicate that the sympathovagal balance was modulated towards increased parasympathetic tone after training-overload in line with the previous work by Le Meur et al. (6). In another study, Le Meur et al. measured stroke volume together with HR at both maximal and submaximal workloads after a three-week training-overload period and found both decreased HR and stroke volume in a functionally overreached group (7). This might indicate that the heart

is fatigued and that the modulation of sympathovagal balance associated with training-overload might avoid excessive damage to the heart. However, downregulation of cardiac function may increase the strain on working muscles and exacerbate muscular fatigue and damage, which in turn would increase recovery requirements and reduce tolerable load.

Furthermore, the reduction in bLa at submaximal workloads following training-overload is in line with previous studies, with clearer effects and greater decreases at higher exercise intensities (4,18). Apart from the post-max stage, neither HR nor bLa responses to the submaximal stages fully normalized back to baseline levels after the taper period. However, HR tended to increase slightly from training-overload, which parallelled other training-overload studies for HR (4,6,7,15) and bLa (4,19). In contrast, HR response to the post-max stage normalized after the taper period, and the bLa response increased beyond baseline levels. However, the distinct effect of the taper period for the post-max stage on HR and bLa are more likely to result from methodological issues, rather than a real effect of the training periods on short-term recovery capacity. The post-max stage was included to investigate if physiological durability was influenced by training-overload. However, different training states influence the ability to respond to both submaximal and maximal workloads, which in turn affects the work and the specific strain they impose on the body. Consequently, the recovery requirement after the maximal exercise test to exhaustion may vary across different training periods. For example, decreased TTE after training-overload may decrease the recovery requirement and exacerbate the reduction in HR during the post-max stage and the increased post-max stage bLa values after taper can be explained by increased bLa after maximal exercise test to exhaustion.

Even though HR and especially bLa at the submaximal stages were similar between training-overload and taper apart from the post-max stage, it does not necessarily imply that the participants were not able to recover during the 1-week taper period. A possible increased HRR response to submaximal workloads, and blunted HR, bLa, and bLa/RPE ratio responses to maximal workload indicate that the blunted HR and bLa responses to submaximal workloads following training-overload were associated with a state of fatigue. Furthermore, normalization of HR, bLa, and bLa/RPE ratio response to maximal exercise and possible increased TTE after taper suggest that the reduced HR and bLa response to submaximal exercise following taper compared to baseline likely are explained by a training effect.

Additionally, RPE during the submaximal stages was slightly lower after taper compared to both baseline and training-overload. While the absence of an increase in RPE following training-overload diverges from the majority of previous training-overload studies (17,20–22), it is concurrent with the findings of others (14,23). Furthermore, this study's results with a lack of increased RPE after training-overload questions the sensitivity of RPE as an indicator of a training-overload state in isolation, at least at an early stage. Nevertheless, RPE can aid the interpretation of other physiological responses to training-overload. Moreover, bLa/RPE responses to submaximal exercise tended to be lower after both training-overload and taper compared to baseline, in line with previous training-overload studies (4,17,22). HR and bLa response to submaximal exercise are used by athletes to monitor both training intensity and training state. However, this may have its pitfalls, if a reduction in HR or bLa is interpreted as a training effect when an athlete is in a fatigued state, may training intensity be increased. Consequently, increasing the training load and unnecessarily exacerbating the state of fatigue. Therefore, submaximal HR and bLa response should be interpreted in conjunction with RPE, physiological responses to either maximal exercise or other training-overload markers.

Hormonal responses

There was no significant effect of the training periods on basal stress hormones, which is in agreement with most previous literature for basal cortisol (14,25–30), ACTH (27,30), hGH (14,27,30), and prolactin (25,27,28). However, the non-significant effect of training-overload was in contrast to Lehmann et al. , who observed decreases in basal cortisol, hGH, and prolactin, and others who have seen decreases in basal ACTH (26,28,31). Even though this study was in line with most previous studies, there still might be a training-overload effect on basal stress hormone response as both this study and most previous studies did not have adequate statistical power.

The negative cortisol response to exercise present after each training period was similar to the cortisol response in the first bout of a two-bout test after training-overload previously seen by Meeusen et al. (27). No significant training period effect on post-exercise and delta cortisol was in line with all previous literature (27,29–31). Rietjens et al. included a combined anterior pituitary test in addition to stress hormone response tests to exercise and found a blunted cortisol

response after training-overload (30). Both this study and previous training-overload studies have sampled blood immediately after maximal exercise cessation and others have shown that blood cortisol concentrations peak 10-20 minutes after stress exposure (44), which could indicate that the real cortisol response to exercise was not captured in the present study. Therefore, a meaningful effect of training-overload on cortisol response could still be true even if no study has been able to show any meaningful effects. Rietjens et al. also included a combined anterior pituitary test in addition to stress hormone response tests to exercise and found a blunted cortisol response after training-overload (30). In contrast to their exercise stress hormone test, Rietjens et al. had repeated measurements of cortisol 100 minutes after stress exposure in the combined anterior pituitary test, and only summed concentrations of cortisol were significantly reduced after training-overload (30). Future training-overload studies should include repeated cortisol samples at different time points after exercise, and this might be easier to implement with salvatory cortisol measurements, which in addition have been shown to better represent the amount of free cortisol than blood cortisol (45).

There was no clear training period effect on either hGH or prolactin response to exercise, which is in line with the results by both Meeusen et al. and Rietjens et al., although in contrast to the blunted hGH and prolactin responses to exercise seen by Lehmann et al. (28). Moreover, there was no significant effect of the training period on either exercise ACTH or delta ACTH in line with the previous work by other training-overload studies (27,30,31). Nonetheless, there are some indications for an increase in post-exercise ACTH after taper compared to baseline, and compared to training-overload, by a small effect size and a large effect size, respectively. The delta ACTH/cortisol ratio was significantly decreased after the training-overload period and both post-exercise and delta ACTH/cortisol ratio increased significantly from training-overload to taper.

Others have proposed blunted ACTH response to stress as a symptom of overtraining syndrome and fatigue (1,13,45). In this context, non-significantly higher post-exercise ACTH and significantly higher ACTH/cortisol ratio after taper compared to baseline can indicate that the participants were in some state of fatigue also at baseline. However, ACTH response to exercise (46) and to insulin tolerance tests (13) have been shown to be higher in healthy trained athletes compared to untrained subjects. Furthermore, Cadegiani et al. have shown that athletes with overtraining syndrome have the same blunted ACTH response as

sedentary subjects (13). In this context, the indicated stronger ACTH response to exercise after taper compared to baseline could represent both higher fitness and a lower degree of fatigue. Furthermore, both the ACTH/cortisol ratio and bLa response to exercise were stronger after tapering and were accompanied by indications of increased performance. This could potentially suggest that the stronger ACTH/cortisol and bLA response can both be attributed to lower level of fatigue and improved fitness. However, the importance of fatigue and fitness on ACTH/cortisol ratio and bLa response to maximal exercise needs to be investigated further.

Moreover, the blunted ACTH/cortisol ratio response observed together with the blunted HR and bLa responses to maximal exercise after training-overload indicate that there might be a connection between adrenal insufficiency and downregulation of HR and bLa responses to exercise. However, neither cortisol nor ACTH response to exercise in isolation was significantly changed by the training period. Therefore, it cannot be inferred from this data which part of the HPA axis undergoes changes responsible for adrenal insufficiency associated with fatigue and training-overload. Regardless, more research is needed to clearly understand the relationship between adrenal insufficiency, sympathovagal modulation, and HR and bLa responses to exercise.

Limitations

This pilot study included only eleven participants at this time point. Therefore, it may lack the necessary statistical power to detect meaningful differences in some of the studied variables. This limitation is particularly relevant to the blood hormone analysis, which was performed on only eight or nine participants due to missing data. Furthermore, there are interindividual variations in how athletes tolerate and respond to a training-overload period. To ensure the athletes were in a similar state after the training-overload period, we used relative training load increases and modulated training load during the training-overload period by the athletes' perceptions of how well they tolerated the training load. However, some participants tolerated the training-overload period better than others. Including these participants in the analysis could obscure clear patterns of training-overload states for the athletes who are the most severely affected. The severely affected athletes are more likely to require a reduction in training load and therefore more crucial to identify in a state of training-overload. Some other training-overload

studies have solved this challenge by categorizing participants by training-overload severity, though, we could not do this with the number of participants present.

Single point measurements of HR were used to calculate HRR, which may have been overly sensitive to temporal variation and to any type of movement or arousal and therefore increased variation in HRR more than necessary and masked any meaningful effect of the training periods on HRR.

Changes in cortisol awakening response by training period may have masked some of the changes in the cortisol response to exercise as fatigue is associated with a blunted cortisol awakening response and all laboratory tests were done in the morning and the cortisol awakening response was not controlled for. Furthermore, we might not have been able to make sure that all participants were in an adequately relaxed state for the blood sample in rest, as a couple of the participants had higher pre-exercise ACTH than expected. Both males and females were included in this study, and as there are sex differences in basal values of hGH (47) could variation in hGH response be greater than it could have been with the inclusion of only one sex. However, sex differences in hGH response to exercise and the effect of training-overload will be investigated later when the data collection has progressed further with more participants. Blunted HR, bLa, and ACTH/cortisol response are all seen in this study to be associated with training-overload. This does not necessarily indicate that there is dysregulation of these responses after a training-overload period, there might only be a reduction in specific physiological stress and that the stress response is blunted, but adequate.

Conclusion

In conclusion, a 2-week training-overload period can be recognized by blunted HR, bLa, RPE, and ACTH/cortisol response to maximal exercise. Furthermore, a 2-week training-overload period may be recognized by blunted HR, and bLa responses to submaximal exercise. However, these submaximal responses must be interpreted in the context of other training-overload markers and the current training period. These responses might therefore serve as early indicators of training-overload together with other training-overload indicators in the context of load management to prevent maladaptation and fatigue in endurance athletes.

References

1. Meeusen R, Duclos M, Foster C, Fry A, Gleeson M, Nieman D, et al. Prevention, diagnosis and treatment of the overtraining syndrome: Joint consensus statement of the European College of Sport Science (ECSS) and the American College of Sports Medicine (ACSM). Eur J Sport Sci. 2013 Jan 1;13(1):1–24.
2. Bellinger P. Functional overreaching in endurance athletes: A necessity or cause for concern? Sports Med. 2020 Jun 1;50(6):1059–73.
3. Aubry A, Hausswirth C, Louis J, Coutts AJ, Buchheit M, Le Meur Y. The Development of Functional Overreaching Is Associated with a Faster Heart Rate Recovery in Endurance Athletes. Med Sci Sports Exerc. 2015 Oct 21;10(10):1769–77.
4. Coutts AJ, Slattery KM, Wallace LK. Practical tests for monitoring performance, fatigue and recovery in triathletes. Journal of Science and Medicine in Sport. 2007 Dec 1;10(6):372–81.
5. Bellinger PM, Sabapathy S, Craven J, Arnold B, Minahan C. Overreaching attenuates training-induced improvements in muscle oxidative capacity. Med Sci Sports Exerc. 2020 Jan 1;52(1):77–85.
6. Le Meur Y, Pichon A, Schaal K, Schmitt L, Louis J, Gueneron J, et al. Evidence of parasympathetic hyperactivity in functionally overreached athletes. Medicine & Science in Sports & Exercise. 2013 Nov;45(11):2061–71.
7. Le Meur Y, Louis J, Aubry A, Guéneron J, Pichon A, Schaal K, et al. Maximal exercise limitation in functionally overreached triathletes: role of cardiac adrenergic stimulation. J Appl Physiol. 2014 Aug;117(3):214–22.
8. Meeusen R, Nederhof E, Buyse L, Roelands B, Schutter G de, Piacentini MF. Diagnosing overtraining in athletes using the two-bout exercise protocol. Br J Sports Med. 2010 Jul 1;44(9):642–8.
9. Stellingwerff T, Heikura IA, Meeusen R, Bermon S, Seiler S, Mountjoy ML, et al. Overtraining syndrome (OTS) and Relative energy deficiency in sport (RED-S): shared pathways, symptoms and complexities. Sports Med. 2021 Nov 1;51(11):2251–80.
10. Mountjoy M, Ackerman KE, Bailey DM, Burke LM, Constantini N, Hackney AC, et al. 2023 International Olympic Committee's (IOC) consensus statement on Relative Energy Deficiency in Sport (REDs). Br J Sports Med. 2023 Sep 1;57(17):1073–97.
11. Bell L, Ingle, Lee. Psycho-physiological markers of overreaching and overtraining in endurance sports: A review of the evidence. Med Sportiva. 2013 Jun 30;17:81–97.
12. Kenttä G, Hassmén P. Overtraining and recovery. A conceptual model. Sports Med. 1998 Jul;26(1):1–16.
13. Cadegiani FA, Kater CE. Hypothalamic-pituitary-adrenal (HPA) axis functioning in overtraining syndrome: findings from endocrine and metabolic responses on overtraining syndrome (EROS)—EROS-HPA Axis. Sports Med Open. 2017 Dec 8;3:45.
14. Bellinger P, Desbrow B, Derave W, Lievens E, Irwin C, Sabapathy S, et al. Muscle fiber typology is associated with the incidence of overreaching in response to overload training. J Appl Physiol. 2020 Oct;129(4):823–36.
15. Ten Haaf T, Foster C, Meeusen R, Roelands B, Piacentini MF, van Staveren S, et al. Submaximal heart rate seems inadequate to prescribe and monitor intensified training. Eur J Sport Sci. 2019 Sep;19(8):1082–91.
16. Lehmann M, Baumgartl P, Wiesenack C, Seidel A, Baumann H, Fischer S, et

al. Training-overtraining: influence of a defined increase in training volume vs training intensity on performance, catecholamines and some metabolic parameters in experienced middle- and long-distance runners. Eur J Appl Physiol. 1992 Mar 1;64(2):169–77.
17. Bosquet L, Léger L, Legros P. Blood lactate response to overtraining in male endurance athletes. Eur J Appl Physiol. 2001 Feb 1;84(1):107–14.
18. Le Meur Y, Hausswirth C, Natta F, Couturier A, Bignet F, Vidal PP. A multidisciplinary approach to overreaching detection in endurance trained athletes. J Appl Physiol. 2013 Feb;114(3):411–20.
19. Fry RW, Morton AR, Garcia-Webb P, Crawford GPM, Keast D. Biological responses to overload training in endurance sports. Eur J Appl Physiol. 1992;64(4):335–44.
20. Le Meur Y, Buchheit M, Aubry A, Coutts AJ, Hausswirth C. Assessing overreaching With heart-rate recovery: what is the minimal exercise intensity required?: Int J Sports Physiol Perform. 2017 Apr;12(4):569–73.
21. Halson SL, Bridge MW, Meeusen R, Busschaert B, Gleeson M, Jones DA, et al. Time course of performance changes and fatigue markers during intensified training in trained cyclists. J Appl Physiol. 2002 Sep;93(3):947–56.
22. Flockhart M, Nilsson LC, Ekblom B, Larsen FJ. A simple model for diagnosis of Maladaptations to Exercise Training. Sports Medicine - Open. 2022 Nov 4;8(1):136.
23. Coates AM, Thompson KMA, Grigore MM, Baker RE, Pignanelli C, Robertson AA, et al. Altered carbohydrate oxidation during exercise in overreached endurance athletes is applicable to training monitoring with continuous glucose monitors. Scand J Med Sci Sports. 2024 Jan;34(1):e14551.
24. Barron JL, Noakes TD, Levy W, Smith C, Millar RP. Hypothalamic sysfunction in overtrained Athletes. J Clin Endocrinol Metab. 1985 Apr 1;60(4):803–6.
25. Coutts AJ, Wallace LK, Slattery KM. Monitoring changes in performance, physiology, biochemistry, and psychology during overreaching and recovery in triathletes. Int J Sports Med. 2007 Feb;28(2):125–34.
26. Poffé C, Ramaekers M, Van Thienen R, Hespel P. Ketone ester supplementation blunts overreaching symptoms during endurance training overload. J Physiol. 2019;597(12):3009–27.
27. Meeusen R, Piacentini MF, Busschaert B, Buyse L, De Schutter G, Stray-Gundersen J. Hormonal responses in athletes: the use of a two bout exercise protocol to detect subtle differences in (over)training status. Eur J Appl Physiol. 2004 Mar 1;91(2):140–6.
28. Lehmann M, Gastmann U, Petersen KG, Bachl N, Seidel A, Khalaf AN, et al. Training-overtraining: performance, and hormone levels, after a defined increase in training volume versus intensity in experienced middle- and long-distance runners. Br J Sports Med. 1992 Dec;26(4):233–42.
29. Uusitalo ALT, Huttunen P, Hanin Y, Uusitalo AJ, Rusko HK. Hormonal Responses to Endurance Training and Overtraining in Female Athletes: Clinical Journal of Sport Medicine. 1998 Jul;8(3):178–86.
30. Rietjens GJWM, Kuipers H, Adam JJ, Saris WHM, van Breda E, van Hamont D, et al. Physiological, biochemical and psychological markers of strenuous training-induced fatigue. Int J Sports Med. 2005;26(1):16–26.
31. Svendsen IS, Killer SC, Carter JM, Randell RK, Jeukendrup AE, Gleeson M. Impact of intensified training and carbohydrate supplementation on immunity and markers of overreaching in highly trained cyclists. Eur J Appl Physiol. 2016;116:867–77.

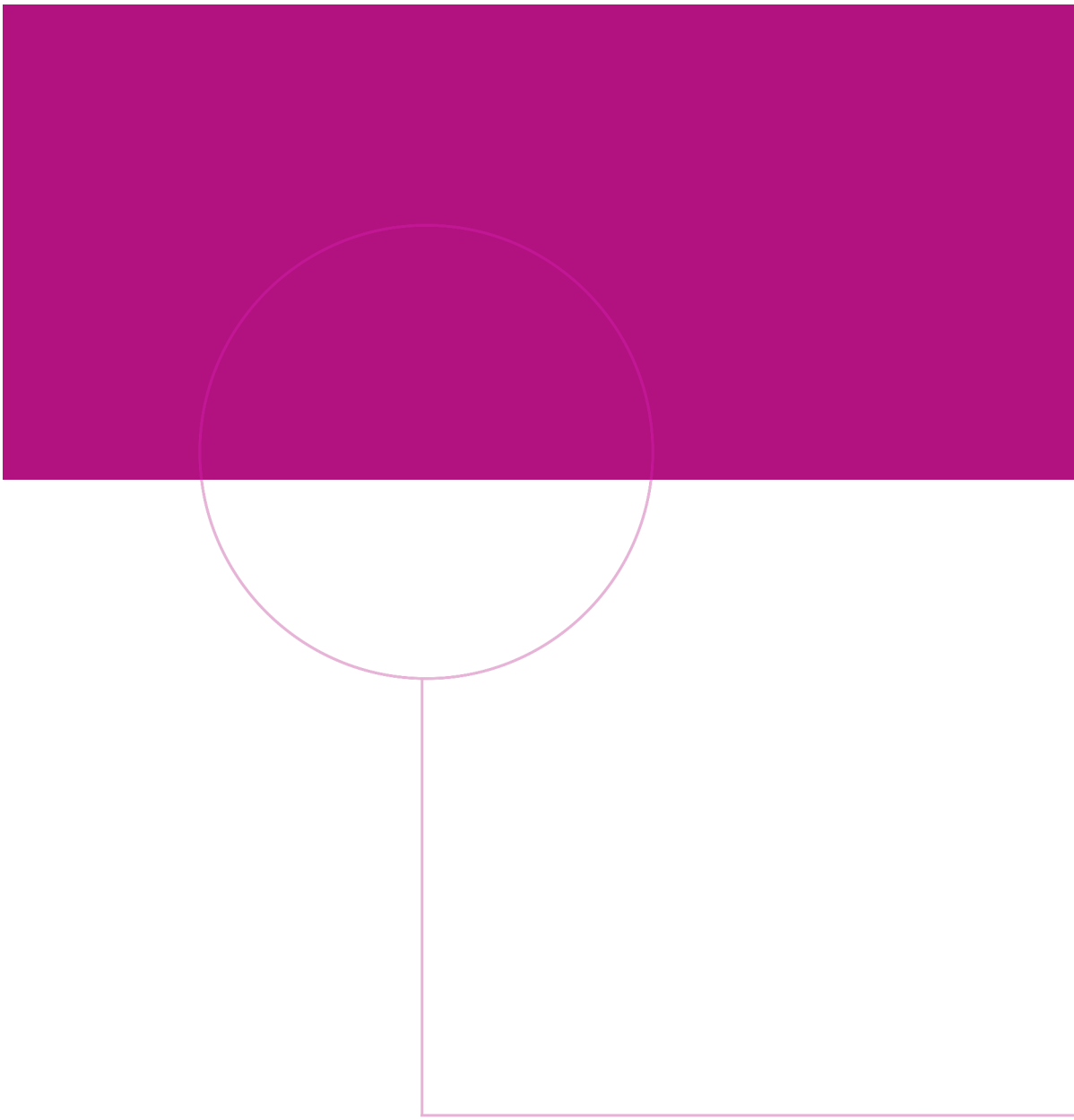
32. Stenqvist TB, Torstveit MK, Faber J, Melin AK. Impact of a 4-Week intensified endurance training intervention on markers of relative energy deficiency in sport (RED-S) and performance among well-trained male cyclists. Front Endocrinol. 2020 Sep 25;11:512365.
33. Buyse L, Decroix L, Timmermans N, Barbé K, Verrelst R, Meeusen R. Improving the diagnosis of nonfunctional overreaching and overtraining syndrome. Med Sci Sports Exerc. 2019 Dec;51(12):2524.
34. Lee SC, Baranowski ES, Sakremath R, Saraff V, Mohamed Z. Hypoglycaemia in adrenal insufficiency. Front Endocrinol. 2023 Nov 20;14:1198519.
35. Mountjoy M, Sundgot-Borgen J, Burke L, Carter S, Constantini N, Lebrun C, et al. The IOC consensus statement: beyond the Female athlete triad—Relative energy deficiency in sport (RED-S). Br J Sports Med. 2014 Apr 1;48(7):491–7.
36. McInnis KC, Ramey LN. High-risk stress fractures: diagnosis and ,anagement. PM&R. 2016 Mar;8(3 Suppl):S113-124.
37. Sim A, Burns SF. Review: questionnaires as measures for low energy availability (LEA) and relative energy deficiency in sport (RED-S) in athletes. J Eat Disord. 2021 Mar 31;9:41.
38. Olympiatoppen. Topptrening [Internet]. [cited 2024 Feb 12]. Available from: https://olt-dagbok.nif.no/
39. Haaf T ten, Staveren S van, Oudenhoven E, Piacentini MF, Meeusen R, Roelands B, et al. Prediction of functional overreaching from subjective fatigue and readiness to train after only 3 days of cycling. International Journal of Sports Physiology and Performance. 2017 Apr 1;12(s2):87–94.
40. Solli GS, Tønnessen E, Sandbakk Ø. The training characteristics of the world's most successful female cross-country skier. Front Physiol. 2017 Dec 18;8:1069.
41. Foster C, Florhaug J, Franklin J, Gottschall L, Hrovatin L, Parker S, et al. A new approach to monitoring exercise training. J Strength Cond Res. 2001 Mar 1;15:109–15.
42. The Norwegian Directorate of Health. Kostholdsplanleggeren [Internet]. [cited 2024 Feb 12]. Available from: https://www.kostholdsplanleggeren.no/
43. Borg G a. V. Psychophysical bases of perceived exertion. Medicine & Science in Sports & Exercise. 1982;14(5):377.
44. Richter SD, Schürmeyer TH, Schedlowski M, Hädicke A, Tewes U, Schmidt RE, et al. Time kinetics of the endocrine response to acute psychological stress. J Clin Endocrinol Metab. 1996 May;81(5):1956–60.
45. Duclos M. A critical assessment of hormonal methods used in monitoring training status in athletes: International SportMed Journal. International SportMed Journal. 2008 Jun;9(2):56–66.
46. Duclos M, Corcuff JB, Rashedi M, Fougère V, Manier G. Trained versus untrained men: different immediate post-exercise responses of pituitary adrenal axis. A preliminary study. Eur J Appl Physiol Occup Physiol. 1997;75(4):343–50.
47. Bunt JC, Boileau RA, Bahr JM, Nelson RA. Sex and training differences in human growth hormone levels during prolonged exercise. J Appl Physiol (1985). 1986 Nov;61(5):1796–801.

Appendix

Appendix 1

Materials, equipment, and methods used for analysis of each hormone.

Hormone	Assay material	Instrument	Assay method
ACTH	EDTA Plasma	Roche cobas pro e801.	Immunoassay, Electrochemiluminescence
Albumin	Serum	Siemens Atellica CH930	Dye-binding assay with bromocresol green, at pH 4.2, and 596 nm light
Cortisol	Serum	Siemens Atellica IM1600	Immunoassay, Chemiluminescence
Free T3	Serum	Siemens Atellica CH IM1600	Immunoassay, Chemiluminescence
hGH	Serum	Roche cobas pro e801	Immunoassay, Electrochemiluminescence
LDL- cholesterol	Serum	Siemens Atellica CH930	Enzymatic assay, with 596 nm light
Prolactin	Serum	Siemens Atellica IM1600	Immunoassay, Chemiluminescence
SHGB	Serum	Siemens Atellica CH930	Immunoassay, Chemiluminescence
Testosterone	Serum	Agilent 1290, Agilent 6465 Triple Quad LC/MS-MS	Liquid-liquid extraction, High-Pressure Liquid Chromatography, Mass spectrometry
Total cholesterol	Serum	Siemens Atellica CH930	Enzymatic assay, with 505 nm light



NTNU

Norwegian University of
Science and Technology