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"Det koster meg ingenting"

- Mølmen

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Summary

Competitive cycling at the elite level is a demanding endurance sport with up to 300 km long races. Elite cyclists annually spend up to ~1000 hrs on the bike, with the majority of training time spent as low-intensity training (LIT) and only ~2-10% in the high-intensity domain (i.e., above the second ventilatory threshold). The decisive moments of cycling (e.g., to establish a break-away or to sprint for the finish line), involve maximal efforts, and top-10 finishers have higher short (<5 min) power outputs, compared to non-top-10 finishers. However, further increasing HIT-load to improve these competition-specific abilities may increase the risk of overreaching and burnout. Sprint training could be an alternative to this, but dedicating entire training sessions to e.g., sprint training might not be a time-efficient strategy. Therefore, including 30-s sprints during LIT-sessions could be an alternative to improve performance (i.e., sprint and endurance performances) after prolonged exercise, which are specific for road races. The aims of this thesis were first, to investigate the acute responses to inclusion of 30-s maximal sprints during a LIT-session (Paper I and II), and second, to investigate adaptations to repeated inclusion of sprints during LIT-sessions in periods of habitual changes in total training load (Paper III and IV). This was studied in three consecutive studies, outlined in four separate papers.

In Papers I and II, we investigated the effects of including sprints during a 4-h LIT-session on acute physiological responses, the subsequent muscular and hormonal responses, as well as the recovery of muscle strength in the following 24 hrs. The effects of including sprints (SPR) were compared to a work-matched LIT-session (CON) in a randomized cross-over design on 12 elite cyclists (maximal oxygen uptake, VO_{2max}: 73±4 $mL \cdot kg^{-1} \cdot min^{-1}$). In Paper I, SPR temporarily changed pedalling technique and muscle activity patterns but did not affect the overall change in gross efficiency (GE) during prolonged cycling, which decreased ~1%-point during the 4-h session in both SPR and CON. Also, repeated sprint performance was maintained during prolonged cycling in SPR. In Paper II, SPR induced more pronounced changes in mRNA levels of markers of fat metabolism (↑PDK4), angiogenesis (↑VEGFA), protein turnover (↑MuRF1 and ↓Myostatin), ion transport (↓Na^+-$K^+\alpha$1, ↓CLC1, and ↓NHE1) and mitochondrial biogenesis (↓PGC-1α) in m. Vastus lateralis compared to CON. Hormonal responses to a LIT-session of habitual duration were overall small in elite cyclists and similar in SPR and CON. However, SPR induced lower responses of growth hormone and sex hormone-binding globulin compared to CON, indicating a generally low endocrine stress response. Importantly, recovery of muscle strength (isokinetic knee extension) was completed 24 hrs after both conditions.

In Paper III and IV, the adaptations to inclusion of sprints during LIT-sessions were investigated in two ~3-wk interventions of habitual changes in total training load in elite cyclists. In Paper III, the effects of including sprints during one weekly LIT-session (SPR, n=7, VO_{2max}: 73±5 $mL \cdot kg^{-1} \cdot min^{-1}$) compared to only LIT (CON, n=9, VO_{2max}: 71±5 $mL \cdot kg^{-1} \cdot min^{-1}$) during a 3-wk transition period of ~60% reduced training load were investigated. Here, SPR improved 30-s sprint mean power 8% more than CON. In addition, after the ~2-h exercise test, 20-min power and fractional utilization of VO_{2max} ($\%VO_{2max}$) were maintained in SPR, while CON reduced these variables, but was not different from SPR. Inclusion of sprints did not affect power output at 4 $mmol \cdot L^{-1}$ [BLa^-] (L_4), which was equally reduced in both groups. However, VO_{2max}, maximal aerobic power (W_{max}), and mental recovery (total burnout) were not affected by the substantially reduced training load in any of the groups.

In Paper IV, the effects of including sprints (SPR, n=9, VO_{2max}: 75±5 $mL \cdot kg^{-1} \cdot min^{-1}$) on 5 LIT-sessions during a 14-d training camp, followed by a 10-d recovery period, compared to LIT only (CON, n=9, VO_{2max}: 75±5 $mL \cdot kg^{-1} \cdot min^{-1}$) were investigated. The training load was increased and decreased by ~50%, respectively, compared to habitual training. SPR improved 30-s sprint and 5-min mean power ~4% more than CON, without affecting total mental stress/recovery. In addition, SPR

maintained protein abundancy of Na^+-$K^+\beta1$ and GE, while Na^+-$K^+\beta1$ was reduced 8% more in CON and GE was reduced after prolonged exercise only in CON. SPR tended to improve $\%VO_{2max}$ during the 5-min test after prolonged exercise, but was not different to CON. However, neither power output and $\%VO_{2max}$ at L_4, VO_{2max}, nor W_{max} were affected by the addition of sprints or by the substantial changes in training load.

In summary, inclusion of sprints during a prolonged LIT-session did not change GE compared to LIT only, but potentially leads to beneficial adaptations in skeletal muscle without impairing muscular performance on the following day. When elite cyclists included a small number (27-51) of sprints during LIT-sessions, 30-s mean power was improved by 4% to 8% more than LIT only, irrespectively of substantial changes in total training load. Furthermore, inclusion of sprints maintained 20-min performance in periods of decreased training load and improved 5-min performance in periods of increased training load, without affecting burnout or mental stress/recovery. The addition of sprints primarily affected performance-related measures after prolonged exercise, whereas measures in the fresh state (e.g., power output at L_4, VO_{2max} and W_{max}) were not altered by sprinting. It is, therefore, suggested that sprints can be included in habitual LIT-sessions to improve competition-specific performance of elite cyclists without affecting mental stress/recovery or increasing the risk of burnout.

Oppsummering

Sykkelkonkurranser på elitenivå er svært krevende og opp til 300 km lange. Elitesyklister bruker opp mot 1000 timer i året på trening, hvor størstedelen er lavintensiv trening (LIT) og kun ~2-10% er høyintensiv trening (HIT, over den andre ventilatoriske terskelen**)**. De avgjørende øyeblikk i sykling, for å komme seg i brudd eller i den avsluttende sprint, innebærer maksimale prestasjoner og syklister i topp 10 på konkurranser har høyere gjennomsnittlig kraft på kortere prestasjoner (<5 min) sammenlignet med syklister utenfor topp 10. Det å øke mengden av HIT for å forbedre disse konkurranse-spesifikke prestasjoner øker dog risken for overtrening og utslitthet. Sprinttrening kan være ett alternativ, men det å legge til økter bare med fokus på sprint er på den andre siden ikke tidsoptimalt i en tettpakket treningsplan for en elitesyklist. Derfor kunne det å legge sprinter til underveis i LIT-øktene være et alternativ for å forbedre ritt-spesifikke prestasjonsmål (ex. sprint og utholdenhetsprestasjon etter langvarig arbeid). Målet med denne avhandling var derfor først å undersøke de akutte tilpasningene når 30-s sprinter ble lagt til underveis i en LIT-økt (Artikkel I og II). Etterfølgende var målet å undersøke treningstilpasningene når sprinter ble lagt til underveis i flere LIT-økter i perioder hvor elitesyklister vanligvis reduserer eller øker treningsbelastningen (Artikkel III og IV).

I artikkel I og II undersøkte vi effekten av å legge til sprinter underveis i en 4-t LIT-økt på akutte fysiologiske responser, muskulære og hormonelle responser og den etterfølgende restitusjonen av muskelstyrke i 24 t etter arbeid. Effekten av å legge til sprinter underveis (SPR) var sammenlignet med en arbeids-matchet LIT-økt (CON) i et randomisert overkryssingsstudie på 12 elitesyklister (maksimalt oksygenopptak, VO_{2max}: 73±4 $mL \cdot kg^{-1} \cdot min^{-1}$). I artikkel I påvirket sprintene sykkelteknikken og muskelaktiveringen midlertidig, men dette påvirket ikke de overordnede endringene i sykkeløkonomien (GE), hvilket reduserte ~1%-poeng i løpet av 4-t økten i både SPR og CON. Derutover ble evnen til å sprinte gjentagende ganger vedlikeholdt underveis i økten i SPR. I artikkel II ledet SPR til større endringer i mRNA-nivåer i markører for fettmetabolisme (↑PDK4), vaskularisering (↑VEGFA), proteinomsetning (↑MuRF1 and ↓Myostatin), iontransport (↓Na^+-$K^+\alpha1$, ↓CLC1, and ↓NHE1) og mitokondriell biogenese (↓PGC-1α) i m. Vastus lateralis sammenlignet med CON. Hormonelle responser til en LIT-økt av vanlig varighet var generelt små for elitesyklister og lik for SPR og CON. Derimot førte SPR til lavere respons i veksthormon og

kjønnshormonbindende globulin sammenlignet med CON, hvilket indikerte en generell lav endokrin stressrespons. Det er viktig å fremheve at muskelstyrke, målt som isokinetisk kneekstensjon, var fullstendig restituert etter 24 t i begge gruppene.

I artikkel III og IV ble effektene av å legge sprinter til underveis i LIT-økter i 3-ukers intervensjoner med vanlige endringer i total treningsmengde undersøkt hos elitesyklister. I artikkel III ble effekten av å legge sprinter til underveis i LIT-økter én gang i uka (SPR, n=7, VO_{2max}: 73±5 $mL \cdot kg^{-1} \cdot min^{-1}$) sammenlignet med bare LIT (CON, n=9, VO_{2max}: 71±5 $mL \cdot kg^{-1} \cdot min^{-1}$) på en 3-ukers transisjonsperiode hvor treningsbelastningen ble redusert med 60%. Her forbedret SPR 30-s sprint 8% mer enn CON. I tillegg ble 20-min sykkelprestasjon og utnyttingsgraden, målt etter ~2 t kontinuerlig sykling, vedlikeholdt hos SPR, mens CON reduserte begge mål, men uten å være forskjellig fra SPR. Effekten målt ved 4 $mmol \cdot L^{-1}$ [BLa^-] (L_4) ble ikke påvirket av sprinter og falt i begge grupper. Derimot var VO_{2max}, maksimal aerob effekt (W_{max}) og mental restitusjon upåvirket i begge grupper av den store reduksjon i treningsmengde.

I artikkel IV ble effektene av å legge sprinter til underveis i 5 LIT-økter (SPR, n=9, VO_{2max}: 75±5 $mL \cdot kg^{-1} \cdot min^{-1}$) på en 14-dagers treningssamling med etterfølgende 10-dagers restitusjonsperiode undersøkt, sammenlignet med kun å kjøre LIT-økter (CON, n=9, VO_{2max}: 75±5 $mL \cdot kg^{-1} \cdot min^{-1}$). Treningsbelastningen var henholdsvis økt og redusert ~50% i forhold til vanlig trening. SPR forbedret både 30-s sprint og 5-min utholdenhetsprestasjon ~4% mer enn CON, uten å påvirke det totale mentale stress- eller restitusjonsnivå. I tillegg til dette vedlikeholdt SPR mengden av proteinet Na^+-$K^+\beta 1$ i skjelettmuskelen samt GE, hvorimot Na^+-$K^+\beta 1$ ble redusert med 8% mer hos CON, samt at GE ble redusert etter langvarig sykling kun hos CON. SPR viste en tendens til å forbedre utnyttingsgraden på 5-min-testen målt etter langvarig sykling, men var ikke forskjellig fra CON. Hverken effekten eller VO_2 målt ved L_4, VO_{2max} eller W_{max} ble påvirket av sprinter eller endringen i treningsbelastning.

Samlet sett, det å legge til sprinter underveis i en LIT-økt endrer ikke på GE sammenlignet med en vanlig LIT-økt og leder potensielt til fordelaktige treningstilpasninger i musklene uten å påvirke restitusjonen av muskelstyrke målt dagen etter. Når elitesyklister legger et mindre antall sprinter (27-51) til underveis i deres vanlige LIT-økter forbedrer de gjennomsnittlig effekt på 30-s sprinter med 4% og 8%, sammenlignet med vanlige LIT-økter, til tross for store endringer i total treningsbelastning. I tillegg vedlikeholdes 20-min utholdenhetsprestasjon i en periode med redusert treningsbelastning, og bedret 5-min utholdenhetsprestasjon i en periode med økt treningsbelastning, uten å føre til utslitthet eller å påvirke mentalt stress/restitusjon. Det å legge sprinter til underveis i langkjøring påvirket primært prestasjon og prestasjonsrelaterte variabler målt etter langvarig arbeid, hvorimot prestasjon i uthvilt tilstand, for eksempel effekten målt ved L_4, VO_{2max} og W_{max}, var upåvirket. Det foreslås derfor at sprinter kan legges til underveis i vanlige LIT-økter for å forbedre konkurransespesifikke prestasjonsmål hos elitesyklister uten å påvirke mentalt stress eller restitusjon, eller øke risken for utbrenthet.

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And now that you have found your name in the acknowledgements and read your praises, please do me the honour of reading a bit further. I might have mentioned you later in the manuscript.

List of papers

This dissertation is based on three separate studies including four research papers which are referred to in the text by their Roman numbering.

I Almquist, N.W., Ettema, G., Hopker, J., Sandbakk, O., Rønnestad, B.R. The Effect of 30-Second Sprints During Prolonged Exercise on Gross Efficiency, Electromyography, and Pedalling Technique in Elite Cyclists. *Int J Sports Physiol Perform. 2019:1-9.*

II Almquist, N.W., Ellefsen, S., Sandbakk, O., Rønnestad, B.R. Effects of including sprints during prolonged cycling on muscular and hormonal responses and recovery in elite cyclists. *In review process in Scand J Med Sci Sports.*

III Almquist, N.W., Løvlien, I., Byrkjedal, P.T., Spencer, M., Kristoffersen, M., Skovereng, K., Sandbakk, O., Rønnestad, B.R. Effects of including sprints in one weekly low-intensity training session during the transition period of elite cyclists. *Accepted for publication in Front Physiol. 07.23.2020.*

IV Almquist, N.W., Wilhelmsen, M., Ellefsen, S., Sandbakk, O., Rønnestad, B.R. Inclusion of 30-s sprints during low-intensity sessions in a high load training camp improves performance in elite cyclists. *Manuscript.*

Abbreviations

ABQ:	Athlete burnout questionnaire
BV:	Blood volume
CI:	Confidence interval
CO_1:	Carbon monoxide
CO_2:	Carbon dioxide
CON:	Control groups/exercises performing only low-intensity training
ddH_2O:	Double-distilled water
DXA-scan:	Dual-energy X-ray absorptiometry scan.
EMG:	Electromyography
ES:	Effect size
F_{max}:	Maximal force
GE:	Gross efficiency
Hb-mass:	Haemoglobin mass
%HbCO:	Carboxy-haemoglobin
HCT:	Haematocrit
HIT:	High-intensity training
HP:	Human pool of skeletal muscle samples collected at Pre
HR:	Heart rate
HR_{max}:	Maximal heart rate
iEMG:	Integrated electromyography
iTRIMP:	Individualized training impulse method
L_4:	Blood lactate concentration of 4 $mmol \cdot L^{-1}$
LIT:	Low-intensity training
LT:	Lactate threshold
LT_1:	Lactate threshold 1
LT_2:	Lactate threshold 2
MIT:	Moderate-intensity training
mRNA:	messenger RNA
NS:	Not significant
O_2:	Oxygen
PL:	Performance-level
P_{max}:	Maximal power
PPO:	Peak power output
PV:	Plasma volume
qPCR:	Quantitative polymerase chain reaction
RBCV:	Red blood cell volume
RCP:	Respiratory compensation point
RER:	Respiratory exchange ratio
RESTQ:	Recovery-Stress Questionnaire for Athletes (RESTQ-36-R-Sport)
RPM:	Rounds per minute
SD:	Standard deviation
SPR:	Sprint groups/exercises including 30-s sprints during low-intensity training
sRPE:	Session rate of perceived exertion
TRIMP:	Training impulse method
TSS:	Training stress score
TT:	Time trial
VCO_2:	Carbon dioxide expired

VE:	Ventilation
VL:	m. Vastus Lateralis
VM:	m. Vastus medialis
V_{max}:	Maximal velocity
VO_2:	Oxygen uptake
VO_{2max}:	Maximal oxygen uptake
$\%VO_{2max}$:	Fractional utilization of VO_{2max}
VT_1:	First ventilatory threshold
VT_2:	Second ventilatory threshold
W_{max}:	Maximal aerobic power output

1. Introduction

1.1. The physiological demands of elite cyclists' competitions

Competitive cycling at the elite level is an arduous endeavour spanning from 1-day races (e.g., The Classics: Milano-Sanremo, Paris-Roubaix) with distances between 180 and 300 km, to multi-stage tours (5 to 10 race-days) and Grand Tours lasting up to 21-22 race-days (e.g., Giro d'Italia, Tour de France or Vuelta a España). A thorough analysis of the different types of professional cycling races has recently given valuable insight into the demands of cycling at the elite level. The Classics are the longest one-day races with the highest average intensity, compared to stages in the Grand Tours and multi-stage races (Table 1) (van Erp and Sanders, 2020).

***Table 1**: Average volume and demands of cycling races/stages in professional male cycling. Data are mean ± SD, from (van Erp and Sanders, 2020).*

	The Classics	Multi-stage Races	Grand Tours
Daily distance (km)	268 ± 19.5	178 ± 36.1	182 ± 40.2
Daily duration (hrs)	6.8 ± 0.5	4.8 ± 0.9	5.0 ± 0.9
Mean power output ($W \cdot kg^{-1}$)	3.3 ± 0.3	3.0 ± 0.4	2.9 ± 0.4
Mean HR (%HR_{max})	75.0 ± 4.5	68.8 ± 4.9	65.8 ± 5.7

HR: Heart rate.

For the majority of the time during competitions, the intensity is low and moderate, i.e., below the second ventilatory threshold (VT_2) and lactate threshold 2 (LT_2), primarily targeting the aerobic system, but with several high-intensity elements (Vogt et al., 2006, Fernandez-Garcia et al., 2000, Rodriguez-Marroyo et al., 2009, Padilla et al., 2001, van Erp and Sanders, 2020). The main performance-determining factors in cycling are therefore maximal oxygen uptake (VO_{2max}) fractional utilization of VO_{2max} (%VO_{2max}), and gross efficiency (GE) (Joyner and Coyle, 2008, Jeukendrup et al., 2000) but power output relative to body mass has also been shown to be of importance in competitions, i.e., mountain stages (Padilla et al., 1999, Lee et al., 2002). To prepare for the strenuous demands of competitions, elite cyclists are known for their very high training volumes of endurance training (Seiler, 2010). However, in the decisive moments of cycling competitions, e.g., in a break-away, to counter-attack or to sprint for the finish line, maximal efforts targeting the anaerobic system are crucial (Menaspa et al., 2015, Peiffer et al., 2018, Abbiss et al., 2013, Fernandez-Garcia et al., 2000). Indeed, top-10 finishers are reported to have higher absolute (W) and relative power output ($W \cdot kg^{-1}$) for short durations (<5 min), compared to non-top-10 finishers, hinting that the maximal aerobic and anaerobic power outputs are essential for success in elite cycling (van Erp and Sanders, 2020).

1.2. The annual training characteristics of elite cyclists

Training volume and "best practice intensity distribution" in annual training of elite cyclists have been thoroughly described during the last ~30 years (Coyle et al., 1991, Lucia et al., 1996, Zapico et al., 2007, Seiler, 2010, Sanders et al., 2017, van Erp et al., 2019b). Exercise intensity can be split into a 3-zone scale representing low-intensity exercise below the first ventilatory threshold (VT_1) or blood lactate threshold 1 (LT_1), moderate-intensity between VT_1/LT_1 and VT_2/LT_2, and high-intensity exercise above VT_2/LT_2, respectively (Figure 1). This is also fairly well reflected in three heart-rate zones representing low-intensity training (LIT: 60-82% of HR_{max}), moderate-intensity training (MIT: 82-87% of HR_{max}) and high-intensity training (HIT: 88-100% HR_{max}) (Seiler, 2010). The extensive nomenclature regarding ventilatory and lactate thresholds has previously been outlined (Binder et al.,

2008, Sjodin and Jacobs, 1981, Hagberg and Coyle, 1983, Davis, 1985, Jamnick et al., 2020) and will not be further discussed here. For clarity, this thesis will use the term L_4, as previously used (Bishop et al., 1998), to denote a blood lactate concentration of 4 mmol·L^{-1}, respectively, although this is also referred to as LT_2 by some. [1]

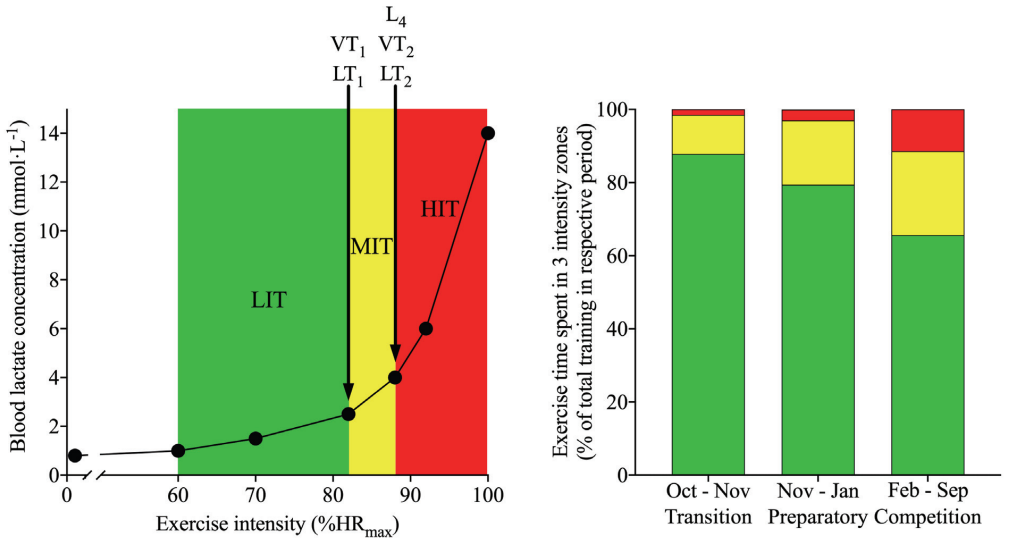

***Figure 1**: The schematic presentation of the 3-zone intensity scale is redrawn from Seiler (2010). The figure shows the representative training intensity distribution (percentage of total training time spent in each HR-zone) through a cycling season of elite cyclists. Periods include the transition period, preparatory period, and the competition period (Lucia et al., 1996, Zapico et al., 2007, Lucia et al., 2000a, Zapico et al., 2010). LIT: Low-intensity training. MIT: Moderate-intensity training. HIT: High-intensity training. VT_1: First ventilatory threshold. VT_2: Second ventilatory threshold. LT_1: Blood lactate threshold 1. LT_2: Blood lactate threshold 2. L_4: Blood lactate concentration of 4 mmol·L^{-1}.*

The annual training cycle for an elite cyclist can be broken into three distinct periods: a preparatory period, a competition period, and a transition period (Mujika et al., 2018). The preparatory period typically starts late autumn (November). It is followed by the competition period with the first competitions starting in the winter (January-March) and lasts through to the beginning of autumn in

[1] *The separation of three training intensities; LIT, MIT, and HIT, respectively, are based on the individual, identifiable, physiological turn-points defined by shifts in blood lactate accumulation, ventilation (VE), oxygen uptake from inspired air (VO_2) and carbon dioxide expiration (VCO_2). The VT_1 is defined by an increase in $VE{\cdot}VO_2^{-1}$ with no increase in $VE{\cdot}VCO_2^{-1}$ and a departure from linearity of VE. This reflects an increased oxygen extraction in the active tissue increasing VE due to the increased CO_2, which stems from the buffering of La^- by bicarbonate. Consequently, the fraction of oxygen in the expired air is lowered. VT_2, also referred to as the respiratory compensation point (RPC), is defined by an increase in both $VE{\cdot}VO_2^{-1}$ and $VE{\cdot}CO_2^{-1}$ (Davis, 1985). This reflects a further increase in VE caused by increasing acidosis and CO_2 from buffering of La^- exiting the muscle cells. The blood lactate thresholds are not defined consistently in the literature. However a common definition of LT_1 is by an increase in the blood lactate concentration of 1 mmol·L^{-1} above mean baseline values measured at exercise intensities of 40-60% of maximal aerobic power output (W_{max})(Hagberg & Coyle, 1983). The LT_1, therefore, roughly matches the occurrence of VT_1. Blood lactate threshold 2 (LT_2) is commonly but not consistently defined by a blood lactate concentration of 4 mmol·L^{-1}, also referred to as OBLA (Sjödin & Jacobs, 1981). Likewise, LT_2 also roughly matches the occurrence of VT_2. A blood lactate concentration of 4 mmol·L^{-1} does not, per se, reflect a distinct threshold. Therefore, and for clarification in this thesis, the power output, VO_2 and $\%VO_{2max}$ at a blood lactate concentration of 4 mmol·L^{-1} will be denoted L_4, and will be used as surrogate performance indicators, representing submaximal performance-related outcomes.*

September. This only leaves a few weeks to recover during the transition period (Lucia et al., 2001). Total annual training volumes are reported to amount to 24,000 to 35,000 km and 800 to >1000 hrs (Chicharro et al., 2000, Lucia et al., 1996, Metcalfe et al., 2017, Zapico et al., 2007). The training volume is generally kept high for the majority of the season. During the preparatory period, training volumes are reported to be ~22 hrs·wk^{-1} or ~700 km·wk^{-1} and are marginally increased in the competition period to ~24-26 hrs·wk^{-1} or ~800 km·wk^{-1} (Chicharro et al., 2000, Lucia et al., 1996, Sassi et al., 2008, Lucia et al., 2000b). However, training volume is substantially decreased in the transition period to ~7 hrs·wk^{-1} or ~250 km·wk^{-1} (Lucia et al., 2000b, Chicharro et al., 2000, Ronnestad et al., 2014). The majority of the training time is spent at relatively low intensity and only smaller fractions are spent in the high-intensity domain above LT_2 and VT_2 (Figure 1) (Zapico et al., 2007, Lucia et al., 1996, Metcalfe et al., 2017, Chicharro et al., 2000). Training intensity distribution varies during these periods. The preparatory period holds a large fraction of LIT (78-83% of exercise time) and small fractions of HIT (1.5-5% of exercise time), while the competition period holds less LIT (50-70%) and more HIT (8-18%) (Lucia et al., 1996, Zapico et al., 2007, Lucia et al., 2000a, Zapico et al., 2010). During the transition period, the training load is substantially decreased. Hence, the fraction of training performed as LIT is typically increased above levels of the preparatory period, while HIT is substantially reduced to ~1.5% (Lucia et al., 2000a) or not performed at all (Sassi et al., 2008, Lucia et al., 1996, Maldonado-Martin et al., 2017).

The interplay between training volume (distance and duration) and the relative intensity makes up the training load, which have been sought quantified and correlated with endurance training adaptations (Sanders et al., 2017, Foster et al., 1996, Manzi et al., 2009). Multiple measures have been used to quantify training load. These are based on relative heart rate measures (training impulse method; TRIMP) (Banister and Calvert, 1980), relative power output measures using on-bike mounted power meters (Training Stress Score™; TSS) (Coggan, 2003), perceived exertion (session rate of perceived exertion; sRPE) and the relationship between individual heart rate reserve and peak blood lactate concentration ([BLa^-]) (individualized training impulse method; iTRIMP) (Manzi et al., 2009). The training load quantification methods using individual, physiological characteristics, (e.g., the TSS and iTRIMP methods) have shown the strongest relationships between training load and endurance training adaptations in competitive cyclists (Sanders et al., 2017).

1.3. Seasonal changes in performance and performance-related measures in elite cyclists

The immense training loads reported in elite cyclists are arguably necessary to reach the high levels of VO_{2max} and $\%VO_{2max}$ during prolonged exercise (Faria et al., 2005, Fernandez-Garcia et al., 2000, Zapico et al., 2007). Most studies of elite cyclists are retrospective, descriptive studies reporting changes in performance and performance-related measures, which probably relates to a reluctance to experiment with their training regimes (Hawley et al., 1997). These studies have revealed seasonal changes in performance, which are summarized in Table 2. Small differences are present between the studies regarding definitions and durations of the three training periods and the changes in training load, which likely relates to the time of the primary competition goals (i.e., peaking performance at a Grand Tour in July). Although most studies have measured performance from the competition period in May-July, competitions usually continue after this. On a general basis, VO_{2max}, W_{max}/peak power output (PPO) and submaximal performance measures (e.g., lactate and ventilatory thresholds) are greatly increased during the preparatory period while small or non-significant increases are reported during the competition period. Consequently, endurance performances are also improved during the preparatory and competition period (Paton and Hopkins, 2005, Ronnestad et al., 2010, Ronnestad et al., 2014). However, not all studies report changes in VO_{2max} (Chicharro et al., 2000, Lucia et al., 2000a) and W_{max} (Chicharro et al., 2000, Lucia et al., 2000a, Ronnestad et al., 2010, Ronnestad et al., 2014) during these periods. This might be due to the timing of testing (e.g., by training load performed

prior to testing, time to recover, motivation), but could also reflect the relatively high performance-level and a limited room for additional training to induce further improvements in elite cyclists. Indeed, after the transition period, both submaximal and maximal performance measures are reported to be substantially lower compared to what is reported in the competition period (Barbeau et al., 1993, Chicharro et al., 2000, Impellizzeri and Marcora, 2007, Lucia et al., 2000a, Lucia et al., 2000b, Paton and Hopkins, 2005, Sassi et al., 2008, Sjogaard, 1984), probably related to the substantial decrease in training volume and intensity.

Seasonal changes in the efficiency of cycling (e.g., gross efficiency, O_2-cost of cycling) are, however, equivocal. Efficiency has been reported not to change in most studies (Lucia et al., 2000a, Impellizzeri and Marcora, 2007, Ronnestad et al., 2014). Yet, some research find efficiency to tend to increase (Sassi et al., 2008) or increase from the preparatory to the competition period, in relation to the volume of HIT in trained cyclists, i.e., a greater volume of HIT led to greater increases in GE (Hopker et al., 2009a). Evidently, efficiency of cycling has been reported to improve during a five year period (Santalla et al., 2009), possibly relating to the accumulated effect of many hours of HIT. The effect of the seasonal changes in training volume and intensity-distribution on GE, therefore, needs further investigation in elite cyclists.

Seasonal changes in the anaerobic performance measures such as sprinting are scarcely investigated in elite cyclists, even though it represents a competition-specific measure to improve (Menaspa et al., 2015, Fortes et al., 2019). Peak and mean power output, obtained on a 30-s all-out Wingate test, is reported to be unchanged during 12 wks of habitual training in the preparatory period (Ronnestad et al., 2010), while systematic sprint training improves sprint performance (Fortes et al., 2019). Specific sprint training does not seem to be a primary focus of elite cyclists, possibly due to the relatively low number of sprint specialists in each team (Menaspa et al., 2015). However, since short maximal efforts are reported to be essential for success in elite cycling (van Erp and Sanders, 2020), it seems plausible to investigate the effects of implementing systematic sprint training in the annual training cycle of elite cyclists.

***Table 2**: Changes in performance and performance-related measures reported after the transition, the preparatory and the competition period in descriptive studies of elite cyclists. Data are calculated percentage changes compared to the previous period's results.*

Transition period (compared to performance reported after competition period)

Authors	Level	n (m,f)	Age (Yrs)	VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	Time of testing	VO_{2max} (%Δ)	W_{max}/PPO (%Δ)	Submaximal power output (%Δ)	Submaximal VO_2 (%Δ)	Endurance performance (%Δ)	GE (%-points Δ)
(Barbeau et al., 1993)	Elite	7	20±2	~74	Nov	0			VT: 0		
(Chicharro et al., 2000)	Prof	11	24±2	73±6	Nov	-2 NS	-3 NS	VT_1: -10 RCP: -10			
(Impellizzeri and Marcora, 2007)	Comp	12	-	64±6	Nov	-6	-7	LT_1: -10 L_4: -10			-0.3 NS
(Lucia et al., 2000a, Lucia et al., 2000b)	Prof	13	24±2	73±5	Nov	-3 NS	0	VT_1: -10 VT_2: -10 LT: -17			0 NS
(Maldonado-Martin et al., 2017)	Comp	10	20±1	79±6	Nov	-11	-7	LT_1: -13 L_4: -12			
(Paton and Hopkins, 2005)	Comp	12	23±7	-	-	-7				4-km TT: -8	
(Sassi et al., 2008)	Prof	13	26±4	75±5	Dec	-10	-10	RCP: -11	RCP: -9		
(Sjogaard, 1984)	Elite	9	26	~71	Dec	-8					

Preparatory period (compared to performance reported after transition period)

Authors	Level	n (m,f)	Age (Yrs)	VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	Duration	VO_{2max} (%Δ)	W_{max}/PPO (%Δ)	Submaximal power output (%Δ)	Submaximal VO_2 (%Δ)	Endurance and sprint performance (%Δ)	GE (%-points Δ)
(Barbeau et al., 1993)	Elite	7	20±2	~74	3 mo (Nov-Feb)	-6 NS		VT_1: -8 NS			
(Chicharro et al., 2000)	Prof	11	24±2	73±6	2 mo (Nov-Jan)	2 NS	1 NS	VT_1: 4 RCP: 5			
(Fortes et al., 2019)	Comp	17	24±2	-	16 wks (-)					30-s peak power: 14 30-s mean power: 12	
(Impellizzeri and Marcora, 2007)	Comp	12	-	64±6	3 mo (Nov-Feb)	6	5	LT_1: 9 L_4: 10			-0.3 NS
(Lucia et al., 2000a, Lucia et al., 2000b)	Prof	13	24±2	73±5	2 mo (Nov-Jan)	2 NS	1 NS	VT_1: 5 VT_2: 4 LT:10			NS
(Paton and Hopkins, 2005)	Comp	12	23±7	-	-	5				4-km TT: 6	
(Ronnestad et al., 2010)	Well-trained	9 (7,2)	30±2	66±2	12 wks (-)	6	2 NS	L_2: NS		40-min: 5	
(Ronnestad et al., 2014)	Well-trained	7	32±8	69±6	16 wks (-)	2	1 NS	L_4: 4 NS		40-min: 3	0 NS
(Ronnestad et al., 2014)	Well-trained	6	30±7	68±5	16 wks (-)	5	1 NS	L_4: 2 NS		40-min: 8	0 NS
(Sanders et al., 2017)	Well-trained	15	22±3	62±4	10 wks (Dec-Feb)	5	3	LT_1: 7 LT_2: 4		8-min: 3	
(Sassi et al., 2008)	Prof	13	26±4	75±5	3 mo (Dec-Mar)	7 NS	8	RCP: 10 NS	RCP: 6 NS		
(Zapico et al., 2007)	Elite	14	20±2	73±2	4 mo (Nov-Feb	6	8	VT_2: 12	VT_2: 5		

Competition period (compared to performance reported after preparatory period)											
(Barbeau et al., 1993)	Elite	7	20±2	~74	5 mo (Feb-Jul)	4 NS			VT_2: 9 NS		
(Chicharro et al., 2000)	Prof	11	24±2	73±6	4 mo (Jan-May)	0	1 NS	VT_1: 6 RCP: 5			
(Impellizzeri and Marcora, 2007)	Comp	12	-	64±6	5 mo (Feb-Jul)	1 NS	2 NS	LT_1: 2 NS L_4: 1 NS			0.6 NS
(Lucia et al., 2000a, Lucia et al., 2000b)	Prof	13	24±2	73±5	4 mo (Jan-May)	1 NS	-1 NS	VT_1: 5 VT_2: 4 LT: 10			NS
(Paton and Hopkins, 2005)	Comp	12	23±7	-	Not specified	2				4-km TT: 2	
(Sassi et al., 2008)	Prof	13	26±4	75±5	3 mo (Mar-Jul)	3 NS	3	RCP: 2 NS	RCP: 3 NS		
(Sjogaard, 1984)	Elite	9	26	~71	5 mo (Feb-Jun)	1-9%					
(Zapico et al., 2007)	Elite	14	20±2	73±2	4 mo (Feb-Jun)	4	-3	VT_2: 0 NS			

Changes in performance or performance-related measures are reported compared to the previous period. For studies where changes were not specified, the respective changes were calculated based on the change in average from before to after a period. Positive numbers represent improvements and negative numbers represent decreases from the prior period. VO_{2max}: Relative maximal oxygen uptake. W_{max}: Maximal aerobic power, the average power output of the last minute of incremental test to exhaustion. PPO: Peak power output on an incremental test to exhaustion with 25 W increments·min^{-1}. LT: Lactate threshold (i.e., highest work-rate not associated with an increase [BLa^-] higher than 0.2 mmol·L^{-1} above baseline). LT_1: Blood lactate threshold 1 (i.e., baseline [BLa^-] + 1 mmol·L^{-1} or + 0.4 mmol·L^{-1}). LT_2: Blood lactate threshold 2 (i.e., mmol·L^{-1} the modified D_{max}-method; the point on the polynomal regression curve that yielded the maximal perpendicular distance to the straight line formed by the lactate threshold and the final lactate point). L_2: Calculated power output or VO_2 at a [BLa^-] of 2 mmol·L^{-1}. L_4: Calculated power output or VO_2 at a [BLa^-] of 4 mmol·L^{-1}. VT_1: Ventilatory threshold 1. VT_2: Ventilatory threshold 2. RCP: Respiratory compensation point when VE/VO_2 and VE/VCO_2 increases. TT: Time trial. NS: Not significant.

1.4. The beneficial effects of sprint training

Sprint training has proven to be a potent training regimen to improve sprint performance in untrained (Gist et al., 2014), trained (Iaia and Bangsbo, 2010), and well-trained athletes (Laursen et al., 2002). The rather rapid training adaptations to sprint training, makes it highly relevant for elite cyclists, in short interventions (e.g., during the transition period or in the preparatory period on training camps). Besides, continuing sprint training for prolonged periods (17 wks) in combination with habitual endurance training, seems to lead to improvements in endurance performance in well-trained athletes (Skovgaard et al., 2017). Hence, sprint training provides an intriguing modality in other parts of the annual training routines of elite cyclists. Adding an intense stimulus such as sprint training in periods of predominantly LIT might yield small performance improvements, relevant for cycling competitions, in cyclists that are already close to their genetic maximum (Psilander et al., 2010). Specifically, the addition of sprint training to habitual LIT has shown to improve both sprint performance (Laursen et al., 2002), and performance at or above intensities eliciting VO_{2max} (Iaia et al., 2009, Bangsbo et al., 2009, Skovgaard et al., 2018b), but also prolonged endurance performance such as 40-min performance in trained and well-trained athletes (Laursen et al., 2002). The utilization of sprint training to improve sprint and endurance performance might relate to peripheral adaptations such as increased enzyme activity (MacDougall et al., 1998, Gunnarsson et al., 2019), and postponement of fatigue through improved ion-transportation (Gunnarsson et al., 2013, Iaia et al., 2011). Even in periods of severe reductions in training volume (-65%), sprint training has proven to maintain VO_{2max}, and 10-km performance in trained runners (Iaia et al., 2008) and maintain enzyme activity and capillary-to-fibre ratio (Iaia et al., 2009), making it highly relevant to include during the transition period.

The long-term mitochondrial adaptations to exercise training are believed to stem from cumulative effects of transient transcriptional responses to each acute exercise bout (Perry et al., 2010). Both sprint and endurance exercise acutely leads to increased mRNA abundance of a regulator of mitochondrial biogenesis, Peroxisome Proliferator-activated receptor gamma Coactivator-1α (PGC-1α), in trained skeletal muscle (Fiorenza 2018, Skovgaard 2016, Brandt 2016). In addition, inclusion of sprints during short (~1 h) LIT-sessions has been shown to increase citrate synthase (CS) protein content and phosphofructokinase (PFK) enzyme activity in trained subjects (Gunnarsson et al., 2019). However, acute muscular responses to inclusion of sprints during low-intensity sessions of more regular durations (~4 hrs) are not investigated in elite cyclists, and little is generally known about their muscular adaptations to short training interventions (Psilander et al., 2010, Sjogaard, 1984, Coyle et al., 1991). The reason might be resentment towards invasive measures such as muscle biopsy sampling among elite athletes, due to an expectancy of pain and decreased exercise ability in the following days. The development of the minimally invasive micro-biopsy technique (Hayot et al., 2005) has opened the avenue for such studies in elite athletes, minimizing pain and risk of adverse effects while simultaneously providing valid and reliable data. The possible beneficial adaptations of adding sprint training to habitual training regimes in elite cyclists should, therefore, be thoroughly investigated. However, dedicating single sessions to sprint training might not be time-efficient, given the extent of the training performed by elite cyclists. Therefore, implementing sprints during habitual LIT-sessions could be an intriguing asset to the regular training regimes of elite cyclists, especially in two periods. First, during the transition period to prevent the reduction of fitness by maintaining intensity without too high costs. Second, during training camps in the preparatory period where LIT-volumes usually are increased, adding or maintaining an intense stimulus in periods of increased load.

1.5. Possible effects of including sprints during the transition period

For elite cyclists, the long competition period is associated with pronounced physical and psychological exertion, which holds an inherent risk of burnout towards the end of the season

(Lemyre et al., 2006, Silva, 1990). The need for a subsequent period of physical and mental recovery is regarded as necessary (Mujika et al., 2018). Consequently, the training load in the subsequent transition period is drastically decreased and an almost sedentary lifestyle has been reported during the first 2 to 3 wks in some elite cyclists (Lucia et al., 2000a, Sassi et al., 2008, Lucia et al., 1996, Maldonado-Martin et al., 2017). However, too long periods of training cessation deteriorates performance (Hammes et al., 2016, Decroix et al., 2016, Mujika and Padilla, 2000b, Mujika and Padilla, 2000a, Maldonado-Martin et al., 2017). Within the first 2 to 4 wks, deterioration of VO_{2max} is primarily mediated by haematological changes (Coyle et al., 1986, Coyle et al., 1984), while continued training cessation further reduces VO_{2max} through decreased oxygen extraction by the muscle tissue (Neufer, 1989). Maintaining a minimum of training load in periods of decreased training volume seems necessary to avoid performance decrements (Mujika, 1998, Bosquet et al., 2007), with HIT playing a key role for maintenance of endurance performance (Garcia-Pallares et al., 2009, Ronnestad et al., 2014, Neufer, 1989). Maintenance of fitness in the transition period might also be crucial for continuous improvement in the following seasons of elite athletes (Mujika et al., 1995). A study on well-trained cyclists showed that performing a HIT-session every 7 to 10 days during an 8-wk transition period, maintained power output at L_4, VO_{2max} and 40-min performance better than LIT only (Ronnestad et al., 2014). However, including sprints during the habitual LIT-sessions might be a beneficial alternative of relatively low strain for elite cyclists to avoid the strenuous HIT-sessions in transition periods where physical and mental recovery is needed. Indeed, short maximal-effort intervals have been reported to be of less strain compared to longer HIT-intervals (Valstad et al., 2018) and could serve as an intensive stimulus, sufficient for maintaining endurance performance and performance-related outcomes in shorter periods of reduced training load (Iaia et al., 2009). However, the acute effects on recovery from daily exercises when implementing sprints during LIT-sessions, needs to be investigated as to avoid a possible development of overreaching and burnout in the transition period.

1.6. Possible effects of including sprints during training camps in the preparatory period

In preparation for the next season's strenuous competitions, training load is gradually increased during the preparatory period (Lucia et al., 2000a, Metcalfe et al., 2017). These immense loads of training reported in elite cyclists seem rather necessary to endure the long competitions. Hence, a wide-spread strategy in elite cycling to manipulate training stimulus is to increase training volume and intensity for short periods (e.g., 1 to 3 wks), typically performed as training camps (Hawley and Stepto, 2001, Saw et al., 2018), and are often followed by periods with less load to avoid overreaching (Hawley and Stepto, 2001, Saw et al., 2018). However, increasing volumes of LIT only, without adding an intensive stimulus during training camps might be too low-intense and monotonous to stimulate further improvements in endurance performance (Costill et al., 1991). Conversely, maintaining or increasing training intensity in periods with increased training volume will drastically increase the total training load, and increase the risk of overreaching (Bellinger, 2020). In fact, several studies investigating concurrent increases in training volume and intensity among trained cyclists and triathletes have led to decreased performance measured in time trials (TT) and performance indices such as VO_{2max} in overreached individuals (Slivka et al., 2010, Halson et al., 2002, Jeukendrup et al., 1992, Le Meur et al., 2013, Le Meur et al., 2014). Adding sprint training during periods of increased LIT provides an intense training stimulus of relatively low load, which has proven beneficial in well-trained cyclists (Laursen et al., 2002). However, dedicating singular sessions during a training camp to sprint training might not be time-efficient and a priority of elite cyclists. A solution to meet these challenges could be to implement sprints during the habitual LIT-sessions during a training camp of increased training load, being a time-efficient way to maintain an intense stimulus during periods of predominantly LIT.

1.7. Rationale for the thesis

The effects of including sprints during LIT-sessions has so far only been investigated in trained subjects during relatively short LIT-sessions (1-1.5 hrs) (Skovgaard et al., 2016, Brandt et al., 2016, Gunnarsson et al., 2019), which might not be sufficient for elite athletes. Therefore, it is first and foremost, important to assess its effects and feasibility during LIT-sessions of regular duration (>3-4 hrs) before such training is advocated for elite cyclists (van Erp et al., 2019b). Thus, it seems expedient to start such an exploration by investigating its acute physiological effects during exercise and the subsequent muscular and hormonal responses, as well as the associated need for post-exercise recovery, before investigating the effects of repeatedly including sprints during habitual LIT-sessions. Inclusion of sprints during LIT-sessions might serve as a performance-maintaining stimulus during periods of habitual reductions in training load (i.e., the transition period) and to improve performance during periods of habitual increases in training load (i.e., on a training camp).

2. Overall purpose

The overall purpose of this thesis was first, to investigate the acute responses to inclusion of 30-s maximal sprints during LIT-sessions of regular duration, and second, to investigate adaptations to repeated inclusion of sprints during LIT-sessions in periods of habitual changes in total training load in elite cyclists. This was studied in three consecutive studies, outlined in four separate papers with the following specific purposes:

2.1. Specific purposes

Paper I: To investigate the acute physiological responses when including sprints during a 4-h LIT-session.

Paper II: To investigate the acute muscular and hormonal responses and subsequent muscular recovery when including sprints during a 4-h LIT-session.

Paper III: To investigate the effects of including sprints during one weekly LIT-session during a 3-wk transition period of reduced training load on sprint and endurance performance as well as the associated changes in physiological capacities, and mental recovery in elite cyclists.

Paper IV: To investigate the effects of including sprints during LIT-sessions during a 14-d training camp of increased training load on sprint and endurance performance after a 10-d recovery period in elite cyclists as well as muscular and haematological adaptations, and stress/recovery measured after the training camp.

3. Methods

This thesis present data from four research papers originating from three separate experimental studies conducted between 2016 and 2019. For clarification, Paper I and II are based on the same acute, randomized cross-over study and Paper III and IV are two separate interventions with randomized experimental and control groups. The methods described here provide a summary, and the reader is referred to the original papers for more detailed descriptions of the methods.

3.1. Participants

To categorize the cyclists in the present studies, the physiological characteristics of male cyclists suggest by (De Pauw et al., 2013) were used (Table 3). Thirty-seven participants were regarded as performance-level 5 athletes and 10 were regarded as performance-level 4 athletes (Table 4). The nomenclature dividing cyclists in different performance-levels and classifications is not equivocal (Jeukendrup et al., 2000, De Pauw et al., 2013, Ansley and Cangley, 2009). However, the performance-levels 4 and 5, fairly reflects two common categories used in the literature, which are based on the fitness-level and training volume: "well-trained/elite cyclists, n=10" and "professional cyclists, n=37" (Table 3). For simplicity, the participants were thus collectively referred to as elite cyclists. The participants were regularly tested in the laboratory as part of the collaboration between our laboratory and the national cycling teams and clubs and were, therefore, accustomed to the testing procedures of maximal sprinting and endurance performance tests.

***Table 3**: Categorization of participants based on definitions by De Pauw et al. (2013) and Ansley and Cangley (2009).*

	Description	VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	W_{max} (W)	W_{max} ($W \cdot kg^{-1}$)	Annual training distance ('000 km)
Definitions by Ansley and Cangley (2009)	Professional	>70	>450	-	>30
	Elite	60-70	375-450	-	15-30
	Club	50-60	275-375	-	5-15
	Recreational	45-50	200-275	-	<5
Definitions by De Pauw et al. (2013)	PL 5/Professional	>71	>350	>5.5	25-35
	PL 4/Well-trained	65-71	380-440	4.9-6.4	15-25
	PL 3/Trained	55-65	320-379	4.6-5.5	3-15
	PL 2/Recreational	45-55	280-319	3.6-4.5	-

PL: Performance-level, VO_{2max}: Maximal oxygen consumption, W_{max}: Maximal aerobic power output the last minute during incremental test to exhaustion.

3.2. Ethical approvals

Before inclusion in an experimental study, the participants received and gave written informed consent to participate and were made fully aware of the possible risks and discomforts associated with the participation. The studies were approved by the local ethics committee at Inland Norway University of Applied Sciences in accordance with the Declaration of Helsinki but without pre-registration in a public database.

***Table 4**: Participants' characteristics and representative training volume (hrs) recorded one month prior to inclusion in studies. Data are mean ± SD.*

Paper	I + II	III	IV
Number of participants (*n*)	12	16	19
Years as a competitive cyclist (Yrs)	5.3 ± 4.1	8.8 ± 3.5	6.5 ± 2.0
Training volume the last month (Hrs)	55 ± 35	51 ± 14	55 ± 12
Age (Yrs)	26.2 ± 6.3	22.4 ± 3.2	21.1 ± 1.5
Body mass (Kg)	76.1 ± 3.2	73.3 ± 6.7	74.4 ± 6.8
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	73.4 ± 4.0	72.2 ± 4.7	75.4 ± 5.1
W_{max} ($W \cdot kg^{-1}$)	6.3 ± 0.3	6.0 ± 0.4	6.4 ± 0.4
Power output at L_4 ($W \cdot kg^{-1}$)	4.3 ± 0.6	4.4 ± 0.4	4.5 ± 0.3
$\%VO_{2max}$ at L_4 (%)	78.0 ± 6.5	83.4 ± 5.3	79.7 ± 4.3
Mean power output on 4 x 30-s maximal sprints ($W \cdot kg^{-1}$)	10.4 ± 0.6	8.9 ± 0.6	9.8 ± 0.5

VO_{2max}: Maximal oxygen consumption, W_{max}: Maximal aerobic power output the last minute during incremental test to exhaustion. L_4: $[BLa^-]$ of 4 $mmol \cdot L^{-1}$.

3.3. Study designs

The three study designs are shortly presented here. For extensive descriptions the reader is referred to papers I-IV.

***Figure 2**: Example of verbal encouragement during prolonged cycling including sprints.*

3.3.1. Papers I and II

For investigation of the acute responses when including sprints during a 4-h LIT-session compared to work-matched low-intensity cycling, the design is outlined in Figure 3.

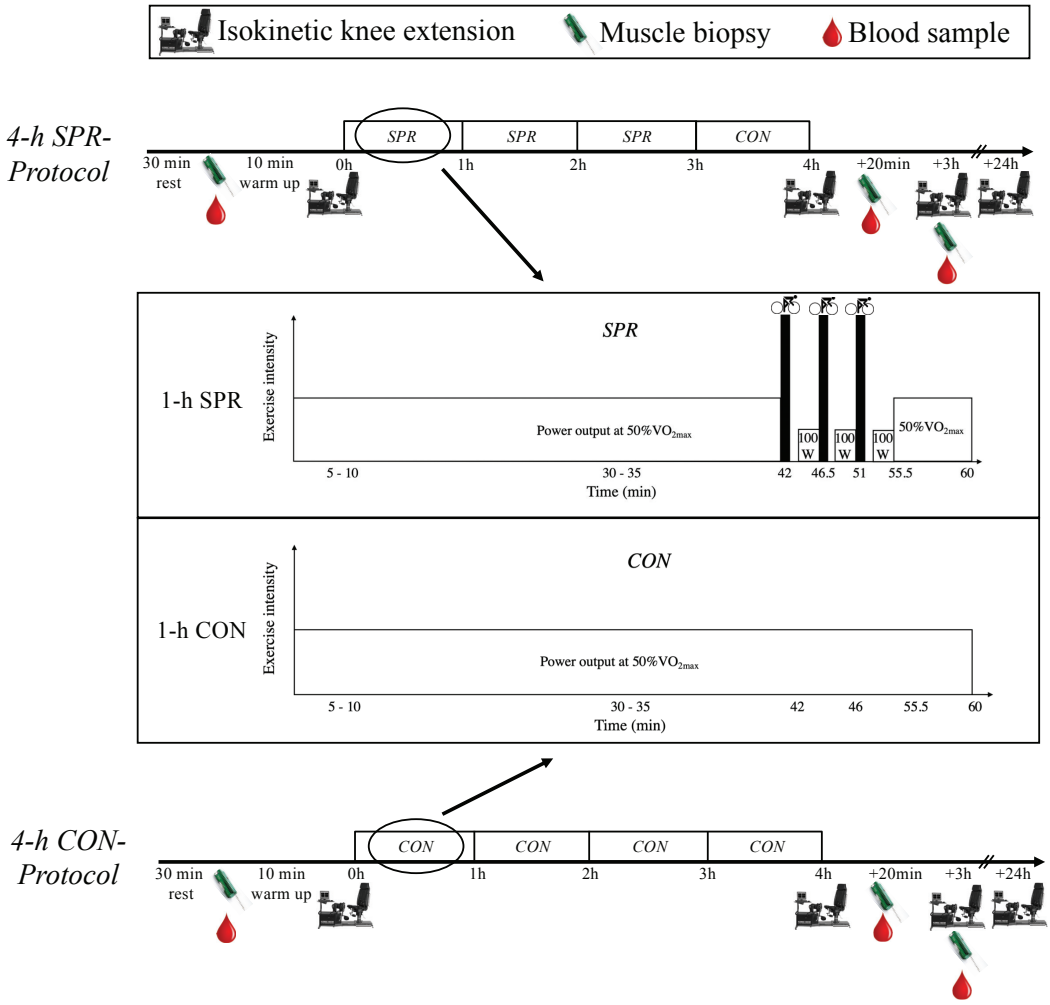

***Figure 3**: Experimental protocols; A 4-h LIT-session including sprints or a work-matched LIT-session. The upper panel shows the SPR-protocol which consisted of three hours of SPR (see 1-h SPR in the middle section for details), followed by 1 h of the CON-protocol. The lower panel shows the CON-protocol which consisted of four hours of CON (see 1-h CON in the middle section for details), which was a work-matched LIT-session with no sprinting. Oxygen uptake (VO_2) and electromyography (EMG) was recorded for three periods during each hour (5-10 min, 30-35 min and 58-60 min, respectively). Black arrows indicate the time point at which rate of perceived exertion (RPE), blood lactate concentration [BLa^-] and heart rate (HR) was registered.*

Participants visited the laboratory on four occasions to perform 1) screening, 2) familiarization, and 3+4) experimental protocols (Figure 3). The screening session consisted of isokinetic knee extension (*see details below; Isokinetic knee extension)*, a 30-s all-out sprint (see *Wingate*), a blood lactate profile test (*see Blood lactate profile test*) and an incremental test to exhaustion to determine VO_{2max} (*see VO_{2max} test*). Familiarization to the experimental protocol consisted of a 4-h bout of low-intensity cycling at a power output equivalent to 50% of VO_{2max} including three 30-s maximal sprints interspersed by 4-min recovery (1 min completely rest and 3-min cycling at 100 W) 42 minutes into every hour of the first, second and third hour (SPR). No sprinting was performed during the last hour. On experimental days, the cyclists performed in a randomized manner *SPR* or 4-hrs of work-matched low-intensity cycling without sprinting (CON). VO_2, electromyography (EMG) and pedalling technique measurements were recorded every hour; from 33^{rd}-35^{th} min and 58^{th}-60^{th} min (6.5 min post sprint). Participants were instructed to keep the same pedalling frequency during these periods. A 5-min break was allowed every hour for the participants to visit the lavatory and to re-calibrate the metabolic system and the cycle ergometer. The change in VO_2, EMG and pedalling technique measurements were expressed relative to baseline values measured during the first hour from 5^{th}-10^{th} min. Perceived exertion, [BLa^-] and HR was registered throughout the experimental protocols (Figure 3). The average power output on SPR and CON was 182 ± 4 W and 182 ± 4 W, respectively. Power output at 50% of VO_{2max} was calculated using submaximal values from the blood lactate profile test together with data from the VO_{2max} test. However, to ensure work-matched protocols, *SPR* involved slightly higher power output during steady-state periods (SPR: 186 ± 5 W vs *E*: 182 ± 4 W), as caused by the 4-min recovery periods between sprints. During familiarization trial the participants consumed water, energy drink and gels without caffeine (Squeezy Sports Nutrition GmbH, Germany) *ad libitum* to prevent dehydration and glycogen depletion. The total consumption and timing of intake was recorded and replicated during experimental protocols. The participants consumed a total of 3.2 ± 0.1 L and 3.2 ± 0.1 L of energy drink and water, during SPR and CON, which including gels amounted to 277 ± 17 g and 274 ± 15 g of carbohydrate, respectively.

3.3.2. Paper III

The design of the study including a 3-wk period of decreased training load in the transition period is outlined in Figure 4.

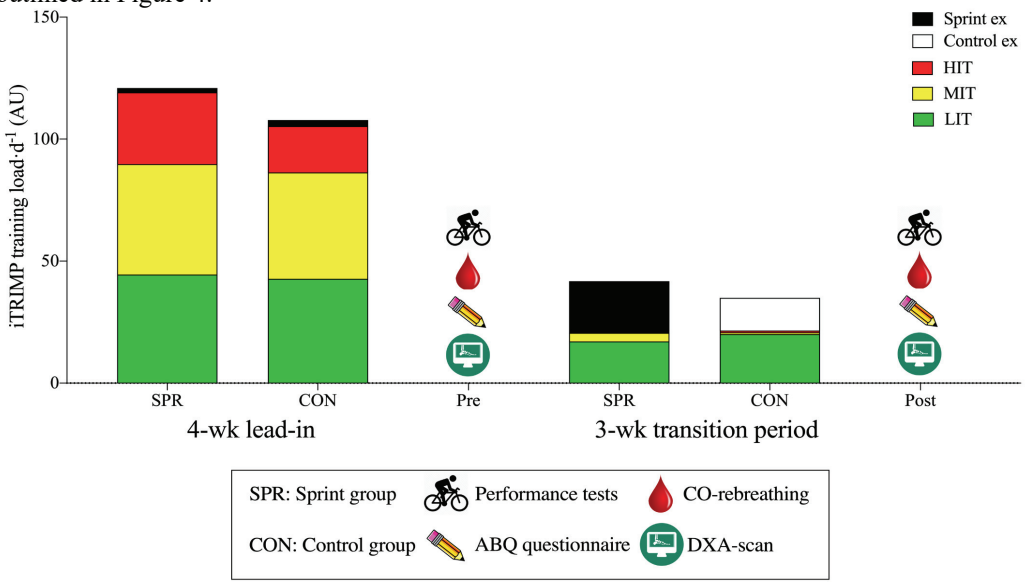

Figure 4*: Design and training load during lead-in and 3-wk transition period of decreased training load quantified using the individualized TRIMP method. HIT: High-intensity training, MIT: Moderate-intensity training. LIT: Low-intensity training. Sprint ex: LIT-session including sprints. Control ex: Time-matched LIT-session. DXA-scan: Body composition by Dual-energy X-ray absorptiometry scan. CO-rebreathing: Haemoglobin mass measurement by CO-rebreathing method. Performance test included; Blood lactate profile test, incremental test until exhaustion (VO_{2max} test), 60 min cycling at 60% of VO_{2max} including four 30-s maximal sprints, concluding with a 20-min test. ABQ: Athlete burnout questionnaire. Bars symbolize average daily training load (AU).*

The intervention was initiated 3 to 5 days after each cyclist's last competition of the season and was carried out over 21.2 ± 0.4 days. During the four weeks prior to the intervention, the cyclists performed on average the same number of training sessions per week (SPR: 6.4 ± 0.7 vs CON: 6.2 ± 1.1 sessions, p=0.803) of which an equal amount was characterized as HIT-sessions (SPR: 15 ± 10% vs CON: 15 ± 9%, p=0.954) and the training load from HIT was not different between groups (p=0.239). After the initial performance test (Pre) the participants were randomly assigned to either a Sprint group (SPR) or a Control group (CON). SPR and CON reduced training load equally (p=0.668) from the competition period to the transition period by 62 ± 9% and 64 ± 11% and only LIT was performed during the intervention (SPR: 13 ± 4 vs CON: 12 ± 3 sessions, p=0.570). However, once a week, SPR included 3 x 3 sets of 30-s maximal sprints during a supervised 90-min LIT-session where CON performed a time-matched supervised session at a power output equivalent to 60% of VO_{2max}. After 3 weeks of reduced training load, another performance test was conducted (Post). The performance test consisted of an incremental leg press test (*see Incremental leg press test*), a submaximal incremental test (*see Blood lactate profile test*), a 6-s all-out sprint test (see 6-s all-out sprint) a maximal incremental test to exhaustion (*see VO_{2max} test*) a 60 min continuous cycling with 4 x 30-s maximal sprints included (*see Prolonged cycling including four repeated 30-s maximal sprints*) and subsequently a 20-min test (*see 5-min and 20-min endurance performance tests*), all conducted as one continuous exercise. Training intensity distribution and individual iTRIMP-load was calculated for each session (Figure 2). A further categorization of the combined sprint and LIT-sessions (Sprint ex) and distance-matched LIT-sessions (Control ex) were also included.

3.3.3. Paper IV

The design of the study including 14-d training camp of increased training load and a subsequent 10-d recovery period is presented in Figure 5.

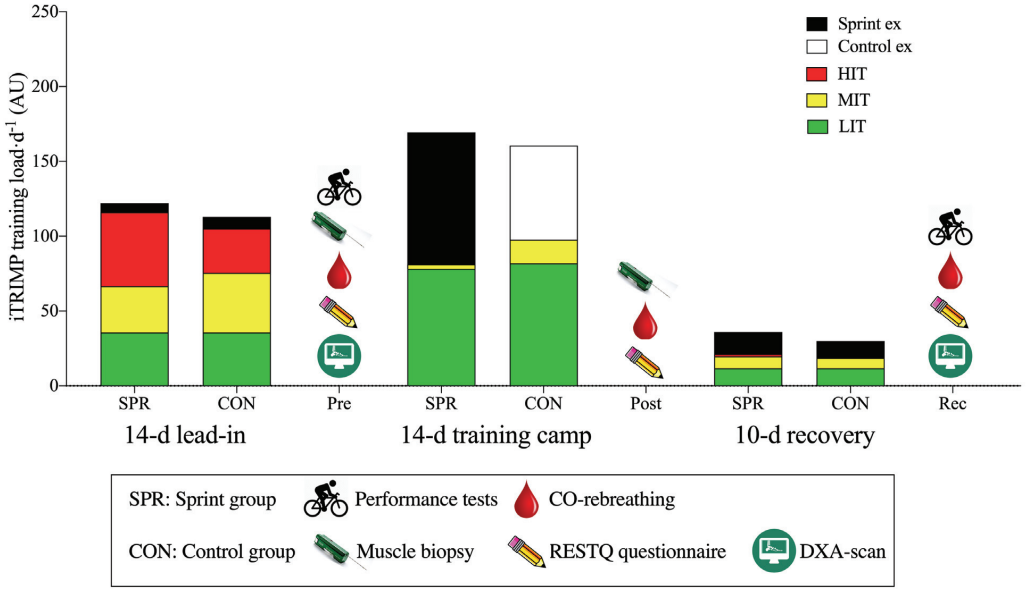

Figure 5*: Study design showing the training load per day in a 14-d lead-in period, 14-d training camp and 10-d recovery period. Training load is divided into HIT: High-intensity training, MIT: Moderate-intensity training. LIT: Low-intensity training. Sprint ex: LIT-session including sprints. Control ex: Distance-matched LIT-session. Muscle biopsy from m. Vastus Lateralis. DXA-scan: Body composition by Dual-energy X-ray absorptiometry scan. CO-rebreathing: Haemoglobin mass measurement by CO-rebreathing method. Performance test included; Blood lactate profile test, incremental test until exhaustion (VO_{2max} test), 60 min cycling at 60% of VO_{2max} including four 30-s maximal sprints, concluding with a 5-min test. RESTQ: Recovery-Stress Questionnaire for Athletes (RESTQ-36-R-Sport) to evaluate recovery and stress during the intervention. Bars symbolize average daily training load (AU).*

During the 14-d lead-in period the participants' individual training was recorded using the subjects' own bicycle computer and heart rate monitors, which was uploaded to the online program (TrainingPeaks, Colorado, USA) for further analysis. A self-administered familiarization trial of the combined sprint and LIT-session, consisting of 1-hr low-intensity endurance cycling and 4 x 30-s sprints, was performed the day prior to Pre- and Rec-testing. Testing was conducted prior to the training camp (Pre) and after a 10-d recovery period (Rec) and included: 1) Dual-energy X-ray absorptiometry (DXA) scan (*see DXA)*, 2) muscle biopsy sampling (*see Muscle biopsy procedure*), 3) performance testing (*see Blood lactate profile test, VO_{2max} test, Prolonged cycling including four repeated 30-s maximal sprints, and 5-min and 20-min tests*) and 4) haemoglobin-mass measurement (*see Hb-mass*). To create as equal groups as possible, the participants were pair-matched based on their total training load, VO_{2max} and sporting discipline/specification (mountain biking or road cycling/sprinter or climber) and assigned to a Sprint-group (SPR) or a Control group (CON). The total training load was calculated using the individualized training impulse method (iTRIMP). The training load during the lead-in was not different between SPR and CON (1707 ± 500 vs 1581 ± 512 AU, respectively, p=0.858), but the total training load from HIT tended to be greater in SPR compared to CON (690 ± 289 vs 414 ± 292 AU, respectively, p=0.061). The 14-d training camp started 5 ± 1 days after Pre-testing, and the daily training load was equally increased between groups (SPR: 50 ±

32%, CON: 47 ± 23%, p=0.806) compared to lead-in. On 5 occasions during the 14-d training camp, SPR performed series of 3 x 30-s maximal sprints interspersed by 4 min of active recovery every hour during a LIT-session of at least 4 hrs in duration. An average of 51 ± 12 sprints was completed during the camp in SPR. CON rode the same course without sprinting and were thereby matched on distance. Other sessions were individualized to reach the personal increase in training load ~50% compared to lead-in but were instructed to keep intensity low. Immediately upon return from training camp, a DXA scan, a resting muscle biopsy and Hb-mass measurement were performed (Post), followed by a recovery period of 10 ± 1 days where daily training load was equally reduced between groups (SPR: -53 ± 32%, CON: -59 ± 10%, p=0.579) compared to lead-in, although frequency-distribution of training was maintained. Performance test, DXA and Hb-mass measurement were performed after the recovery period (Rec). There was no difference in training load between SPR and CON in any part of the study and changes in load during the intervention were equal. Training intensity distribution was calculated as described above (Paper III).

3.4. Exercise protocols

In all studies, participants were instructed to refrain from caffeine, beta-alanine and bicarbonate 24 hrs prior to testing. Participants were also instructed to register and duplicate food intake and time of consumption 24 hrs prior to testing and all testing was performed on the same time of the day in a controlled environmental condition (16-18°C and 20-35% relative humidity) with a fan ensuring air circulation around the rider. All cycling tests were performed on the same electromagnetic braked cycle ergometer, measuring power output at 6 Hz (Lode Excalibur Sport, The Netherlands). The fixed modus was used during continuous cycling allowing the cyclists to freely choose frequency with a fixed resistance.

3.4.1. Wingate test (Papers I and II)

The Wingate modus was used for sprints with the resistance set to 0.8 $nm \cdot kg^{-1}$ body mass. A standardized 20 min warm-up including 3 x 20-s, submaximal sprints were performed prior to an all-out 30-s Wingate test. Sprints were started from 80 revolutions per minute (RPM), in a seated position with verbal encouragement throughout. Mean power output, representing the 30-s average power output sustained throughout the Wingate test was recorded.

3.4.2. Blood lactate profile test (Papers I-IV)

A schematic illustration of the exercise protocols used in paper III and IV is outlined in Figure 6.

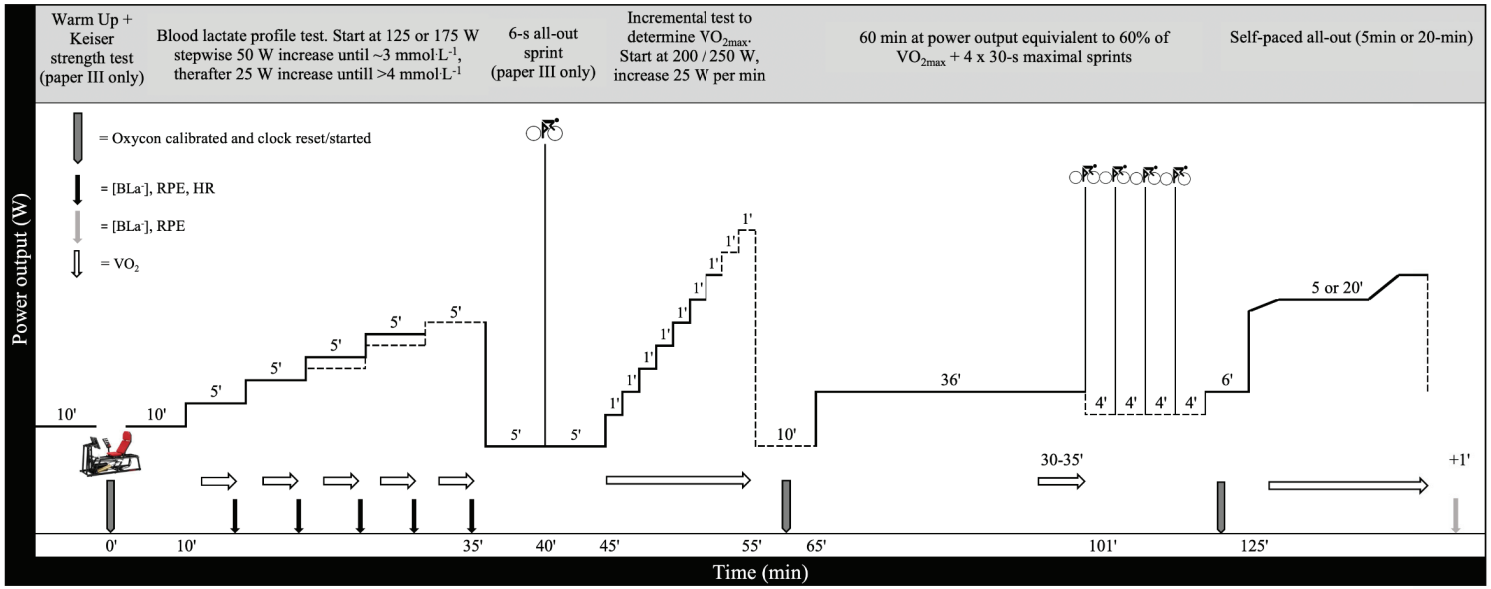

Figure 6: *Schematic illustration of the test protocol used in paper III and IV including strength test (paper III only), blood lactate profile test, 6-s all-out sprint (paper III only), incremental test to exhaustion, 60 min continuous cycling including 4 x 30-s maximal sprints and 5-min or 20-min test.*

To determine the relationship between power output, VO_2 and [BLa^-], a submaximal incremental test was performed. Briefly, participants cycled for 5 min starting at 125 or 175 W depending on previous results in the laboratory, followed by 50-W increments every 5 min until a blood lactate concentration [BLa^-] of 3 mmol·L^{-1}, after which increments were 25 W. The test was terminated at a [BLa^-] of 4 mmol·L^{-1} or higher. VO_2 was measured from 2 to 4.5 min in every increment and an average VO_2 was calculated during the last 2 min of this period. VO_2 was measured using a computerized metabolic system with mixing chamber (Oxycon Pro, Erich Jaeger, Hoechberg, Germany) with standard calibration procedures. Cadence was freely chosen but the participants were instructed to stay seated and keep an even cadence throughout VO_2-measures. Blood was sampled from the fingertip at the end of each 5-min bout and analysed for whole blood [BLa^-] using a lactate analyser (Biosen C_line, EKF Diagnostic, Germany). From these steady-state periods gross efficiency, referred to "the fresh state" was calculated (see gross efficiency). Power output at 4 mmol·L^{-1} [BLa^-], referred to as L_4 and the corresponding $\%VO_{2max}$ in relation to VO_{2max}, were also calculated using straight-line interpolation between the two last increments below and above 4 mmol·L^{-1} [BLa^-], respectively.

3.4.3. 6-s all-out sprint (Paper III)

After 5 min of active recovery at ~100 W, a 6-s all-out sprint was performed in the seated position with a stationary start and a resistance of 0.8 Nm·kg^{-1} body mass. Peak power output was defined as the highest value achieved during the 6-s all-out.

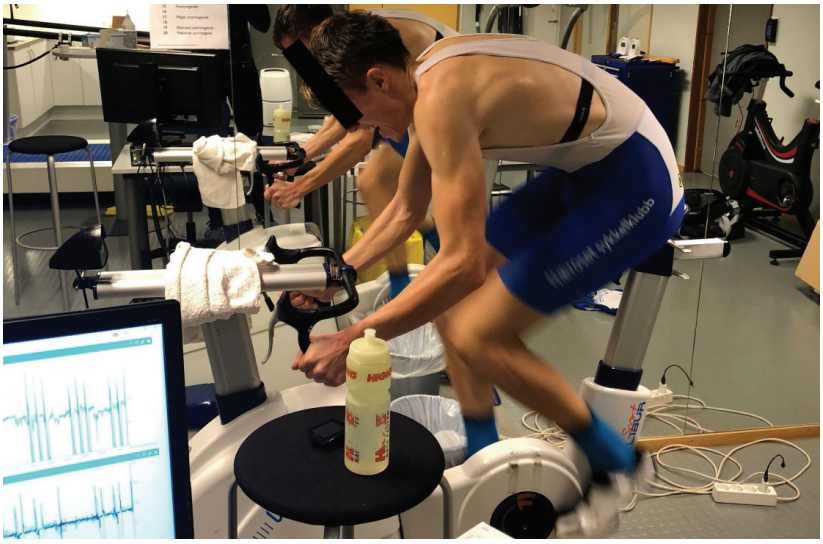

Figure 7*: Example of sprint in the seated position on a Lode Excalibur Sport electromagnetic braked cycle ergometer.*

3.4.4. VO_{2max} test (Papers I-IV)

After 10-min recovery, an incremental test to exhaustion to determine VO_{2max} was initiated with 1-min increments, starting at 200 or 250 W depending on previous results. Power output increased by 25 W every minute until the RPM dropped below 60·min^{-1} despite audible encouragement from test leader. VO_{2max} was calculated as the highest average of a 1-min moving average using 5-s VO_2-measurements. W_{max} was calculated as the mean power output during the last minute of the incremental test.

3.4.5. Prolonged cycling including repeated 30-s maximal sprints (Papers III and IV)
Ten min after the incremental test, a 60-min continuous cycling at an intensity equivalent to 60% of VO_{2max} was performed. The intensity was calculated from power output and VO_2-measures obtained on the blood lactate profile test and VO_{2max} test using straight line interpolation. Four repeated 30-s maximal sprints separated by 4 min active rest (100 W) were included between 36-50 min and the test was concluded by a self-paced 5-min or 20-min test. During sprints, the resistance was set to 0.8 $Nm \cdot kg^{-1}$ using the Wingate-modus and the test started at 80 RPM and was performed in the seated position. Strong verbal encouragement was given throughout all sprints. To avoid glycogen depletion during such prolonged exercise protocols, gels (Enervit Sport Gel, Sweden) and energy-drink (Squeezy, Norway) without caffeine was provided ad libitum after the incremental test to exhaustion and throughout (Paper III and IV). The intake of gels and beverages was recorded Pre and repeated at Post/Rec tests. Mean power output during 30-s sprints were recorded as the 30-s average power output sustained throughout every sprint. VO_2 was recorded from 34-36 min and used as a measure of GE in the "fatigued state" and participants were instructed to keep the same cadence as during the lactate profile test of similar power output. Further, VO_2 was measured throughout the 5-min test (Paper IV) and in 1-min periods during the 20-min test from 4-5, 9-10, 14-15, and 19-20 min (Paper III) and expressed relatively to VO_{2max} to calculate $\%VO_{2max}$. Recording of VO_2 started 30-s prior to every VO_2-measure to ensure steady-state measurements.

3.4.6. 5-min and 20-min tests (Papers III and IV)
Before engaging in 5-min or 20-min tests, the test leader conferred with the participant on what power output he intended to start the test on. Participants were blinded to the average power output during tests but adjusted the resistance themselves by 1-W increments. To ensure the same pacing conditions in Pre and Post settings, the initial power output was replicated, and the controller was placed next to the participant who then freely could adjust the power output. The time was always visible to the participants and strong verbal encouragement was given throughout the tests. Immediately after the tests, the participants rated their perceived exertion on the 6-20 Borg-scale (Borg et al., 1987) and 1 min after blood was sampled from the fingertip to determine $[BLa^-]$.

3.5. Measurements and calculations

3.5.1. EMG (Paper I)
To evaluate muscle fibre recruitment during prolonged exercise, EMG measurements via a wireless EMG-module (Ergotest Innovation as, Norway) using MuscleLab system (Pantaray Research Ltd. version 10.5.51.4221, Israel) was performed using surface electrodes (DUO-TRODE, Myotronics Inc, Kent, U.S.A) on m. Vastus Lateralis and m. Vastus Medialis placed according to recommendations (Konrad, 2006). Raw EMG-data were captured at 1000 Hz and smoothed using a moving average with a 20-sample window width, repeated 20 times. Integrated EMG (iEMG) was calculated as the average of the smoothed EMG data over 60 crank cycles and expressed relative to the baseline (8th – 9th min within 4 hrs of cycling). The frequency distribution to obtain median frequency was calculated in Matlab (R2016b) using its PSD routine ('periodogram' function) with default settings with a frequency resolution of 1 Hz.

3.5.2. Pedalling technique (Paper I)
Pedalling technique measurements were recorded using the Lode Ergometry Manager Software (Lode, version 10.4.5, Netherlands). The torque generated perpendicular to the crank axle was recorded at every 2°. Crank angle was referenced to 0° at the top dead center and 180° at the bottom. Angle of peak torque (in degrees) was recorded as the mean of the highest propulsive torque during the downstroke phase. Mechanical effectiveness was defined as mean of the highest resistive torque

during the upstroke phase (force acting negatively on the propulsive force) expressed relative to the mean torque (in percentage).

3.2.3. Gross efficiency (Papers I, III, and IV) and %VO_{2max} (Papers III and IV)
During periods of VO_2-measurement, participants were asked to maintain the same pedalling frequency throughout both in the fresh and fatigued state. GE was defined as the ratio between the mechanical power output, and the metabolic power input was calculated from steady-state periods, using the oxygen equivalent (Peronnet and Massicotte, 1991) and respiratory exchange ratio (RER): Power input = VO_2 $L \cdot s^{-1}$ · (4840 $J \cdot L^{-1}$ · RER + 16,890 $J \cdot L^{-1}$). Oxygen consumption was recorded and fractional utilization of VO_{2max} was calculated from VO_2-measurements obtained during the blood lactate profile test and 5-min test and expressed relatively to VO_{2max} (%VO_{2max}).

3.5.4. Hb-mass (Papers III and IV)
Participants rested for 20 min in a semi-recumbent position and Hb-mass was determined using a modified version of the carbon monoxide (CO) rebreathing technique, as described elsewhere (Siebenmann et al., 2012) using an OpCO (WGT, Austria). Briefly, the participant breathed 100% chemically pure O_2 for 3.5 min before a blood sample (125 µL) was drawn from the fingertip (Paper IV) or from the antecubital vein (Paper III) using pre-heparinized syringes (PICO50 80IU, Radiometer, DK) and immediately analyzed in quadruplicate for carboxy-Hb (%HbCO) on a hemoximeter (ABL800, Radiometer, Copenhagen, Denmark). Subsequently, the subjects rebreathed a bolus of chemically pure CO (Multigas SA, Domdidier, Switzerland) corresponding to 1.5 $mL \cdot kg^{-1}$, mixed with O_2 for 9 min 25 s. A sensor registered and regulated the O_2-level during the rebreathing. After rebreathing, blood was sampled and analyzed for %HbCO in quadruplicate. The change in %HbCO between first and second measurement was used to calculate Hb-mass and the remaining CO in the system was quantified by the OpCO. Total red blood cell volume (RBCV), total blood volume (BV), and plasma volume (PV) were calculated from Hb-mass and haematocrit (Hct) measured in blood samples collected prior to any physical tests, using the following calculations as described earlier (Siebenmann et al., 2015):

$$Hb_{mass} = 644 \times nCO_{abs} \times 25/\Delta HbCO$$

where $\Delta HbCO$ is the change in %HbCO between the blood sample before and after administration of CO-dose.

$$RBCV\ (mL) = Hb_{mass} \times Hct/[Hb]$$
$$BV\ (mL) = RBCV \times 100\ /Hct$$
$$PV\ (mL) = BV - RBCV$$

Figure 8*: Example of the CO-rebreathing technique using the OpCO with antecubital venous blood sampling.*

3.5.5. DXA (Papers III and IV)

After an overnight fast, a DXA scan on a Lunar Prodigy (GE Healthcare, Chicago, Illinois, USA) was performed to determine body mass, lean body mass and body fat percentage using the encore software (GE Healthcare v.17). All DXA-scans were performed by the same technician using standardized procedures and the technician was blinded for Pre and Post/Rec measures when analysing the images. In paper IV, a DXA-scan was performed on a subset of the participants immediately upon return from training camp but did not show any changes in body mass, lean body mass or body fat in either SPR or CON of differences between groups and were, therefore, not included in this thesis. For a detailed summary of changes in body composition, the reader is referred to Paper IV.

3.6. Muscle biopsy procedure and muscle analyses (Papers II and IV)

3.6.1. Muscle sampling and homogenization

After at least 2 hrs of fasting, participants rested for 30 min in a supine position before a muscle sample was collected from m. Vastus Lateralis of a randomized leg using the micro biopsy technique (Bard Magnum, Bard Nordic, Helsingør, Denmark), using 14-gauge needles (Medax medical devices, Poggio Rusco, Italy) under local anaesthesia (2-3 mL Lidocaine, Mylan Dublin, Ireland). The first biopsy was sampled at one third of the distance from the patella to anterior superior iliac spine with subsequent biopsies sampled approximately 2 cm proximal to the previous sample from the same leg. Biopsies were frozen within ~10 s in -80°C liquid isopentan and transferred to an Eppendorf tube for storage in a -80°C freezer. Muscle samples, ~1.0-4 mg d.w. were freeze dried in a Christ Alpha 1-2 LDplus freeze dryer, (Vakuum-Service A.S, Norway) and dissected free from blood and connective tissue before homogenization for western blotting and enzyme activity assays. For quantitative polymerase chain reaction analyses (qPCR) w.w. biopsies were used.

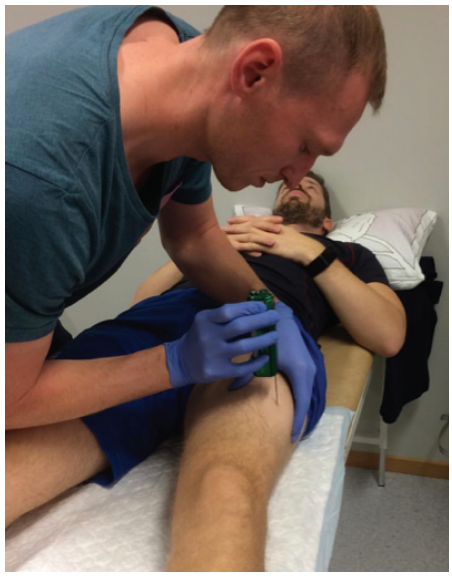

Figure 9*: Example of the micro biopsy procedure from m. Vastus Lateralis using the Bard Magnum micro biopsy gun.*

3.6.2. qPCR (Paper II)

Total RNA was extracted from muscle tissue using a combination of phase separation and silica-column clean-up. Muscle tissue (~30 mg) was homogenized in 200 µl of TRIzol® Reagent (Invitrogen, Life Technologies AS, Oslo, Norway), using 0.5 mm RNAse-free Zirconium Oxide beads and a bead homogenizer (Bullet Blender, Next Advanced, Averill Park, NY, USA), according to manufacturer's instructions, as previously described (Almquist et al., 2020). Following homogenization, TRIzol® Reagent was added to a total volume of 1 mL and the homogenate was vortexed and incubated at room temperature for 5 min, after which 200 µL of chloroform was added, followed by 3 min incubation and subsequent phase separation by centrifugation (12000 g, 10 min, 4°C). Four-hundred µL of the aqueous phase mixed with an equal volume of 100% ethanol and incubated 10 min at room temperature on a silica spin-column (Zymo-Spin™ IIC, Zymo Research, Irvin, USA). Following brief centrifugation, the flow-through was discarded and the column was washed by centrifugation once with RWT buffer and twice with RPE-buffer (Qiagen Nordic, Oslo, Norway). RNA was eluted from the column in TE buffer heated to 60°C by centrifugation. RNA purity and quantity were assessed by evaluation of absorbance at 230, 260, and 280 nm using a micro-volume spectrophotometer (Nanodrop 2000, Thermo Scientific, USA).

Samples were reverse transcribed in duplicates (500 ng total RNA) using SuperScript® IV Reverse Transcriptase (Invitrogen, Life technologies AS, Oslo, Norway), using anchored Oligo-dT and random hexamer primers (Thermo Scientific, Life Technologies AS, Oslo, Norway), according to manufacturer's instructions. Real-time RT-PCR was performed on 2 µL cDNA (1:50 dilution) in a 10 µL reaction volume, using 2X SYBR® Select Master Mix (Applied Biosystems, Life Technologies AS, Oslo, Norway) and specific primers added at a 0.5 µM final concentration, using a fast-cycling real-time detection system using Applied Biosystems™ QuantStudio 5 Real-Time PCR System (Thermo Fischer Scientific). Cycling consisted of 40 cycles; three seconds at 95°C followed by 30 seconds 60°C. Melt-curve analysis was performed for all reactions to verify single product amplification. Real-time RT-PCR parameters are presented in Table 5.

Quantification cycles (Cq) were determined using the second derivate method from raw fluorescence data, exported from the Applied Biosystems software and analyzed using the qpcR-

library (Ritz and Spiess, 2008). Primer efficiency values were estimated from single reactions and averaged for each primer pair (Ritz and Spiess, 2008). Gene abundance data was efficiency corrected and analysis was done using log-transformed values. In order to control for RNA quantity in cDNA synthesis and subsequent dilution, suitable reference genes were determined from systematic evaluation of 12 transcripts (sequences available on request) with modification for the present repeated-measures study design. Beta-2-microbulin (B2m), TATA-box-binding protein (TBP) and Peptidyl-prolyl cis-trans isomerase A (PPIA), as their abundance did not change with sampling time-points or exercise condition. Normalization of target genes were thus performed using the geometric average of these three internal reference genes as described elsewhere (Vandesompele et al., 2002). Sequences of primers are presented in Table 5.

Table 5. *Sequences of qRT-PCR primers utilized for analysis of mRNA abundance. Average primer efficiencies and Cq-values for all reactions are given. Values are mean ± SD.*

	Primers for qRT-PCR		*Efficiency*	*Cq*
Gene	*Forward primer*	*Reverse primer*	*Mean ± SD*	*Mean ± SD*
β2-m	*TGACTTTGTCACAGCCCAAGA*	*CGGCATCTTCAAACCTCCATGA*	*1.98 ± 0.01*	*23.2 ± 0.3*
CLCN1	*TTCAGCGCCTTTGTGTTTCG*	*AATCCCGATGGCAGCAAAAG*	*1.82 ± 0.01*	*29.7 ± 1.2*
IGF1	*ATGTATTGCGCACCCCTCAA*	*GTACTTCCTTCTGGGTCTTGGG*	*1.86 ± 0.01*	*28.9 ± 0.6*
MSTN	*AGGAGAAGATGGGCTGAATCC*	*CCCTTCTGGATCTTTTTGGTGTG*	*1.91 ± 0.01*	*32.4 ± 0.9*
ATP1A1	*ATCCTTGAGTACACCTGGCTTG*	*TTTCCTTGCCATGCGTTTGG*	*1.62 ± 0.01*	*32.6 ± 0.8*
ATP1B1	*ATTTTGGACTGGGCAACTCC*	*ATTTGGGCTGCAGGAGTTTG*	*2.09 ± 0.01*	*23.6 ± 0.4*
ATP1A2	*TTCCTCGGGGCTTCAAATTC*	*ATGAGCCCCACAAAGCAAAG*	*2.16 ± 0.01*	*23.1 ± 0.6*
SLC9A1	*TCCATGCAAGTGCTGTTTGG*	*TTCTTCTGTACAGGCAGCAGAG*	*1.87 ± 0.01*	*31.0 ± 0.6*
PDK4	*CCAGACCAACCAATTCACATCG*	*TTCAACTGTTGCCCGCATTG*	*2.06 ± 0.01*	*25.2 ± 2.0*
PGC1αs1	*TATGGAGTGACATCGAGTGTGC*	*ACCCAGAAAGCTGTCTGTATCC*	*1.94 ± 0.01*	*26.2 ± 0.6*
PGC1αs4	*TGTGCCATATCTTCCAGTGACC*	*TGCAGTTCCAGAGAGTTCCAC*	*2.06 ± 0.01*	*27.5 ± 1.3*
PPIA	*AAGGGTTCCTGCTTTCACAG*	*TGTGAAGTCACCACCCTGAC*	*1.66 ± 0.01*	*25.6 ± 0.4*
TBP	*AACAGGTGCTAAAGTCAGAGCAG*	*ACGTCGTCTTCCTGAATCC*	*1.87 ± 0.01*	*30.8 ± 0.4*
TFAM	*AAAGCTCAGAACCCAGATGC*	*AATCAGGAAGTTCCCTCCAACG*	*2.06 ± 0.01*	*27.0 ± 0.3*
THBS1	*AACAAACAGGTGTGCAAGCC*	*ACTTGGCGTTCTTGTTGCAG*	*1.93 ± 0.01*	*29.5 ± 2.0*
VEGFA	*CCTGCAAAAACACAGACTCG*	*CTCGGCTTGTCACATCTGC*	*1.99 ± 0.01*	*26.2 ± 0.9*

β2-m: β2 microgrobulin. CLC-1: Chloride voltage-gated channel 1. IGF1: Insulin-like growth factor 1. ATP1A1: Na^+-K^+α1. ATP1A2: Na^+-K^+α2. ATP1B1: Na^+-K^+β1. SLC9A1/NHE1: Sodium-hydrogen exchanger 1. PDK4: Pyruvate dehydrogenase kinase 4. PGC-1αs1: Peroxisome proliferator-activated receptor gamma coactivator-1α splice 1. PGC-1αs4: Peroxisome proliferator-activated receptor gamma coactivator-1α splice 4. RPL32: Ribosomal protein L32. TBP: TATA-box binding protein. TFAM: Mitochondrial transcription factor A. THBS1: Thrombospondin. VEGFA: Vascular endothelial growth factor A.

3.6.3. Western blotting (Paper IV)

Samples were homogenized for ~120 s using a plastic pestle in 80 $\mu L \cdot mg^{-1}$ fresh lysis buffer [2mM HEPES (pH 7.4), 1mM EDTA (pH 7.0), 5mM EGTA (pH 7.5), 10mM MgCl2, 1% Triton-X-100, phosphatase and protease inhibitors]. Subsequent to homogenization the samples were rotated end-over-end for 1 h and centrifuged for 10 min at 10000 g to separate undissolved tissue from the supernatant. Afterwards, the supernatant was carefully separated from the pellet and stored at -80°C until further analysis.

Protein concentration was determined using the Pierce Detergent Compatible Bradfor Assay Kit #23246. Briefly, 5 µL samples were diluted 1:10 in ddH2O and loaded in triplicates onto a 96-well micro titer plate, mixed with 250 µL Pierce Detergent Compatible Bradford Assay Reagent, and measured spectrophotometrically at 595 nm using a Multiscan FC microplate reader (Thermo Fisher Scientific), using the SkanIt software 2.5.1 for Multiscan (Thermo Scientific). Pierce Serum Albumin standards with protein concentrations ranging from 0.025 to 2.0 $mg \cdot mL^{-1}$ was used to create a standard curve. Protein concentrations were calculated from the standard curve after correction for the absorbance of the ddH2O.
The lysates were normalized to a protein concentration of 2.0 $\mu g \cdot \mu L^{-1}$ in fresh HEPES. The lysates were prepared with a 4 x Laemmli sample buffer (Bio-Rad Laboratories AB, Oslo, Norway) with 10% 2-Mercsptoethanol and heated for 5 min at 95°C. Proteins samples (15 µg of total protein) were separated at 300 V for 60 min on an Invitrogen gel (Novex™ 4-20% Tris-Glycine Plus Midi) followed by a wet transfer to a PVDF membrane (0.2 µm Immun-Blot, Bio-Rad) at 400 mA for 1 h. All samples from each subject were loaded on the same gel in technical duplicates. Membranes were then stained using a reversible total protein stain (Pierce Reversible Protein Stain, Thermo Fischer Scientific) to ensure appropriate protein transfer and to control loading. Membranes were then blocked for 1 h at room temperature in 3% Bovine Serum Albumin in Tris-buffered Saline including 0.1% Tween-20 (TBST) before an overnight incubation in primary antibody on a rocking table at 4°C. Membranes were then washed 2 x 5 min in TBST before a 1 h incubation in horseradish-peroxidase conjugated secondary antibody diluted in 5% skimmed milk in TBS-T at room temperature. The membranes were then washed 4 x 5min in TBS-T and band were visualized using chemiluminescent detection (SuperSignal, West Femto Maximum Sensitivity Substrate, ThermoFischer Scientific) and recorded with a digital camera (G:BOX, Syngene) with the software GENESys, Chemi-XR5. Band intensity was quantified using Image Lab 6.0.1 (Bio-Rad, Laboratories), adjusted for background intensity. Samples were expressed relative to total protein stain and normalized to a human standard containing equal amounts of all Pre-samples which was loaded on each gel in duplicate. The antibodies applied for these analyses were purchased from Abcam; Anti-Citrate synthase, 1:2000 (ab96600), anti-HADH, 1:8000 (ab154088), Santa Cruz Biotechnology; PFK-1, 1:500 (sc166722), and Thermo Fischer Scientific; Na^+-K^+β1, 1:1000 (MA3-930).

3.6.4. Enzyme activity (Paper IV)
CS and phosphofructokinase (PFK) activity were assayed in muscle lysates using commercially available kits (CS: CS0720, PFK: MAK093, St. Louis, MO, Sigma-Aldrich) according to the manufacturer's instructions as described previously (Meinild Lundby et al., 2018). All activities were normalized to protein concentration as described above and expressed in international $mU \cdot mg^{-1}$ protein.

3.7. Blood sampling and hormone analyses (Paper II)

After at least 2 hrs of fasting, participants rested for 30 min in a supine position before an antecubital venous blood sample was collected in 9 mL vacuettes containing Z Serum Clot Activator for serum (Greiner bio-one GmbH, Austria). Blood samples were collected before (Pre), 20-min after (Post) and 3 hrs after (3h) each experimental protocol. After 30 min, the blood was spun at 2600 rpm for 15 min on a KUBOTA 2420 (JZ4725-M000, Tokyo, Japan) and serum were separated from red blood cells, frozen and stored at -80°C until further analysis. Serum concentrations of total cortisol, testosterone, GH, sex hormone-binding globulin (SHBG) and IGF1 were measured using an Immulite 1000 ExUs edition (Siemens, USA) using kits from the Immulite Immunoassay System Menu (Siemens Medical Solutions Diagnostics, NY, USA). The ratio between free testosterone and SHBG was calculated from this.

3.8. Strength and power measures (Humac, Keiser) (Papers I, II, and IV)

3.8.1. Isokinetic knee extension (Paper II)

To evaluate recovery of muscle strength (Coutts et al., 2007) prior to and after exercise, a one-legged isokinetic knee extension test was performed Pre, Post, 3 hrs and 24 hrs after (24h) experimental protocols, using the leg that was not exposed to biopsy sampling. Isokinetic torque was evaluated at 60, 180 and 240°·s^{-1} using an isokinetic dynamometer (Humac Norm, Computer sports Medicine Inc. USA). Subjects performed 3 familiarization trials on both legs prior to experimental protocols. Peak torque of the best repetition was analysed using the software (HUMAC 2015 v.15, Computer Sports Medicine Inc).

***Figure 10**: Example of one-legged isokinetic knee extension test performed 24 hrs after the 4-h experimental protocol using the Humac Norm dynamometer.*

3.8.2. Incremental leg press test (Paper III)

After a 10-min cycling warm-up at self-selected power output (150-200 W) a predetermined 10-repetition incremental leg press test set to 250 kg for all participants on a Keiser AIR300 horizontal leg-press (Keiser Sport health equipment INC., Fresno, CA) was initiated. The Keiser AIR300 uses pneumatic resistance to measure force and velocity in each repetition. The incremental test was performed in the seated position with a 90° knee-joint angle, starting at 41 kg and increasing to 250 kg at the 10th repetition with increased and standardized rest-periods between repetitions. If the participant exceeded 250 kg, the test continued with 60-s rest between attempts until failure. The participants were instructed to push as explosively as possible until failure. The theoretical, maximal velocity (V_{max}), maximal force (F_{max}) and maximal power (P_{max}) was then calculated based on the second-order polynomial relationship between force and power (Colyer et al., 2018).

Figure 11*: Example of the incremental leg-press test using a Keiser horizontal leg-press.*

3.9. Questionnaires (Papers III and IV)

3.9.1. ABQ (Paper III)

To evaluate mental recovery, the 15-item sport-specific Athlete Burnout Questionnaire (ABQ) was used (Raedeke and Smith, 2001). Athletes were asked to rate "How often do you feel this way?" 15 different statements to evaluate their participation motives in their sport on a 5-point likert-scale from 1 = *almost never* to 5 = *almost alway*s. The ABQ has three 5-item subscales assessing three key dimensions of burnout: (1) Reduced sense of accomplishment (e.g., "It seems that no matter what I do, I don't perform as well as I should"); (2) Emotional and physical exhaustion (e.g., "I feel so tired from my training that I have trouble finding energy to do other things") and (3) Devaluation of sport participation (e.g., "The effort I spend participating in my sport would be better spent doing other things"). A total summarized score for the ABQ is achieved by averaging all three subscale scores. The questionnaires were completed at Pre and Post.

3.9.2. RESTQ and Borg-scale questionnaires (Paper IV)

To evaluate the stress-recovery state at Pre, three times during training camp as well as Rec, the short version of the Recovery-Stress Questionnaire for Athletes (RESTQ-36-R-Sport) was used (Michel Nicolas, 2016). The 36 questions are divided in 12 subscales with 3 items for each subscale. The participants filled out the questionnaire in the mornings and answered the questions on the background of the past 3 days/nights, using a 7-point scale ranging from 0 (never) to 6 (always). On the day after Sprint- or Control sessions the participants were asked after breakfast to evaluate the global intensity of yesterday's session using rate of perceived exertion (sRPE) (Foster, 1998) evaluated on a modified version (1-10) of the original Borg-scale (Borg et al., 1987) and their motivation to exercise by answering "how motivated are you to exercise today"? on a 9-point scale going from very, very motivated to very, very demotivated.

In Paper IV, the RESTQ was filled out at two occasions during the training camp, after two and four of the LIT-sessions including sprints or distance-matched LIT-sessions, respectively. However, no changes were observed in neither stress nor recovery during the training camp in SPR

or CON and there were no differences between groups. These data are, therefore, not included in the figures in the present thesis, but a detailed summary can be viewed in Paper IV.

3.10. Training load

Training load was quantified using the iTRIMP as described elsewhere (Manzi et al., 2009), by weighting exercise intensity according to an individual's own HR vs [BLa^-] relationship, calculated by line of best fit from the lactate profile and VO_{2max} test. iTRIMP uses the weighting factor y_i, which increases exponentially based on the HR vs [BLa^-] relationship to weight every HR. An accumulated iTRIMP score was calculated by the following equation:

$$\text{iTRIMP (arbitrary units (AU))} = D \text{ (min)} \times \Delta HR_{ratio} \times y_i$$

where ΔHR_{ratio} is calculated from $(HR_{work}\text{-}HR_{rest})/(HR_{max}\text{-}HR_{rest})$, and D is time spent exercising.
To clarify training intensity distribution, and calculate individual training load from each type of session, training logs were analysed and categorized based on the 3-zone model (Sylta et al., 2014) into sessions focusing on LIT (60-82% of HR_{max}), moderate-intensity training (83-87% of HR_{max}) and HIT (88-100% HR_{max}). A further categorization of the combined sprint and LIT-sessions (Sprint ex) and distance-matched LIT-sessions (Control ex) were also included.

3.11. Statistical analyses

3.11.1. Paper I

Differences in physiological variables within and between conditions were evaluated by a marginal-model approach using the SPSS-software version 23 (SPSS, IBM). Time and condition were specified as fixed effects. Repeated effects were specified by subject. A significant main effect or interaction was further evaluated by a multiple-comparison approach with Sidak adjustment. A significance level of 0.05 was applied and p-values >0.05 and <0.1 were described as tendencies. Hopkins' effect sizes (ES) (Hopkins et al., 2009) using pooled SD was calculated to compare the practical significance of differences in changes between conditions. Interpretations of the magnitude of ES were as follows: <0.2 trivial, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large and 2.0-4.0 very large difference.

3.11.2. Paper II

For Log2 fold-changes in mRNA abundance and blood hormone levels, a marginal model was applied, wherein effects of time (Pre, Post, 3h) and exercise condition (SPR, CON) and their interaction were assessed using SPSS-software version 23. Time and condition were specified as fixed effects. Repeated measures were specified by subject. To compare changes between conditions, a marginal model with baseline values as a co-variate was used. A significant main effect or interaction was further evaluated by a multiple-comparison approach with Sidak adjustment. A significance level of 0.05 was applied and data were expressed as mean ± 95% confidence interval (CI). Gene abundance data are presented as Log2 fold-change from pre-exercise ± 95% CI. Hopkins' effect sizes (ES) (Hopkins et al., 2009) using pooled SD was calculated to compare the practical significance of differences in changes between conditions. Interpretations of the magnitude of ES were as follows: <0.2 trivial, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large and 2.0-4.0 very large difference.

3.11.3. Paper III

Variables were tested for normal distribution using Shapiro-Wilk test. A mixed linear model was applied to compare relative changes between groups in physiological-, performance-, and strength measures with group (and sprint) defined as fixed effects and corrected using Pre- values as a covariate using the software SPSS v.25. To compare main effects of time a mixed linear model was applied with fixed effects defined by group and time and random effects were defined by subject.

Data are presented as mean ± SD. Whenever a significant main effect was obtained a Sidak post hoc analysis was performed with an alpha-level of 0.05. *P*-values >0.05 and <0.1 were described as tendencies. Hopkins' ES using pooled SD was calculated to highlight the practical significance of differences in performance changes between groups. Interpretations of the magnitude of ES were as follows: <0.2 trivial, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large and 2.0-4.0 very large difference (Hopkins et al., 2009).

3.11.4. Paper IV

All variables were tested for normal distribution using Shapiro-Wilk test and were log-transformed to obtain normality if not. To compare relative changes from Pre to Post between groups in physiological-, performance-, muscular and haematological measures, a mixed linear model was applied with group (and sprint) defined as fixed effects and corrected using Pre-values as a covariate using the software SPSS v.25. To compare main effects of time and group a mixed linear model was applied with fixed effects defined by group and time and random effects were defined by subject. Stress-recovery measures were tested for normal distribution by a Shapiro-Wilk test and main effects of time, group and interaction was tested using a 2-way ANOVA for repeated, dependent measures with an alpha-level of 0.05. Data are presented as mean ± SD unless otherwise stated. Whenever a significant main effect was obtained a Sidak post hoc analysis was performed with an alpha-level of 0.05 and p-values >0.05 and <0.1 were described as tendencies. Hopkins' effect sizes (ES) using pooled SD was calculated to compare the practical significance of differences in changes between conditions (Hopkins et al., 2009). Interpretations of the magnitude of ES were as follows: <0.2 trivial, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large and 2.0-4.0 very large difference.

4. Results

4.1. Acute responses to inclusion of sprints during a LIT-session

4.1.1. GE, pedalling technique, and EMG (Paper I)

GE was reduced to the same extent (p=0.809, ES: 0.3) in SPR (-1.0 ± 0.8%-points, p<0.001) and CON (-0.9 ± 0.8%-points, p<0.001) from the beginning (8-10 min) to the end (238-240 min) of the 4-h exercise (Figure 12).

The mechanical effectiveness decreased more in SPR than in CON (p=0.034, ES: 0.2, Figure 12). Specifically, only SPR decreased from the beginning to the end of exercise (SPR; -2.2 ± 2.1%-points, p=0.044 vs CON; -1.3 ± 2.4%-points, p=0.841). However, the decrease in mechanical effectiveness did not correlate with the reduction in GE in either SPR (r=0.08) or CON (r=0.22).

Angle of peak torque during the pedal stroke did not change in SPR (p=0.402) or CON (p=0.221) from beginning to end of exercise and there was no difference between conditions (p=0.128, ES: 0.1).

iEMG increased in VL (p=0.025) and VM (p<0.001) in both SPR and CON from beginning (9-10 min) to the end of exercise (238-239 min, Figure 12). There was no effect of condition in VL (p=0.261, ES: 0.2), whereas CON overall tended to increase more in VM, compared to SPR (p=0.057, ES: 0.4) and was increased more in the last hour of exercise (p=0.008 and p=0.020, respectively). Median frequency did not change during exercise in either condition in VL (SPR; -2.9±4.9 vs CON; -2.3±5.0 Hz, p=0.775) or VM (SPR: -2.7±3.4, p=1.0 vs CON: -1.3±3.4 Hz, p=0.345) and no difference between conditions was observed (VL: p=0.334, ES: 0.3, VM: p=0.207, ES: 0.1).

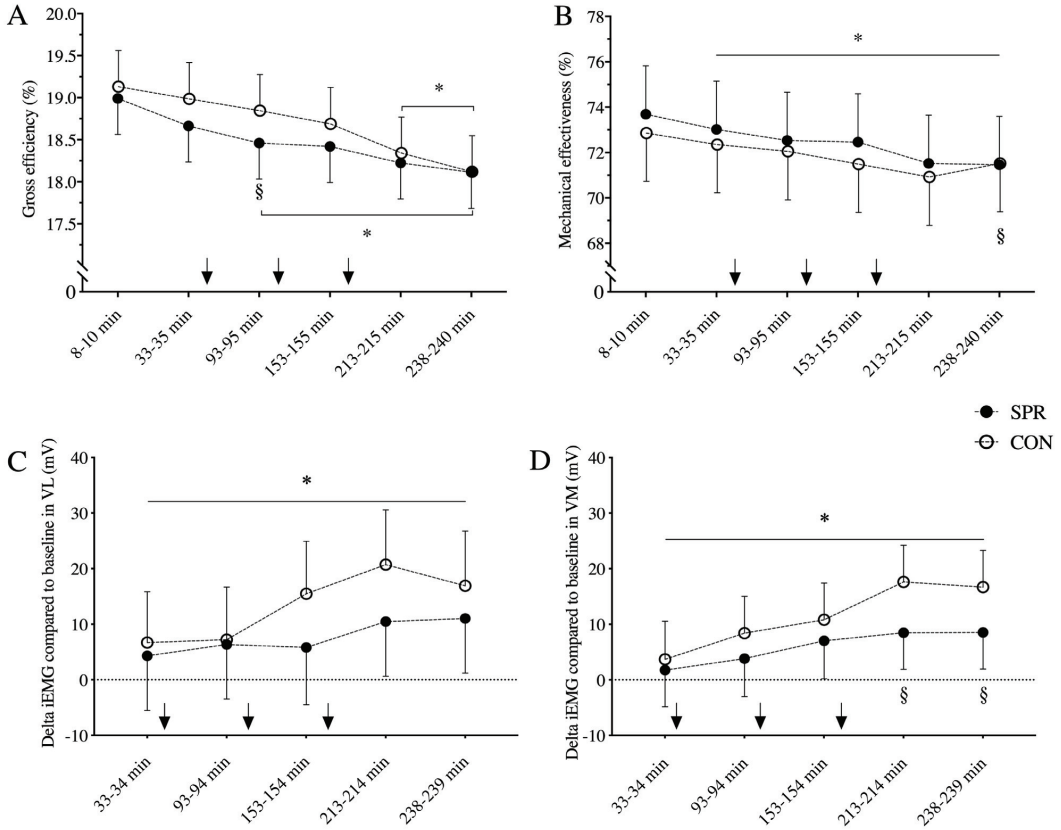

***Figure 12**: Gross efficiency (panel A), mechanical effectiveness (panel B), and changes in iEMG in vastus lateralis (panel C) and vastus medialis (panel D) measured in steady-state periods during a 4-h low-intensity session with (SPR) or without sprints (CON). Arrows indicate time points of each set of 3 x 30-s sprints during SPR. * indicates significant ($p<0.05$) effect of time compared to beginning of exercise, § indicates significant ($p<0.05$) difference in response between conditions. Mean ± 95%CI.*

4.1.2. Acute muscular responses (Paper II)

SPR led to different changes in abundance of mRNA for markers of mitochondrial function, angiogenesis, ion-transport and protein synthesis compared to CON (Figure 13A). For markers of mitochondrial function, SPR was associated with reduced PGC-1αs1 mRNA levels compared to CON (Figure 13B+C), measured as changes from Pre to Post (SPR: 1.2 ± 0.2 fold vs CON: 1.5 ± 0.3 fold, p=0.008, ES: 0.9) and from Pre to 3h (SPR: 0.9 ± 0.2 fold vs CON: 1.2 ± 0.3 fold, p=0.001, ES: 1.2). For PDK4, responses were more pronounced in SPR at both Post (SPR: 11.9 ± 8.7 fold vs CON: 2.8 ± 2.6 fold, p<0.001, ES: 2.2) and 3h (SPR: 6.1 ± 3.9 fold vs CON: 2.6 ± 2.4 fold, p=0.02, ES: 1.3, Figure 13B+C). There was no differences between conditions for TFAM (Figure 13A).

For markers of angiogenesis, VEGFA responses were more pronounced in SPR at 3h (SPR: 2.8 ± 0.6 fold vs CON: 2.3 ± 0.5 fold, p=0.014, ES: 0.9, Figure 13B+C).

For markers of ion transportation, Na^+-K^+α1 mRNA levels were lower in SPR compared to CON at 3h (SPR: 1.3 ± 0.4 fold vs CON: 2.1 ± 1.2 fold p=0.021, ES: 0.8, Figure 13B+C). CLC1 mRNA levels were lower in SPR compared to CON at 3h (SPR: 0.6 ± 0.4 fold vs CON: 1.1 ± 0.6 fold p<0.001, ES: 1.3, Figure 13B+C). Neither of the two exercise modalities affected Na^+-K^+α2 or Na^+-K^+β1 mRNA abundances and there were no differences between conditions, although Na^+-K^+α2 mRNA levels tended to be reduced in SPR compared to CON at Post (SPR: 1.0 ± 0.1 fold vs CON: 1.1 ± 0.1 fold, p=0.050, ES: 0.7).

For markers of protein synthesis, only SPR was associated with reduced myostatin mRNA abundances compared to CON at Post (SPR: 0.3 ± 0.2 fold vs CON: 1.3 ± 0.8 fold, p=0.005, ES: 1.4) and 3h (SPR: 0.3 ± 0.2 fold vs CON: 2.1 ± 2.6 fold, p=0.008, ES: 1.2), and increased MuRF1 mRNA abundances at Post (SPR: 4.5 ± 1.2 fold vs CON: 2.3 ± 0.9 fold, p=0.001, ES: 1.3, Figure 13A).

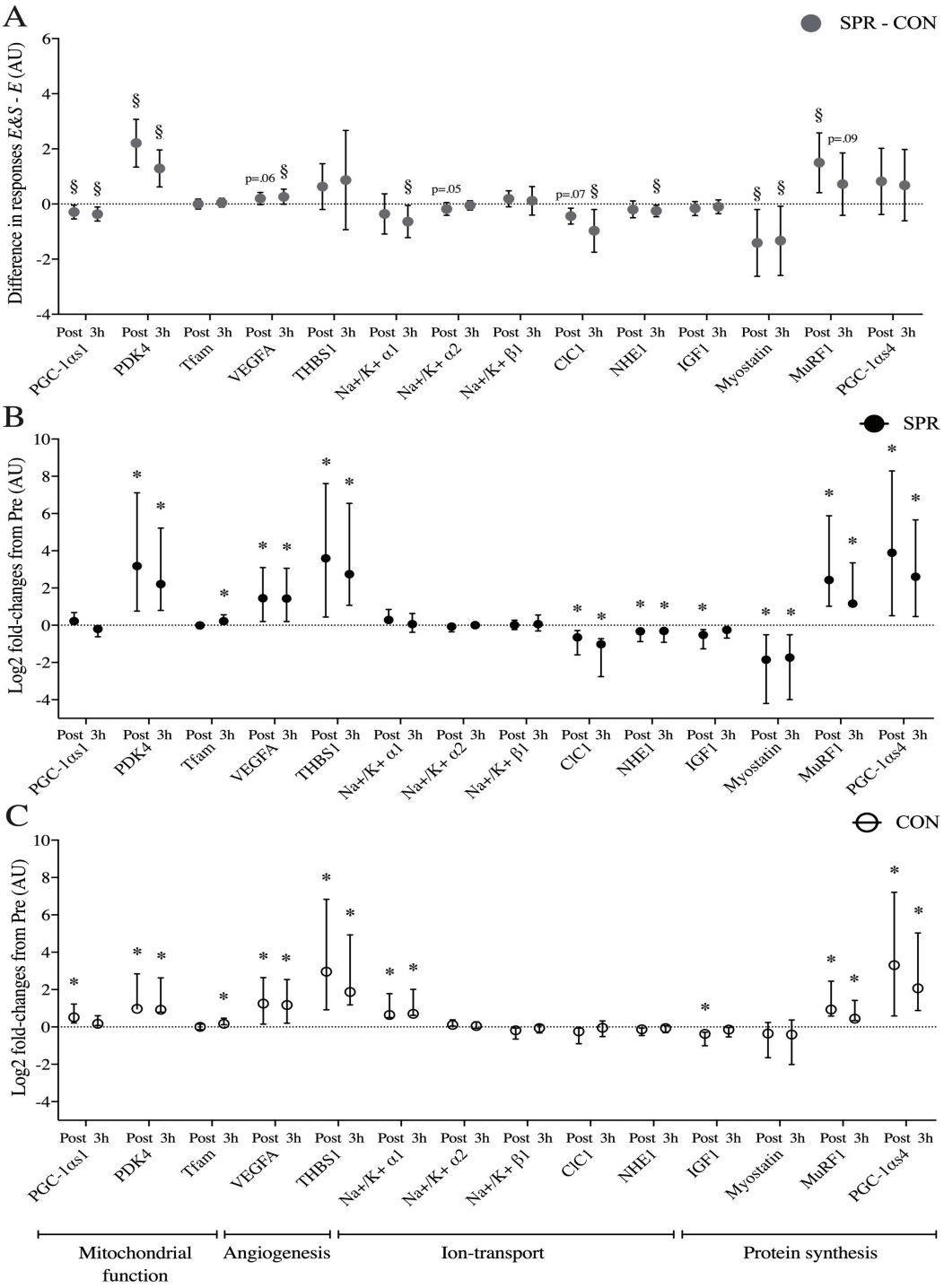
A
SPR - CON
Difference in responses E&S - E (AU)
B
SPR
Log2 fold-changes from Pre (AU)
C
CON
Log2 fold-changes from Pre (AU)
Post 3h
PGC-1αs1
PDK4
Tfam
VEGFA
THBS1
Na+/K+ α1
Na+/K+ α2
Na+/K+ β1
ClC1
NHE1
IGF1
Myostatin
MuRF1
PGC-1αs4
p=.06
p=.05
p=.07
p=.09
Mitochondrial function
Angiogenesis
Ion-transport
Protein synthesis

***Figure 13**: Effects of a 4-h low-intensity session with (SPR, panel B) or without sprint intervals (CON, panel C) on mRNA abundances of markers of mitochondrial function, angiogenesis, ion transport and protein turnover in m. vastus lateralis of elite cyclists, measured directly after (Post) and 3 hrs after exercise (3h). Panel A) Differences in responses between SPR and CON. Markers of mitochondrial function: Peroxisome proliferator-activated receptor gamma coactivator-1α splice 1 (PGC-1αs1), Pyruvate dehydrogenase kinase 4 (PDK4), Mitochondrial transcription factor A (TFAM), angiogenesis: Vascular endothelial growth factor A (VEGFA) and Thrombospondin (THBS1). Markers of ion transport: Na^+-K^+ α1 (ATP1A1), Na^+-K^+ α2 (ATP1A2) and Na^+-K^+ β1 (ATP1B1), Chloride voltage-gated channel 1 (CLC-1), Sodium-hydrogen exchanger 1 (SLC9A1/NHE1). Markers of protein synthesis regulation: Insulin-like growth factor 1 (IGF1), myostatin, muscle ring finger 1 (MuRF1), Peroxisome proliferator-activated receptor gamma coactivator-1α splice 4 (PGC-1αs4). * indicates significant ($p<0.05$) difference from pre exercise, § indicates significant ($p<0.05$) difference in response between conditions, tendencies to difference in responses are indicated by p-values. Values are log2-fold changes with 95% CI, n = 12.*

4.1.3. Acute blood lactate and hormonal responses (Paper II)

In SPR, [BLa^-] was increased after the first (16.3 ± 1.4 mmol·L^{-1}), second (15.8 ± 1.4), and third set of sprints (15.4 ± 1.4 mmol·L^{-1}), and remained elevated compared to CON until after the last set of sprints ($p<0.001$). There were no differences between conditions in [BLa^-] at the beginning of exercise, and [BLa^-] tended to be higher at the end of exercise in SPR compared to CON ($p=0.084$). In CON, [BLa^-] remained unchanged throughout exercise ($p=1.000$).

Overall, SPR and CON had limited impact on blood hormone concentrations, with only cortisol decreasing in both conditions from Pre to Post and 3h with no difference between conditions ($p=0.846$, Figure 14A). For, IGF1 and testosterone, SPR and CON resulted in similar blood concentration profiles (Figure 14A). For GH, SPR led to blunted responses at Post, contrasting the 4.0 ± 6.1 μg·L^{-1} greater increase observed in CON ($p=0.042$, Figure 14B). For SHBG, no effect was seen of either condition on blood concentrations (Figure 14A), though SHBG response was 2.3 ± 3.4 nmol·L^{-1} lower in SPR compared to CON ($p=0.035$, Figure 14B), without affecting testosterone:SHBG ratios.

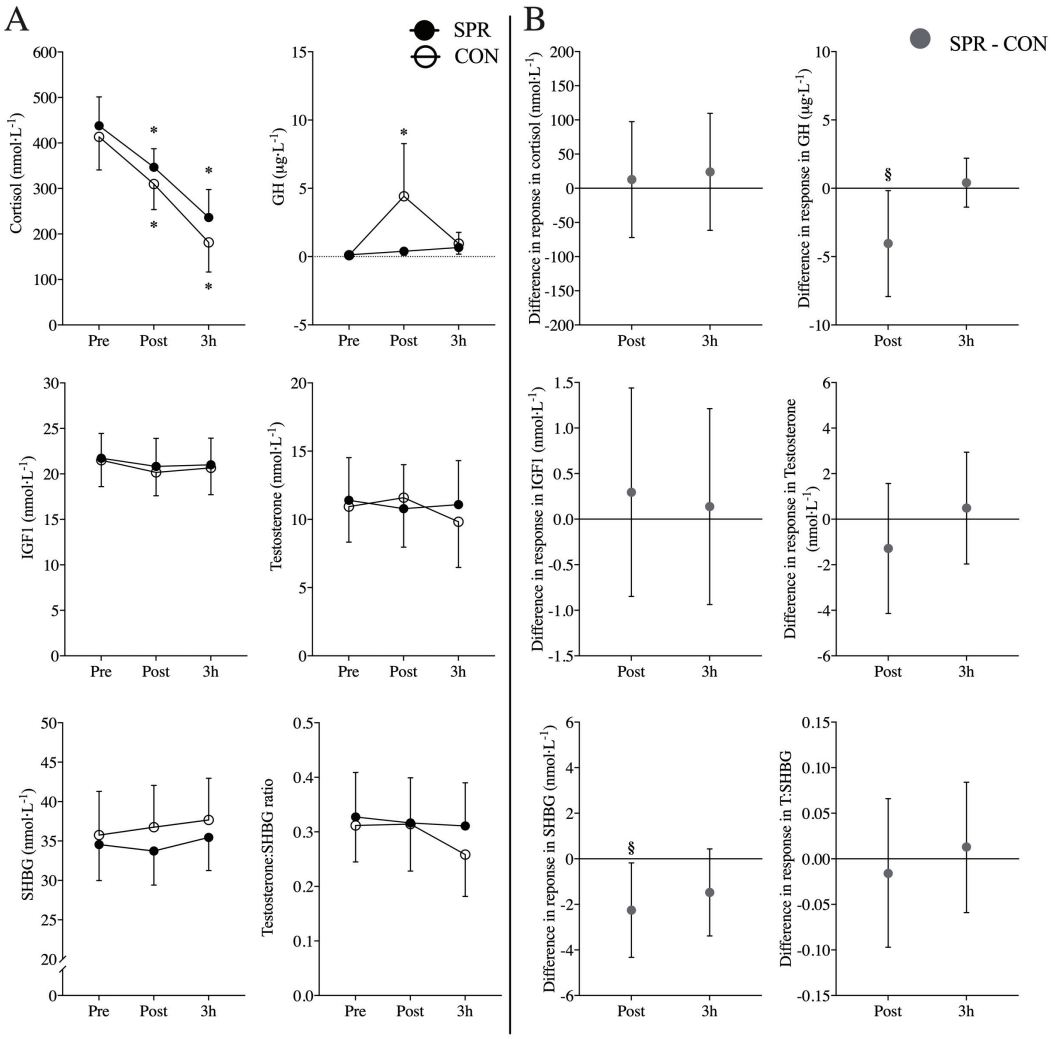

Figure 14*: Blood hormone responses to a 4-h low-intensity session with (SPR) or without sprints -(CON). A) Hormone concentrations in blood measured before (Pre), 20-min after (Post) and 3 h after exercise (3h). B) Differences in absolute changes in blood hormone concentrations between Pre and Post, and Pre and 3h (values are SPR – CON). Cortisol, Growth hormone (GH), Insulin-like growth factor 1 (IGF1), Testosterone, sex hormone-binding globulin (SHBG) and Testosterone:SHBG ratio. * indicates significant (p<0.05) difference from pre exercise, § indicates significant (p<0.05) difference in response between conditions. Mean ± 95% CI, n = 12.*

4.1.5. Isokinetic knee extension

Immediately after SPR and CON (Post), participants displayed similar reductions in isokinetic knee extension torque at 180°·s^{-1} compared to Pre ($p<0.05$, Figure 15), with no changes being evident at 60°·s^{-1} or 240°·s^{-1}. Torque at 180°·s^{-1} was recovered 3h exercise in both SPR and CON but torque at 60°·s^{-1} was lower in SPR compared to CON ($p=0.050$, ES: 0.2) and 240°·s^{-1} ($p=0.009$, ES: 0.2). For both conditions, torque was equally recovered 24 hrs after exercise at all velocities.

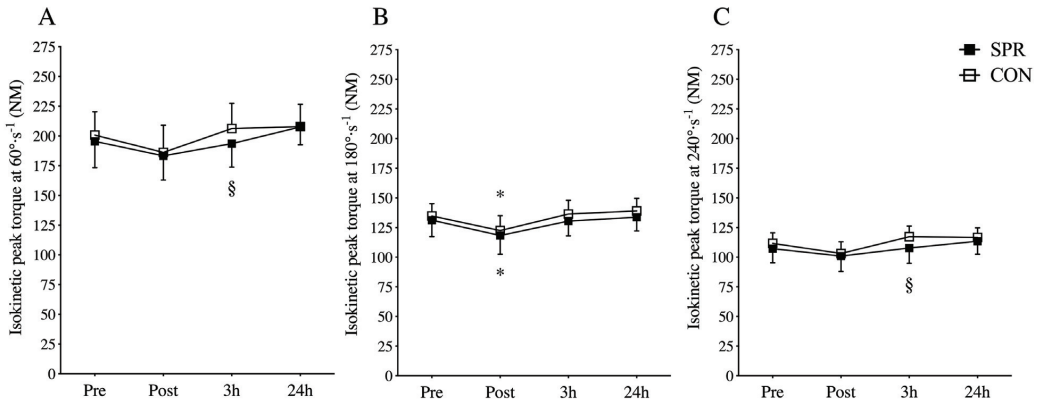

Figure 15*: Isokinetic knee extension torque before (Pre), after (Post), 3 hrs after (3h) and 24 hrs after (24h) a 4-h low-intensity session with (SPR) or without sprints (CON). Knee extension was performed at three different speeds: Panel A; 60°·s^{-1}, Panel B; 180°·s^{-1} and Panel C; 240°·s^{-1}. * indicates significant ($p<0.05$) difference from pre exercise, § indicates significant ($p<0.05$) difference between conditions. Mean ± 95% CI, n = 12.*

4.2. Adaptations to repeated inclusion of sprints during LIT-sessions

4.2.1. Sprint and endurance performances (Papers III and IV)

After the 3-wk transition period of reduced training load (Paper III), SPR had a larger increase in 30-s sprint mean power than CON from Pre to Post (8 ± 11%, p=0.010), with ES on changes between groups being moderate to large on the four sprints (ES: 0.6 – 1.7, Figure 16A). Overall, a positive effect of time was observed for 30-s sprint mean power in SPR (4 ± 5%, p=0.035) and a negative effect was observed for CON (-4 ± 5%, p<0.001). Peak power output during the 6-s all-out sprint did not change differently between groups (p=0.587, ES: 0.0) and neither SPR (Pre: 18.7 ± 2.8 vs Post: 18.8 ± 2.6 $W \cdot kg^{-1}$, p=0.921) nor CON (Pre: 17.6 ± 1.4 vs Post:17.5 ± 1.3 $W \cdot kg^{-1}$, p=0.980) changed during a transition period of reduced training load.

After the 14-d training camp of increased load and subsequent 10-d recovery period (Paper IV), 30-s sprint mean power increased 4 ± 4% more in SPR compared to CON (p=0.001, Figure 16B) and the changes were considered small to moderate between groups (ES: 0.3-1.0). On average, SPR improved the four sprints by 3 ± 2% from Pre to Rec (p<0.001) whereas CON was unchanged (-1 ± 2%, p=0.124).

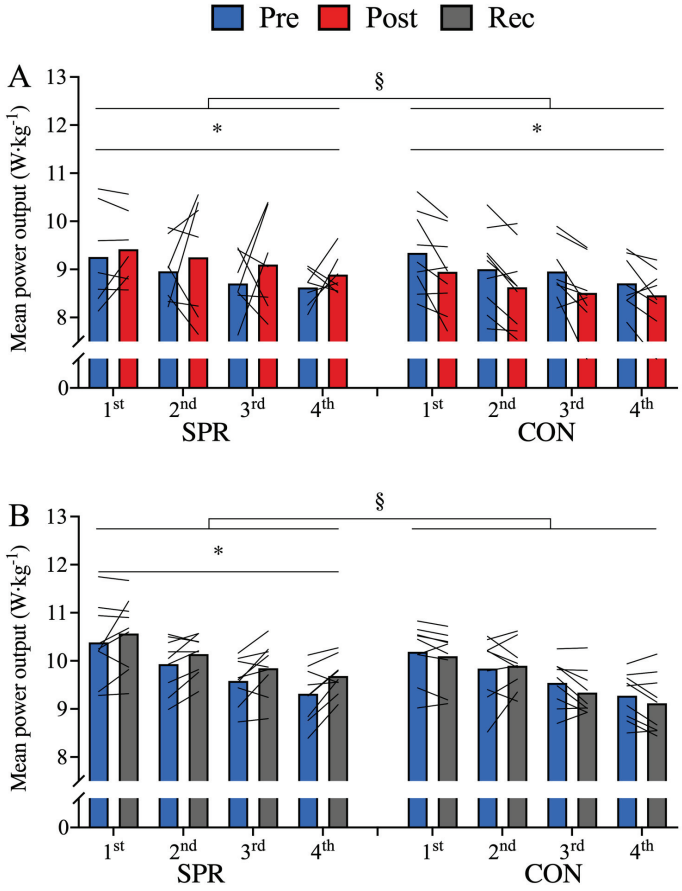

***Figure 16**: Mean power output of repeated 30-s sprints during prolonged exercise before (Pre) and after (Post/Rec) a 3-wk transition period (Panel A, Paper III) and a 14-d training camp and subsequent 10-d recovery (Panel B, Paper IV) including sprints during LIT-sessions (SPR) or performing LIT only (CON). * indicates main effect of time (p<0.05). § indicates main effect of group on changes from Pre to Post/Rec (p<0.05). Mean and individual values.*

After the 3-wk transition period (Paper III), 20-min power did not change differently between SPR and CON (p=0.633, ES: 0.1, Figure 17A). However, 20-min power was maintained from Pre to Post in SPR (-1 ± 5%, p=0.374), whereas a small decline was observed in CON (-3 ± 5%, p=0.039).

After the 14-d training camp and subsequent 10-d recovery (Paper IV), the 5-min power was increased more in SPR compared to CON from Pre to Rec (4 ± 8%, p=0.045, ES: 0.5, Figure 17A). However, neither SPR (2 ± 4%, p=0.144), nor CON (-2 ± 4%, p=0.144) changed from Pre to Rec.

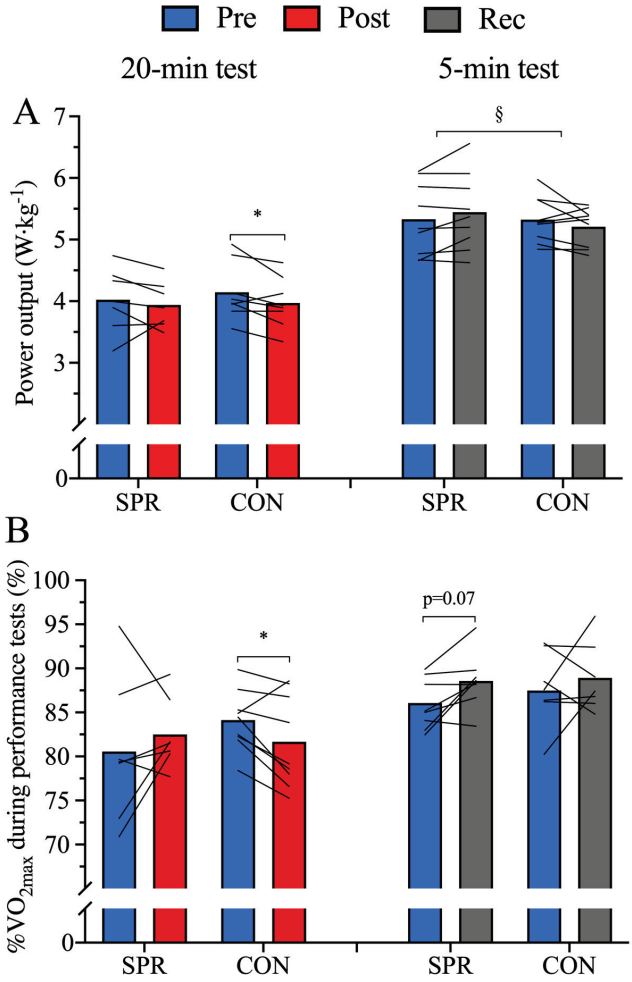

***Figure 17**: 20-min and 5-min mean power (Panel A) and fractional utilization of VO_{2max} (Panel B) after a 3-wk transition period (left panels, Paper III) and a 14-d training camp and subsequent 10-d recovery (right panels, Paper IV) including sprints during LIT-sessions (SPR) or performing LIT only (CON). * indicates main effect of time (p<0.05) and tendencies are denoted with p-values. § indicates main effect of group on changes from Pre to Rec (p<0.05). Mean and individual values.*

4.2.2. Performance-related measures (Papers III and IV)

%VO_{2max}, L_4, and body composition

After the 3-wk transition period (Paper III), %VO_{2max} during the 20-min test, was not changed differently between groups (p=0.185), but the difference in change was considered moderate (ES: 0.8, Figure 17B). Specifically, SPR maintained utilization from Pre to Post (1.9 ± 6.1 %- points, p=0.175) whereas CON decreased moderately (-2.5 ± 2.9 %-points, p=0.015). Relative power output at L_4 decreased similarly in SPR (-4 ± 4%) and CON (-5 ± 5%) from Pre to Post (p=0.825, ES: 0.1, Table 6). %VO_{2max} at L_4 did not change differently between groups but the ES was considered moderate (p=0.159, ES: -1.0). Specifically, SPR maintained %VO_{2max} at L_4 (p=0.692) while CON tended to decrease (p=0.092). Body mass increased similarly between groups from Pre to Post (p=0.925, ES: 0.0). Specifically, body mass tended to increase in SPR (p=0.069) and increased in CON from Pre to Post (p=0.046). Lean body mass and body fat did not change differently between groups (p=0.259, p=0.840, respectively), however, lean body mass decreased in CON (p=0.011) while lean body mass and body fat were unaltered in SPR. There was no difference in changes between groups in VO_{2max} (p=0.426) or W_{max} (p=0.496) and both groups remained unchanged from Pre to Post.

***Table 6**: Performance-related measures during blood lactate profile test and VO_{2max} test and body composition before (Pre) and after (Post) 3-wk transition period (Paper III) including sprints during LIT-sessions (SPR) or performing LIT only (CON). For DXA-measures before and after decreased training load, n=4 in SPR and n=5 in CON.*

	SPR				CON			
	Pre *n*=7	Post *n*=7	%Δ	*p*	Pre *n*=9	Post *n*=9	%Δ	*p*
VO_2 at L_4 ($mL \cdot min^{-1}$)	4350 ± 634	4330 ± 725	-0.5 ± 8.5	0.873	4480 ± 593	4276 ± 578	-4.4 ± 5.9	0.089
%VO_{2max} at L_4 (%)	80.6 ± 5.8	81.6 ± 6.5	1.0 ± 6.6	0.692	85.6 ± 3.9	81.7 ± 6.4	-3.9 ± 6.6	0.092
Power output at L_4 (W)	328 ± 66	316 ± 61	-3.5 ± 5.1	0.072	321 ± 41	308 ± 42*	-4.0 ± 5.0	0.033
Power output at L_4 ($W \cdot kg^{-1}$)	4.4 ± 0.5	4.2 ± 0.4*	-4.1 ± 3.5	0.021	4.4 ± 0.4	4.2 ± 0.4*	-4.8 ± 5.2	0.005
VO_{2max} ($mL \cdot min^{-1}$)	5396 ± 678	5278 ± 537	-1.7 ± 5.0	0.264	5222 ± 532	5236 ± 570	0.3 ± 5.0	0.879
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	73.4 ± 4.9	71.4 ± 4.0	-2.5 ± 5.7	0.172	71.3 ± 4.5	71.0 ± 4.8	-0.5 ± 4.0	0.771
W_{max} (W)	439 ± 58	445 ± 49	1.7 ± 5.0	0.543	442 ± 48	440 ± 39	-0.1 ± 5.0	0.811
W_{max} ($W \cdot kg^{-1}$)	6.0 ± 0.3	6.0 ± 0.3	1.1 ± 6.5	0.682	6.0 ± 0.5	6.0 ± 0.4	-0.9 ± 4.9	0.576
Body mass (kg)	73.6 ± 9.0	74.2 ± 9.4	0.7 ± 1.0	0.069	73.1 ± 4.8	73.7 ± 4.9*	0.8 ± 1.0	0.046
Lean body mass (kg)	63.0 ± 6.1	61.6 ± 6.9	-2.3 ± 2.8	0.291	63.6 ± 6.1	60.3 ± 5.7*	-5.1 ± 4.3	0.011
Body fat (%)	11.0 ± 1.5	11.6 ± 1.4	0.6 ± 0.7	0.268	12.1 ± 5.3	12.6 ± 5.9	0.5 ± 1.2	0.293

%VO_{2max} at L_4: *Fractional utilization of VO_{2max} at [BLa^-] 4 $mmol \cdot L^{-1}$. VO_{2max}: Maximal oxygen uptake. W_{max}: Maximal aerobic power output. * indicates main effect of time (p<0.05) with respective p-values denoted. § indicates main effect of group on changes from Pre to Post (p<0.05). Mean ± SD.*

After the 14-d training camp and subsequent 10-d recovery (Paper IV), %VO_{2max} during the 5-min test, did not change differently between groups from Pre to Rec (p=0.814, ES: 0.5, Figure 17B), but tended to increase in SPR (p=0.068), whereas it remained unchanged in CON (p=0.352). No differences were observed for any measure from the blood lactate profile test or VO_{2max} test or in body composition between SPR and CON from Pre to Rec and neither group changed from Pre to Rec (Table 7). However, VO_2 at L_4 increased in both SPR (p=0.036) and CON (p=0.031) from Pre to Rec but the change was not different between groups (p=0.860, ES: 0.0).

***Table 7**: Performance-related measures during blood lactate profile test and VO_{2max} test and body composition before (Pre) and after (Rec) a 14-d training camp and subsequent 10-d recovery period (Paper IV) including sprints during LIT-sessions (SPR) or performing LIT only (CON).*

	SPR				CON			
	Pre *n*=9	Rec *n*=9	%Δ	*p*	Pre *n*=9	Rec *n*=9	%Δ	*p*
VO_2 at L_4 ($mL \cdot min^{-1}$)	4394 ± 555	4494 ± 50*	2.5 ± 3.3	0.031	4423 ± 385	4520 ± 399*	2.2 ± 3.0	0.036
$\%VO_{2max}$ at L_4 (%)	79.3 ± 3.2	79.9 ± 4.5	0.7 ± 3.7	0.596	79.5 ± 5.4	80.9 ± 4.9	1.4 ± 3.7	0.269
Power output at L_4 (W)	329 ± 47	332 ± 35	1.3 ± 4.1	0.650	330 ± 42	336 ± 32	2.3 ± 4.1	0.329
Power output at L_4 ($W \cdot kg^{-1}$)	4.5 ± 0.3	4.5 ± 0.2	0.4 ± 3.7	0.976	4.4 ± 0.4	4.5 ± 0.3	1.5 ± 3.7	0.376
VO_{2max} ($mL \cdot min^{-1}$)	5538 ± 631	5629 ± 639	1.8 ± 5.4	0.359	5564 ± 370	5594 ± 495	0.5 ± 5.4	0.760
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	75.4 ± 5.2	75.9 ± 6.7	0.6 ± 6.7	0.666	75.1 ± 5.4	75.0 ± 6.7	-0.1 ± 6.7	0.985
W_{max} (W)	476 ± 49	479 ± 43	1.0 ± 3.2	0.521	475 ± 41	477 ± 39	0.5 ± 3.2	0.734
W_{max} ($W \cdot kg^{-1}$)	6.5 ± 0.5	6.5 ± 0.4	-0.2 ± 2.8	1.000	6.4 ± 0.3	6.4 ± 0.4	-0.1 ± 2.8	0.857
Body mass (kg)	74.1 ± 7.5	74.2 ± 8.1	0.0 ± 1.8	0.999	74.4 ± 4.9	74.5 ± 4.7	0.2 ± 1.8	0.994
Lean body mass (kg)	62.0 ± 6.2	62.1 ± 5.1	0.0 ± 1.8	1.000	60.8 ± 4.1	60.6 ± 3.8	-0.3 ± 1.8	0.936
Body fat (%)	12.6 ± 1.5	12.6 ± 2.1	0.0 ± 1.1	0.999	14.6 ± 2.6	15.0 ± 3.1	0.4 ± 1.1	0.732

*$\%VO_{2max}$ at L_4: Fractional utilization of VO_{2max} at $[BLa^-]$ 4 $mmol \cdot L^{-1}$. VO_{2max}: Maximal oxygen uptake. W_{max}: Maximal aerobic power output. * indicates main effect of time ($p<0.05$) with respective p-values denoted. § indicates main effect of group on changes from Pre to Rec ($p<0.05$). Mean ± SD.*

GE in the fresh and semi-fatigued state

After the 3-wk transition period, GE in fresh and semi-fatigued state did not change differently between SPR and CON from Pre to Post (Table 8). However, before the transition period, SPR experienced a decrease in GE from fresh to semi-fatigued state (-1.0 ± 1.0%-points, p=0.016), but this was not the case after the transition period and no other changes were observed between groups or states. After the 14-d training camp and subsequent 10-d recovery GE in fresh and semi-fatigued state did not change differently between groups or from Pre to Rec (Table 8). However, after the training camp, GE tended to decrease in the fresh (p=0.078) and decreased in the semi-fatigued state (p=0.005) only in CON from Pre to Rec.

***Table 8**: Gross efficiency (GE) in the fresh state during the blood lactate profile test and in the semi-fatigued state during the 60 min continuous cycling, measured before (Pre) and after the 3-wk transition period (Post, upper panel, Paper III) and the 14-d training camp (Rec, lower panel, Paper IV) including sprints during LIT-sessions (SPR) or performing LIT only (CON).*

	SPR				CON			
Paper III	Pre *n*=7	Post *n*=7	Δ	*p*	Pre *n*=9	Post *n*=9	Δ	*p*
GE fresh (%)	19.9 ± 1.0	19.5 ± 1.0	-0.4 ± 1.0	0.276	19.1 ± 1.0	19.2 ± 1.0	0.1 ± 1.0	0.699
GE semi-fatigued (%)	18.9 ± 1.0	18.9 ± 1.0	-0.1 ± 1.0	0.884	19.1 ± 1.0	19.3 ± 1.0	0.2 ± 1.1	0.568
Paper IV	Pre *n*=9	Rec *n*=9	Δ	*p*	Pre *n*=9	Rec *n*=9	Δ	*p*
GE fresh (%)	19.7 ± 0.7	19.5 ± 0.8	-0.2 ± 0.6	0.354	19.8 ± 0.5	19.4 ± 0.6	-0.4 ± 0.6	0.078
GE semi-fatigued (%)	19.1 ± 0.8	18.9 ± 0.7	-0.4 ± 0.6	0.225	19.4 ± 0.7	18.8 ± 0.7*	-0.7 ± 0.6	0.005

*** indicates main effect of time ($p<0.05$) with respective p-values denoted. Mean ± SD.*

Haematological measures

After the 3-wk transition period (Paper III), neither of the haematological measures changed differently between SPR and CON and both groups were unchanged from Pre to Post (Figure 18A-D). After the 14-d, training camp and subsequent 10-d recovery (Paper IV), neither BV or PV changed differently between groups and was unaltered from Pre to Post and Rec (Figure 18B+C). Hb-mass did not change differently between groups (p=0.157, ES: 0.1) and was unaltered in SPR, whereas it tended to increase in CON from Pre to REC (2.3 ± 3.1%, p=0.067, Figure 18A). RBCV increased in both groups (p=0.003) and the increase was not different between groups (p=0.394, ES: 0.1). Specifically, RBCV tended to increase in SPR (2.6 ± 4.7%, p=0.065) and increased in CON (3.9 ± 4.5%, p=0.023, Figure 18D) from Pre to Rec.

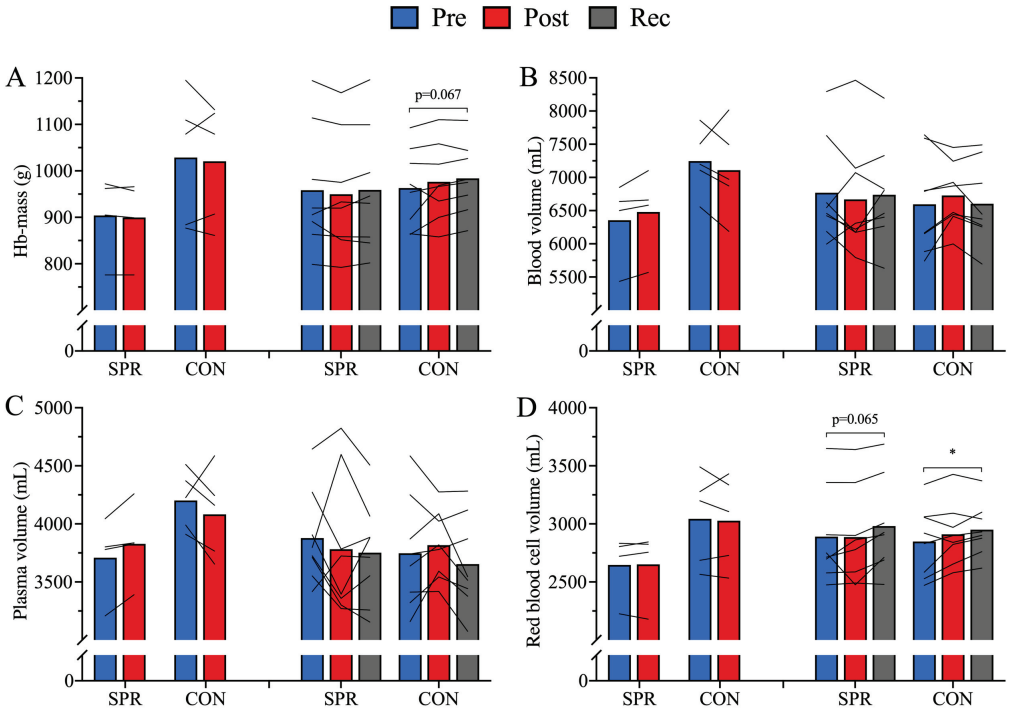

Figure 18*: Panel A: Haemoglobin mass (Hb-mass), panel B: Blood volume (BV), panel C: Plasma volume (PV) and panel D: Red blood cell volume (RBCV) before (Pre) and after (Post) a 3-wk transition period (left-side panels, Paper III) and before (Pre), after (Post) and after 10-d recovery (Rec) from a 14-d training camp (right-side panels, Paper IV) including sprints during LIT-sessions (SPR) or performing LIT only (CON). * indicates main effect of time (p<0.05) and tendencies are denoted with p-values. Mean and individual values.*

Skeletal muscle adaptations

After the 14-d training camp (Paper IV), protein contents of CS (p=0.12, ES: 0.6), PFK (p=0.70, ES: 0.4) and HAD (p=0.95, ES: 0.3) did not change differently between SPR and CON (Figure 19A-C). Specifically, protein content of CS was unchanged in SPR (2 ± 18%, p=0.960), whereas it tended to decrease in CON (-9 ± 8%, p=0.062, Figure 19A) from Pre to Post. Protein content of PFK was reduced in both SPR (-14 ± 13%, p=0.023) and CON (-17 ± 12%, p=0.002, Figure 19B) from Pre to Post. For HAD, the protein content was unchanged in both SPR (-1 ± 33%, p=0.584) and CON (5 ± 38%, p=0.969, Figure 19C) from Pre to Post. Protein content of Na^+-K^+ β1 decreased more in CON compared to SPR from Pre to Post (-8 ± 14%, p=0.035, ES: 0.6, Figure 19D). Specifically, in SPR,

protein content of Na^+-K^+ β1 was maintained (2 ± 7%, p=0.526) ,whereas it decreased in CON (-6 ± 7%, p=0.021). The enzyme activity of CS (p=0.158, ES: 0.6) and PFK (p=0.955, ES: 0.6) did not change differently between groups and were not changed in either SPR (CS: 20 ± 40%, p=0.281, PFK: 7 ± 30%, p=0.366) or CON (CS: -2 ± 17%, p=0.783, PFK: -6 ± 10%, p=0.372) from Pre to Post (Figure 19E+F).

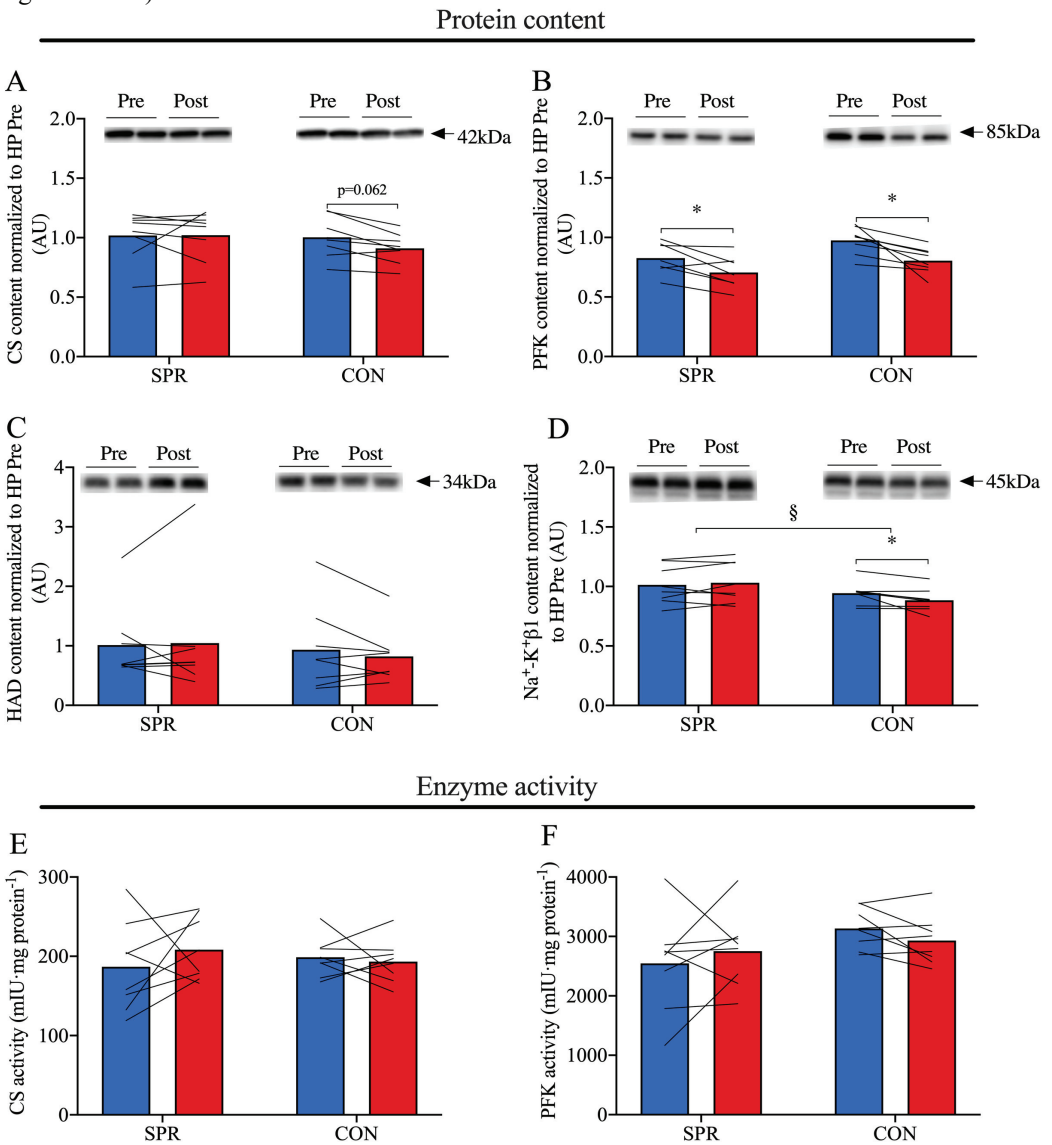

***Figure 19**: Muscle protein quantity of; Panel A: Citrate synthase (CS), panel B: β-hydroxyacyl (HAD), panel C: phosphofructokinase (PFK), panel D: Sodium-potassium pump β1 (Na^+-K^+ β1) and enzyme activity of; panel E: Citrate synthase (CS), panel F: phosphofructokinase (PFK) quantified before and after a 14-d training camp (Paper IV) including sprints during LIT-sessions (SPR, n=8) or performing LIT only (CON, n=8). Individual band-intensities were expressed relative to total protein stain and normalized to a human pool (HP) containing equal amounts of all Pre-samples. * indicates main effect of time (p<0.05) and tendencies are denoted with p-values. § indicates main effect of group on changes from Pre to Post (p<0.05). Mean ± 95% CI.*

4.2.3. Leg extension strength (Paper III)

After the 3-wk transition period (Paper III), strength-related variables V_{max} (p=0.127, ES: 0.5), F_{max} (p=0.645, ES: 0.3), and $P_{max,}$ (p=0.362, ES: 0.2) did not change differently between SPR and CON (Table 9). Specifically, F_{max} and $P_{max,}$ did not change within either group from Pre to Post, but V_{max} was increased by 14 ± 19% from Pre to Post in CON only (p=0.018).

Table 9*: Strength parameters measured using Keiser leg extension apparatus from before (Pre) to after (Post) a 3-wk transition period (Paper III) in elite cyclists including sprints during a low-intensity training session once a week (SPR, n=7) or only performing low-intensity training (CON, n=9).*

	SPR			CON		
	Pre	Post	*p*	Pre	Post	*p*
V_{max} ($M \cdot S^{-1}$)	4.0 ± 0.8	4.1 ± 0.9	p=0.870	3.8 ± 08	4.2 ± 0.5*	p=0.018
F_{max} (N)	3030 ± 441	2971 ± 528	p=0.742	3400 ± 902	3095 ± 725	p=0.108
P_{max} (W)	1516 ± 332	1524 ± 460	p=0.880	1553 ± 251	1611 ± 310	p=0.247

*V_{max}: Maximal velocity. F_{max}: Maximal force. P_{max}: Maximal power. * indicates main effect of time (p<0.05) with respective p-values denoted. Mean ± SD.*

4.2.4. Mental stress/recovery, ratings of sRPE, and motivation to exercise (Papers III and IV)

After the 3-wk transition period (Paper III), mental recovery, measured by total burnout score, did not change differently (p=0.493) between SPR and CON and both groups were unchanged from Pre to Post (Figure 20A). However, for the subscale "reduced sense of accomplishment" the groups tended to change differently (p=0.085, Figure 20B). SPR did not change (Pre: 2.3 ± 0.5 vs Post: 2.2 ± 0.5, p=0.622) but CON increased from "rarely" towards "sometimes" (Pre: 2.5 ± 0.7 vs Post: 2.8 ± 0.5, p=0.040). For "devaluation", the groups tended to change differently (p=0.085) but neither SPR (Pre: 1.6 ± 0.6 vs Post: 1.8 ± 0.7, p=0.263) nor CON (Pre: 1.7 ± 0.4 vs Post: 1.5 ± 0.4, p=0.151) changed from Pre to Post (Figure 20C). No group-differences or within-group changes were observed for "emotional and physical exhaustion" (Figure 20D).

After the 14-d camp and subsequent 10-d recovery, total stress and total recovery was not differently affected by SPR and CON and did not change in either group (Figure 20E+F). After the 14-d training camp, the participants were asked to rate the sRPE of yesterday's sprint- or control-session and their motivation to exercise the following day. SPR rated the workout as heavier or tending to be heavier compared to CON after the first four workouts (p=0.002, p=0.001, p=0.001, and p=0.077, respectively, Figure 20G), while after the 5th (p=0.401) workout, the two training modalities were rated to be equally strenuous. In SPR, sRPE decreased from the first to 4th (p=0.042) and 5th (p=0.002) workout. Motivation to exercise was not different between SPR and CON on the morning after sprint- or control-workout, though it decreased in CON (p=0.014) from the first to the last workout, whereas motivation was maintained at a high level during the training camp in SPR (Figure 20H).

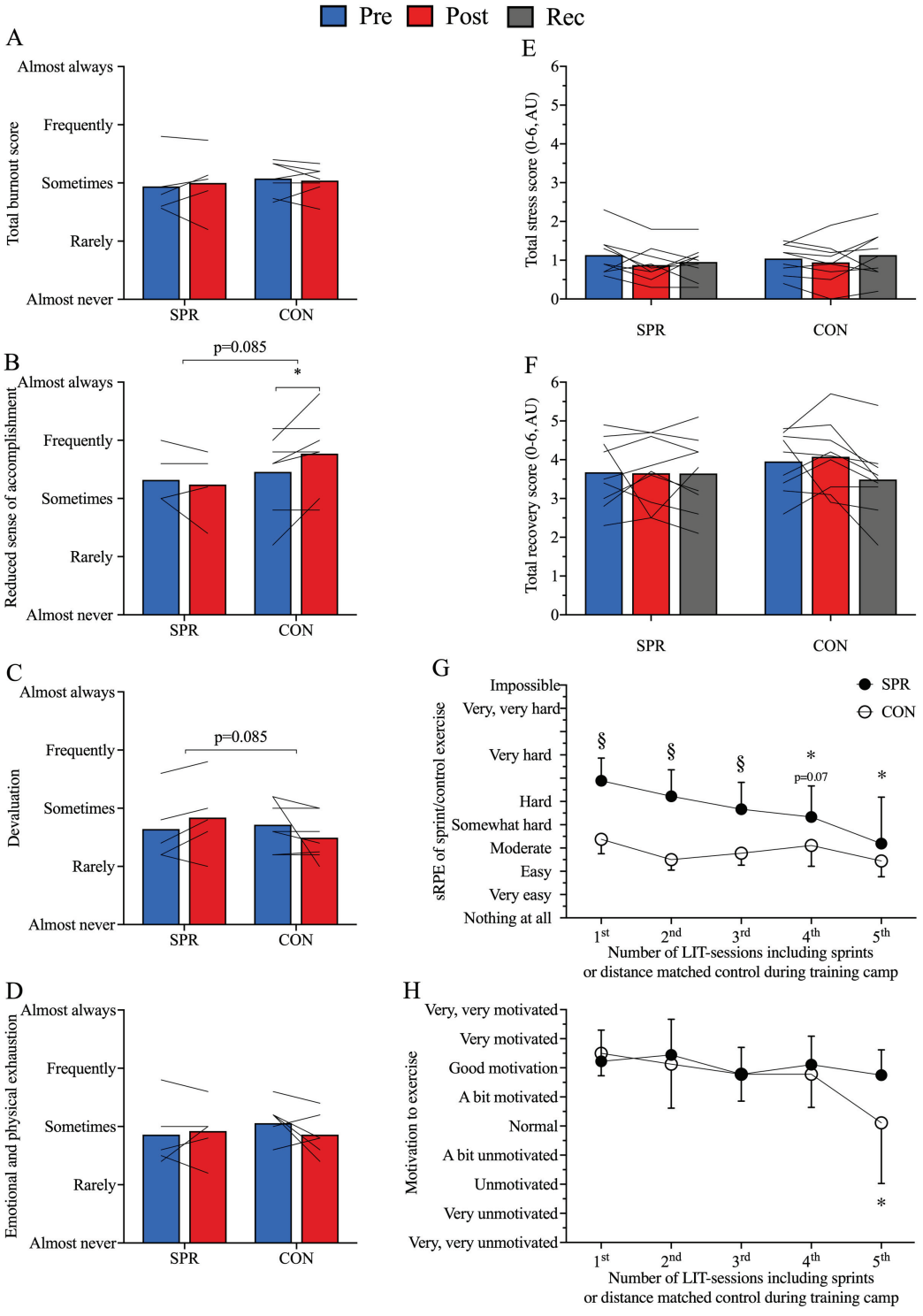
Pre
Post
Rec
A
Almost always
Frequently
Sometimes
Rarely
Almost never
Total burnout score
SPR
CON
B
p=0.085
*
Reduced sense of accomplishment
C
Devaluation
D
Emotional and physical exhaustion
E
Total stress score (0-6, AU)
F
Total recovery score (0-6, AU)
G
Impossible
Very, very hard
Very hard
Hard
Somewhat hard
Moderate
Easy
Very easy
Nothing at all
sRPE of sprint/control exercise
§
p=0.07
1st
2nd
3rd
4th
5th
Number of LIT-sessions including sprints
or distance matched control during training camp
H
Very, very motivated
Very motivated
Good motivation
A bit motivated
Normal
A bit unmotivated
Unmotivated
Very unmotivated
Very, very unmotivated
Motivation to exercise

***Figure 20**: Panel A: Total burnout score, panel B: Reduced sense of accomplishment, panel C: Devaluation, panel D: Emotional and physical exhaustion before (Pre) and after (Post) a transition period (left-side panels, Paper III) including sprints during LIT-sessions (SPR, n=5) or performing LIT only (CON, n=7). Panel E: Total stress score, panel F: Total recovery before (Pre), after (Post) and after 10-d recovery (Rec) from a 14-d training camp (right-side panels, Paper IV) including sprints during LIT-sessions (SPR, n=9) or performing LIT only (CON, n=9). Panel G: Yesterday's session's rate of perceived exertion (sRPE), H: Motivation to exercise. * indicates main effect of time ($p<0.05$). § indicates main effect of group and tendencies are denoted with p-values. Mean and individual values. For sRPE and Motivation to exercise, values are mean ± 95%CI.*

5. Discussion

5.1. Main findings

In Papers I and II, the acute responses to inclusion of 30-s sprints during a 4-h LIT-session (SPR) were compared to a work-matched LIT-session (CON). In Paper I, the inclusion of sprints temporarily changed pedalling technique and muscle activity patterns, but this did not affect GE, which decreased ~1% in both SPR and CON from the start to the end of the session. Also, repeated sprint performance was maintained during prolonged cycling in SPR. In Paper II, SPR induced greater mRNA responses for markers of fat metabolism (PDK4), angiogenesis (VEGFA) and protein turnover (MuRF1 and Myostatin) in m. Vastus lateralis compared to CON, but decreased mRNA levels for markers of ion transport (Na^+-$K^+\alpha1$, CLC1, and NHE1), and mitochondrial biogenesis (PGC-1α). Hormonal responses to a LIT-session of habitual duration were overall small in elite cyclists and similar in SPR and CON. However, SPR induced lower responses of GH and SHBG compared to CON, indicating a generally low endocrine stress response. Recovery of muscle strength, measured as isokinetic knee extension torque, was reduced 3 hrs after exercise in SPR compared to CON but was equally and fully recovered 24 hrs after both conditions.

In Papers III and IV, the adaptations to inclusion of sprints during LIT-sessions were investigated in two ~3-wk interventions of habitual changes om total training load (i.e., decreased training load (Paper III), and increased training load (Paper IV), respectively. In Paper III, SPR improved 30-s sprint mean power 8% more than CON from Pre to Post. In addition, 20-min power and fractional utilization of VO_{2max} (%VO_{2max}) were maintained from Pre to Post in SPR, while CON reduced these variables, but was not different from SPR. SPR and CON reduced power output at L_4 by the same extent from Pre to Post. However, VO_{2max}, W_{max}, and total burnout were not affected by the substantially reduced training load in any of the groups. In Paper IV, SPR improved 30-s sprint and 5-min mean power ~4% more than CON from Pre to Rec. Furthermore, protein content of Na^+-$K^+\beta1$ was maintained from Pre to Post in SPR, whereas it decreased 8% more in CON compared to SPR. No other differences were observed between groups in protein abundance or enzyme activity. The increased training load during the training camp was associated with similar increases in RBCV and VO_2 at L_4 in both groups from Pre to Rec. However, no changes occurred in %VO_{2max} at L_4, VO_{2max}, W_{max} or other haematological measures and stress and recovery measures were not affected by the intervention in any of the groups.

5.2. Acute responses to inclusion of sprints during a LIT-session

5.2.1. GE, pedalling technique and EMG

Paper I showed that GE decreased during a 4-h LIT-session in elite cyclists both when including sprints (SPR) and when cycling at low intensity only (CON), indicating that the prolonged duration of exercise is mainly responsible for the reduced GE. This is supported by the findings of earlier studies where VO_2 increases progressively during prolonged low-intensity exercise (2-3 hrs) in untrained to well-trained subjects (Hopker et al., 2017, Ronnestad et al., 2011, Mullins et al., 2015). Several factors may explain the reduced GE during prolonged exercise, for example changes in mechanical effectiveness, pedalling technique and recruitment pattern of thigh muscles, all of which might affect cycling performance. Efficiency of cycling and pedalling technique has previously been reported to improve over many seasons of cycling, likely yielding a highly efficient propulsion probably improving cycling performance (Santalla et al., 2009, Coyle et al., 1991). In well-trained cyclists, an improved mechanical effectiveness has been reported together with an improved 5-min performance after 12 wks of combined endurance and heavy strength training (Hansen et al., 2012). In Paper I, mechanical effectiveness was only reduced in SPR (-2%-points) and not in CON (-1%-point) during the 4-h LIT-session. Mechanical effectiveness has been reported to decrease in

competitive cyclists riding on a relatively high power output (80% of maximum power output) to exhaustion (Sanderson and Black, 2003). This could indicate that changes in mechanical effectiveness primarily relate to the relative intensity of exercise. However, in line with previous findings (Korff et al., 2007), the change in mechanical effectiveness in the present study was not correlated to the reduction in GE, which, evidently, decreased by the same extent in both SPR and CON. Changes in pedalling technique might also affect GE, as a correlation between an earlier occurrence of peak torque during the pedal stroke and improved 40-min TT has been found in a study on combined strength and endurance training in highly-trained cyclists (Ronnestad et al., 2015). In theory, an earlier peak torque could reduce the time of blood flow obstruction to the working muscle during the downstroke phase, which is highest at peak torque (Takaishi et al., 2002). However, mean angle of peak torque during the down stroke phase did not change in SPR or CON during the 4-h LIT-sessions and did, therefore, not seem to affect GE. Hence, changes in pedalling technique does not seem to explain the reduction in GE during prolonged low-intensity cycling.

During the 4-h LIT-session (Paper I), iEMG increased in VL and VM in both SPR and CON, which indicates a gradual recruitment of additional motor-units with a concurrent reduction in GE. The increasing iEMG may indicate a decreasing efficiency of already recruited fibres, as reported earlier during both low-intensity (Hausswirth et al., 2010) and supramaximal intensities (Vanhatalo et al., 2011). However, without changes in fibre type recruitment, since mean power frequency was unchanged in both SPR and CON. Sprinting was associated with temporal increases in iEMG in SPR while the prolonged effect on iEMG was small and patterns returned to baseline prior to the next set of sprints. The level of variance in the recruitment pattern of lower limb muscles is rather high, even in professional cyclists (Hug et al., 2004), but the fact that iEMG was not affected differently by SPR compared to CON, supports the notion (Hug et al., 2004), that the nervous system has multiple ways of accomplishing a given motor task in both the fresh and gradually fatiguing state. The increasing iEMG in concert with decreasing GE suggests a peripheral fatigue development, possibly related to progressive mitochondrial or contractile inefficiency (Hopker et al., 2017). A common pattern of pedalling technique might not be obvious in elite cyclists (Hug et al., 2004), despite a high degree of expertise and could, therefore, question the specificity of our EMG-measures of only two thigh muscles.

Taken together, GE decreases as a function of time during a 4-h LIT-session and the inclusion of maximal repeated sprints did not affect the changes in GE compared to work-matched, low-intensity cycling. Changes in pedalling technique and muscle activity patterns measured during and directly after sprints, occurred together with a temporarily reduced GE, but did not affect overall physiological changes during prolonged cycling. Most importantly, these changes in cycling efficiency, technique and muscular activation did not seem to negatively affect subsequent repeated sprint performance in elite cyclists. Therefore, including repeated 30-s maximal sprints during a prolonged LIT-session did not lead to greater reductions in performance-related measures compared to low-intensity cycling only and repeated sprint performance was not reduced.

5.2.2. Acute muscular responses

Paper II showed that SPR was associated with more pronounced plasticity-associated responses in muscle cells compared to CON. Specifically, markers of fat metabolism (PDK4), angiogenesis (VEGFA) and muscle hypertrophy (myostatin and MuRF1) mRNA levels were augmented more in SPR compared to CON, while SPR was associated with decreased levels of markers of ion transport (Na^+-$K^+\alpha 1$, CLC1 and NHE1) and blunted responses for a marker of mitochondrial biogenesis (PGC-1α) compared to CON.

5.2.2.1. Mitochondrial function and biogenesis. SPR induced greater acute increases in a marker of fat metabolism, PDK4 mRNA, compared to CON, supporting previous findings in trained

subjects performing a shorter (~1.5 hrs) SPR-protocol (Skovgaard et al., 2016). PDK4 impairs oxidation of carbohydrates through negative regulation of the pyruvate dehydrogenase complex and may act to shunt metabolism towards fatty acid metabolism (Herbst et al., 2014). This was probably potentiated by excessive glucose utilization during sprinting (Herbst et al., 2014), but since we did not measure muscle glycogen content, this could not be confirm. However, in support of this notion, combined sprint and endurance exercise has previously shown larger depletion of glycogen stores compared to endurance exercise only (Brandt et al., 2016). An interrelationship between PDK4 expression and glucose availability, therefore, seems likely. Furthermore, sprint exercise acutely increases several genes involved in fat metabolism, including PDK4 (Rundqvist et al., 2019), and 6 wks of sprint training has led to increased activity of enzymes involved in fat metabolism in trained subjects (Skovgaard et al., 2018a). It is thus plausible to suggest that SPR increases the capacity of muscle tissue to metabolize fatty acids, despite the continuous carbohydrate feeding during the 4-h LIT-session.

In contrast to previous studies of short (1-1.5 hrs) SPR-protocols in trained subjects (Skovgaard et al., 2016, Brandt et al., 2016), the PGC-1α mRNA levels in the present study were less increased in SPR compared to CON, and the increases were, overall, small (1.5-fold), compared to those of less fit individuals (~6-10 fold) (Skovgaard et al., 2016, Brandt et al., 2016). Previously, lactate administration in mice has shown to increase PGC-1α mRNA levels (Kitaoka et al., 2016) in skeletal muscle. Therefore, inclusion of sprints during a LIT-session increasing [BLa^-] should, expectedly, lead to greater abundances of PGC-1α mRNA than low-intensity cycling alone. The generally minor responses in PGC-1α mRNA and lesser responses in SPR compared to CON might be due to several factors. First, the higher fitness level of the elite cyclists in the present study compared to those of previous studies (Gunnarsson et al., 2019, Brandt et al., 2016, Skovgaard et al., 2016), may explain the relatively low responses, since acute PGC-1α mRNA responses to exercise in previously untrained individuals are reported to decrease from 7-fold to ~2-fold after as few as <14 endurance exercises (Granata et al., 2018). Second, PGC-1α activity is interconnected with carbohydrate availability and, therefore, increases in response to decreased muscle glycogen levels (Psilander et al., 2013). As SPR-protocols in general seem to induce greater glycogen depletion than low-intensity exercise alone (Brandt et al., 2016), this may have contributed to the previously observed greater increases in PGC1-α mRNA levels in SPR-protocols compared to CON (Skovgaard et al., 2016, Brandt et al., 2016). In the present study, we sought to mimic habitual practise by allowing participants to ingest exogenous glucose *ad libitum* during exercise to avoid pronounced glycogen depletion, thus possibly explaining the relatively low PGC-1α mRNA responses. Third, PGC-1α mRNA responses in a study by Gunnarsson et al. (2019), did not differ between a short (1-h) SPR-protocol and CON-protocols in trained subjects (Gunnarsson et al., 2019), which supports our findings. Furthermore, including sprints during LIT-sessions in an 8-wk intervention still led to increased mitochondrial protein content (Gunnarsson et al., 2019). This supports the notion that acute PGC-1α mRNA abundance does not necessarily coincide with longitudinal changes in PGC-1α protein (Cochran et al., 2014), since changes in protein content are not exclusively a results of changes in mRNA availability, but also greatly depends on translation and stabilisation (Christiansen, 2019, Makhnovskii et al., 2020). Finally, PGC-1α induces positive regulation of PDK4 and VEGFA mRNA levels (Olesen et al., 2010), which makes the blunted PGC-1α mRNA responses in SPR compared to CON in the present study difficult to explain, as both PDK4 and VEGFA increased more in SPR compared to CON. Hence, these small acute responses to SPR and CON in PGC1-α mRNA warrant further investigation into the chronic adaptations in mitochondrial biogenesis and function in elite athletes.

5.2.2.2. Angiogenesis. For VEGFA, the response after SPR were more pronounced than after CON, suggesting that including sprints during prolonged low-intensity exercise may exert greater effects on angiogenic processes than low-intensity exercise alone, possibly leading to greater vascularization over time. Notably, the augmented VEGFA response seen after SPR in the present study are in contrast to data from recent studies (Skovgaard et al., 2016, Brandt et al., 2016), wherein no beneficial effects were seen of including sprints during a LIT-session in trained subjects. In these studies, the duration of the exercise was much shorter than in the present study (1.5 h vs. 4 h) (Skovgaard et al., 2016, Brandt et al., 2016), suggesting that the potentially positive effects of SPR-protocols on angiogenesis may depend on the overall duration/load of the exercise. Indeed, both SPR and CON induced marked angiogenic responses in muscle, evident as increased abundances of VEGFA and THBS1 mRNA, resembling typical observations made after endurance exercise, eventually leading to capillary growth after training in untrained subjects (Hoier et al., 2012).

5.2.2.3. Ion transport. In contrast to what might be expected, SPR induced moderate to large reductions in mRNA levels of Na^+-$K^+\alpha1$, NHE1 and CLC-1 compared to CON. As previously reviewed (Hostrup and Bangsbo, 2017, Christiansen, 2019), most studies find increased ion-transport capacity in muscle after a period of sprint training, both with and without changes in protein content. While the decreased mRNA levels of ion transporters after SPR could be interpreted to lead to a general reduction in ion transport capacity, this may be an invalid biological interpretation. The timing of muscle biopsies in the present study, performed ~3.5 hrs (Post) and ~7.5 hrs (3h) after the first set of sprints in SPR, might explain these seemingly contradictory findings. For example, exercise-induced increases in NHE1 mRNA levels tend to peak in a delayed manner after exercise (24-48 h) in recreationally active men, with only small increases being detected during the initial 9 hrs (McGinley and Bishop, 2016). Furthermore, mRNA levels of Na^+-K^+-ATPases are not universally reported acutely to increase in response to sprint exercise (Christiansen et al., 2018). However, 6-wks sprint training did lead to increases in a number of Na^+-K^+-ATPases in the same study. In addition, Na^+-$K^+\alpha2$ protein content has also been reported to increase after 7 wks of HIT (Nielsen et al., 2004), despite acute mRNA levels were unaltered after the same exercise protocol (Nordsborg et al., 2003). Indeed, the capacity of ion transport is regulated on more levels than that of mRNA, as recently reviewed (Christiansen, 2019). While Na^+-K^+-ATPases and NHE1 play important roles in the housekeeping of muscle cells, contributing to resetting homeostasis after bursts of electrical and metabolic activity, CLC-1 plays a more direct role in regulation of muscle excitability by clamping the membrane potential, predominantly in type II muscle fibres during contractions to fatigue (Pedersen et al., 2009). In humans, CLC-1 content is higher in type II fibres, and generally lower in muscle of trained subjects compared to untrained, with protein abundances correlating negatively with exercise performance (Thomassen et al., 2018). The decreased CLC-1 mRNA levels in SPR might thus relate to excessive activation of type II muscle fibres during sprints. However, Thomassen et al. (2018) did not report any changes in CLC-1 protein content in response to 4 wks of SPR training. It is thus reasonable to question the biological significance of our results. Compared to previous studies on CLC-1 (Thomassen et al., 2018), our SPR-protocol was more physiologically demanding (<1 h vs. 4 hrs) and given the plausible role of CLC-1 in regulating muscle excitability (Pedersen et al., 2009). It remains an intriguing possibility that SPR, and perhaps type II muscle fibre-activating endurance training in general, alters CLC-1 biology over time, possibly explaining the lower levels of CLC-1 in trained subjects compared to untrained. Taken together, these preliminary alterations in mRNA levels of ion transporting proteins does not give a clear-cut indication of altered ion transport capacity. The adaptations to repeated SPE should indeed be investigated during longer interventions in elite athletes given its relevance in maximal performances (Hostrup and Bangsbo, 2017).

5.2.2.4. Muscle protein turnover. SPR decreased mRNA levels of myostatin and increased levels of MuRF1 more than CON. Reduced myostatin levels typically promote muscle protein

synthesis (Ruas et al., 2012), and increased MuRF1 levels increase rates of proteolysis (Heras et al., 2019, Louis et al., 2007), suggesting an increase in protein turnover in SPR compared to CON. Proteolytic responses seem to dominate over hypertrophic responses after endurance exercise (Louis et al., 2007), hypothetically explaining the smaller muscle fibre size seen in endurance-trained athletes (Trappe et al., 2006). In contrast, activation of type II muscle fibres during sprinting (Edgett et al., 2013) might have augmented myostatin and MuRF1 responses to SPR. Hypothetically, this could indicate a greater protein turnover in SPR compared to CON, which might affect the rebuilding of skeletal muscle during recovery (Dohm et al., 1985, Tipton et al., 2018). However, the effects of repeated sessions of SPR needs to be investigated on changes in muscle mass to confirm this notion in elite cyclists.

5.2.3 Acute blood hormonal responses

In Paper II, blood hormonal responses were quite small, and only SHBG and GH showed slightly reduced responses to SPR immediately after the exercise compared to CON. Otherwise, SPR and CON led to similar changes in blood concentrations of cortisol, IGF1 and testosterone.

The decreased SHBG levels observed immediately after SPR compared to CON may have affected testosterone biology. SHBG binds free testosterone, and a decrease in SHBG-levels after SPR might hypothetically result in an augmented testosterone-induced anabolic signalling (Florini, 1987). Inclusion of sprint intervals during low-intensity exercise may thus counteract the reduced testosterone levels typically seen in athletes performing large volumes of endurance training (Hackney et al., 2017). Furthermore, increased free testosterone may have beneficial effects on physiological responses to training, facilitating muscle plasticity (Florini, 1987) and erythropoiesis (Shahani et al., 2009). However, neither of these notions can be confirmed, as free testosterone was not measured in the present study, nor did we find clues to support alterations in free testosterone levels in blood, since testosterone:SHBG ratios remained unchanged after both exercise protocols. In addition, testosterone did not change in either condition, which may seem surprising, as it contrasts previous findings in trained athletes, who display acute increases in testosterone after both LIT exercise (2 hrs at ~55% VO_{2max}) and repeated 30-s sprinting (Wahl et al., 2013). However, the high fitness level of our participants may have affected blood hormonal responses, as higher fitness increases the threshold intensity at which these responses occur (Viru, 1992). Indeed, elite cyclists seem to display only small degrees of metabolic stress after habitual cycling for 4 h, as compared to exhaustive endurance exercise (Anderson et al., 2016).

[BLa^-] was greatly increased during SPR compared to CON, suggesting a greater metabolic strain when including sprints during a LIT-session (Brooks, 2018). GH and cortisol levels in blood are also regarded as biomarkers of such metabolic strain (Stokes et al., 2004, Viru and Viru, 2004), and hence tend to increase in an intensity-dependent manner (Wahl et al., 2013). It was thus surprising that GH concentrations in blood were unaltered after SPR, while they increased after CON (~5 $ug \cdot L^{-1}$), the latter response resembling that observed after a 2-h LIT-session (~4 $ug \cdot L^{-1}$) (Wahl et al., 2013). The blunted GH-response in SPR compared to CON, contrasts previously reported effects of repeated sprinting, which typically leads to elevated GH levels (Rundqvist et al., 2019, Esbjornsson et al., 2009). Arguably, this aberrancy may be due to the timing of blood sampling, as blood was collected ~90 min after the last set of sprints. Sprint-induced increases in GH levels typically return to baseline within 60-80 min (Rundqvist et al., 2019, Wahl et al., 2013), hence, temporal fluctuations in markers of metabolic strain could have occurred during the SPR protocol. As for cortisol, blood levels decreased steadily throughout the 4-h LIT-session after both SPR and CON. This may also reflect the timing of blood sampling, since cortisol is reported to be highest after high-intensity and sprint exercise compared to prolonged low-intensity exercise (Wahl et al., 2013, Wahl et al., 2010). Elevated levels of cortisol for prolonged periods has earlier been suggested to have an impairing effect on

training adaptations (Adlercreutz et al., 1986). However, a more recent study suggests that cortisol may act as important signalling cue for recycling damaged proteins, resulting in a pool of free amino acids, which in turn may facilitate protein synthesis in the anabolic phase (Viru and Viru, 2004). The overall reduction in cortisol levels in both SPR and CON, are more likely a result of the duration of the exercise protocol applied and hence, the time of the day. Cortisol levels varies in a circadian manner and with food intake, whereby it increases in periods of fasting, (e.g., during the night), and decreases throughout the day (Hackney and Viru, 1999). Hence, the steadily decreasing cortisol levels were likely associated with the continuous intake of carbohydrates during exercise, along with its prolonged duration, starting at 8 a.m. and lasting for seven hrs (corresponding to blood sampling at 3h).

Overall, the low responses of GH and the decreasing levels of cortisol measured after SPR and CON suggest that LIT-sessions of habitual duration were associated with relatively low degrees of metabolic stress in elite cyclists, with no evidence for a prolonged stress response in the three hours following exercise. However, a temporal increase in physiological stress during SPR cannot be ruled out and the more chronic effects of such training, therefore, needs further investigation.

5.2.4. Recovery of isokinetic torque

In Paper II, SPR and CON led to similar reductions in isokinetic knee extension torque measured immediately after the 4-h LIT-session (Post), and both SPR and CON were associated with complete recovery the following day (24h). In trained athletes, reductions in peak torque are typically observed after periods of relatively large loads of aerobic endurance exercise with inadequate periods of recovery (Coutts et al., 2007). Notably, three hrs after exercise, torque was reduced at both the lowest ($60°\cdot s^{-1}$) and highest ($240°\cdot s^{-1}$) contraction velocity in SPR compared to CON, suggesting greater muscular fatigue in both slow and fast motor units during the initial phase of the recovery period (Coutts et al., 2007). However, this reduction was temporary and not evident after 24 hrs, where both SPR and CON were fully and equally recovered. This suggests that 30-s sprint intervals included in LIT-sessions did not lead to excessive need for recovery in elite cyclists, a perspective that is supported by the lack of marked endocrine stress responses. Therefore, it seemed to be a feasible training strategy that did not reduce muscle performance on consecutive days, though this needs to be confirmed by studies investigating the effects of repeated SPR-sessions over longer interventions.

5.3. Adaptations to repeated inclusion of sprints during LIT-sessions

5.3.1. Sprint performance and strength-related measures

Sprint training has proven to be a potent training modality to improve sprint performance in both untrained and trained participants (Gist et al., 2014, Ross and Leveritt, 2001). Recently, the positive effects of regular sprint training on sprint performance has been reported in elite cyclists, although no control group was included in this study (Fortes et al., 2019). The Papers III and IV in this thesis are, therefore, the first studies to show the potency of sprint training in highly specialized elite cyclists when including sprints during habitual LIT-sessions in controlled studies with control groups included. Specifically, sprint performance was improved by 8% more in SPR compared to CON after a transition period of reduced training load. Likewise, SPR improved sprint performance 4% more than CON after a training camp of increased training load. These changes were large and small, respectively, and suggest that inclusion of a relatively small number of sprints (27 to 51 x 30-s), can improve sprint performance in elite cyclists, which arguably is relevant in cycling competitions (Menaspa et al., 2015, van Erp and Sanders, 2020).

Improvements in sprint performance in SPR-groups were quite similar between Paper III (4%) and IV (3%) despite substantial differences in total training load and almost twice the number of sprints performed in Paper IV compared to Paper III (51 vs 27). In support of our findings, 30-s sprint

performance has also been reported to improve during longer periods (12 wks) when sprints are included in habitual training routines of elite cyclists (Fortes et al., 2019). In the study by Fortes et al (2019), sprint performance was even further improved after a 2-wk step-wise taper, reducing training load by 15% and 30% in wk 1 and 2, respectively (Fortes et al., 2019). However, continuing tapering for 4 wks, reducing training load by 45-60%, deteriorated sprint performance, despite maintaining regular sprint training (Fortes et al., 2019). This indicates that substantial changes in total training load, also might affect sprint performance. While a 60% decrease in training load and absence of sprint training deteriorated sprint performance by 4% in CON (Paper III), increasing training load by ~50% during a training camp did not affect sprint performance in CON (-1%, Paper IV). To improve sprint performance in elite cyclists, the addition of sprint training seems necessary, since 12 wks of habitual, high-load training including LIT, MIT and HIT-sessions, does not seem to alter 30-s sprint performance in well-trained cyclists (Ronnestad et al., 2010). The findings of Paper III and by Fortes et al. (2019) underline the potency of sprint training during short periods (2-3 wks) of reduced training load, while longer periods (≥4 wks) of substantially reduced training load seem to deteriorate sprint performance in elite cyclists. As one might expect, sprint training improves sprint performance during short periods (2-3 wks) of both reduced and increased training load, but might also affect other performance-related measures in elite cyclists during periods of habitual changes in total training load.

After the transition period (Paper III), neither peak power during a 6-s sprint nor V_{max} in leg-extension changed differently between SPR and CON, while V_{max} was improved in CON only. The unaltered peak power and strength measures in SPR contrasts previous studies where short periods (2 to 4 wks) of reduced training volume improved peak power output (Fortes et al., 2019) and muscle strength (Martin et al., 1994) in elite cyclists and runners. While the intensity of training was primarily low except for the small amount of sprints in Paper III, the maintained intensity-distribution in Martin et al. (1994) and Fortes et al. (2019), might explain this discrepancy in strength-related measures for SPR. Prolonged sprint training (>6 wks) has also led to changes in muscle fibre distribution, increasing the portion of type IIA fibres (Allemeier et al., 1994, Andersen et al., 1994, Jacobs et al., 1987, Jansson et al., 1990), while shorter interventions (4 wks) has not (Esbjornsson Liljedahl et al., 1996). The short interventions and small number of sprints included in the present studies were probably unlikely to induce significant changes in muscle fibre-type distribution. However, drastically reducing activation of higher-order motor units, as when performing only LIT in CON, might have affected muscle fibre-characteristics and hence V_{max}.

The substantially reduced training load in combination with sprint training (Paper III) might, however, also affect muscle mass and cross-sectional area of muscle fibres. Interestingly, after the 3-wk transition period of reduced training load, only CON reduced lean body mass by -5%, although not different from SPR who maintained lean body mass (-2%). As previously discussed, inclusion of sprints during a LIT-session, acutely alters markers of protein turnover more than LIT only (Paper II). In accordance with these acute responses, sprint training has previously been shown to load to insignificant (6-12%) (Allemeier et al., 1994) and significant (5-50%) increases in muscle fibre cross-sectional area (Linossier et al., 1997, Sleivert et al., 1995), with a concomitant increase in muscle mass of the thigh muscle (Linossier et al., 1997). Furthermore, a greater cross-sectional area is reported in sprinters compared to endurance athletes (Hakkinen and Keskinen, 1989), thereby hinting of a possible hypertrophic stimulus of sprint training, particularly in the sprint-activated type II muscle fibres (Ross and Leveritt, 2001). This notion could in part explain the maintained lean body mass in SPR during a period of drastically reduced training load. Unfortunately, gold-standard measures of muscle fibre cross-sectional area (i.e., immunohistochemistry) and muscle mass alterations (i.e., magnetic resonance imaging) were not applied in the present studies to confirm this. Furthermore, it cannot be excluded, that the observed changes in lean body mass also could have been

caused by differences in strength training or training loads between groups, albeit insignificant. In contrast, when increasing training load during the training camp (Paper IV), no changes occurred in lean body mass in either SPR or CON, arguing against a hypertrophic response of sprint training. However, the increased volume of LIT may have blunted this response, since proteolytic responses dominate above hypertrophic responses after prolonged low-intensity endurance exercise (Louis et al., 2007). Therefore, sprint training might, possibly, act to maintain muscle mass of elite cyclists through hypertrophic-like responses in periods of decreased training load, although not leading to increased muscle mass per se. However, inclusion of sprints during LIT-sessions does not seem to increase muscle mass of elite cyclists when performed together with habitual high loads of LIT. Longer studies investigating the additive and maintaining effects of regular sprint training on muscle mass in elite cyclists are, indeed, needed to clarify these notions. In this case, the micro biopsy procedure applied in the present studies, would prove a valuable tool to access this in elite athletes, without compromising habitual training routines.

The improved sprint performance seen in SPR compared to CON in both Paper III and Paper IV, suggests improvement of anaerobic capacity. This should, expectedly, lead to improved short high-intensity endurance performance, such as W_{max}. However, W_{max} was unchanged in the present studies, which contrasts previous studies where W_{max} was improved when sprint training was added to regular LIT (Laursen et al., 2002) or implemented in LIT-sessions (Gunnarsson et al., 2019). While this discrepancy could relate to a difference in the total number of sprints (27 and 51 x 30-s sprints in Paper III and IV, respectively, vs. 96 and 144 x 30-s sprints in Laursen et al. (2002) and Gunnarsson et al. (2019), respectively), it could also relate to differences in total training load. Training cessation leads to substantial decreases in W_{max} (Maldonado-Martin et al., 2017), while smaller reductions in training load (-40%) maintains W_{max} in well-trained cyclists (Ronnestad et al., 2014). The latter is in line with findings in Paper III, where W_{max} was maintained after a 60% decrease in training load, irrespectively of inclusion of sprints. Furthermore, the addition of an intense training stimulus e.g., HIT once a week (Ronnestad et al., 2014) or sprints during a transition period does, therefore, not affect W_{max} when training load is decreased. However, including HIT during short periods (2 to 3 wks) of increased training load improves W_{max} in elite cyclists (Ronnestad and Vikmoen, 2019, Jeukendrup et al., 1992) while the inclusion of a small number of sprints (51 x 30-s sprints) during a training camp was insufficient to alter W_{max} in Paper IV. Intuitively, this discrepancy could relate to the difference in accumulated time at high intensities between the short 30-s sprints and longer HIT-sessions.

Taken together, including sprints during LIT-sessions improves sprint performance, despite substantial changes in total training load, while an absence of sprint training together with a decreased training load, deteriorates 30-s sprint performance. The combined effects of sprint training and altered training load on changes in muscle fibre-type distribution, lean body mass and strength-related characteristics remain inconclusive, but the small number of sprints in the present studies did not seem to affect W_{max}. The improved sprint performance in SPR, arguably, indicates an improved anaerobic capacity, and despite the principle of specificity applies to sprint training, the adaptations hereto could also affect other competition-specific performance measures such as 5-min and 20-min all-out performances after prolonged exercise.

5.3.2. 20-min and 5-min performance and performance-related measures

In Paper III, including sprints during LIT-sessions in a transition period of reduced training load, maintained 20-min performance in SPR (-1%), whereas a small decline of ~3% was observed in CON, although not different from SPR. In Paper IV, inclusion of sprints during LIT-sessions during a training camp of increased training load, induced a small to moderate increase in 5-min performance which was 4% larger in SPR compared to CON. The prolonged test-protocol applied in Papers III

and IV included a ~2-h exercise of low to moderate intensities including maximal efforts (e.g., VO_{2max}-test and repeated 30-s sprints), before concluding with an endurance performance test. This protocol was designed to induce a semi-fatigued state in the elite cyclists, thereby mimicking the physical requirements of cycling competitions, possibly making the sprint and endurance performances more competition-specific compared to efforts made in the fresh state.

<u>$\%VO_{2max}$, VO_{2max}, L_4, and haematological measures</u>

Although the changes in 20-min performance did not differ between SPR and CON in Paper III, the relevance of avoiding a decrease in 20-min performance after prolonged cycling, would arguably be of importance in cycling competitions (van Erp et al., 2019a). Endurance performance, such as a 20-min test, is mainly determined by $\%VO_{2max}$, VO_{2max}, efficiency and to a lesser fraction anaerobic capacity (Joyner and Coyle, 2008, Jeukendrup et al., 2000). In SPR, the maintained 20-min performance was coincided by a maintained $\%VO_{2max}$ during the test, contrasting the ~3%-points decrease in CON. However, neither VO_{2max}, W_{max} or GE in fresh or semi-fatigued state changed in any group. Thus, the moderate difference (ES: 0.8) in development pattern in $\%VO_{2max}$ within groups was probably the main explanation for the changes in 20-min performance. However, changes in anaerobic capacity indicated by altered sprint performance possibly also played a role.

In Paper III, the reductions in submaximal exercise measures in CON (e.g., $\%VO_{2max}$ during the 20-min test and power output at L_4), are possibly related to a decreased oxidative capacity (Coyle et al., 1984). Oxidative capacity has been reviewed to decline with reduced training in a volume-dependent fashion (Neufer, 1989). To counteract these declines, maintaining or increasing intensity of exercise seems important (Neufer, 1989, Ronnestad et al., 2014), and, probably, explains the unchanged $\%VO_{2max}$ in SPR. However, power output at L_4 decreased similarly in SPR and CON and indicates that inclusion of sprints, does not affect performance-related measures in the fresh state, but rather pose a protective effect on these measures after prolonged exercise. Generally, reducing training load decreases power output at L_4 (Maldonado-Martin et al., 2017, Ronnestad et al., 2014) and time to exhaustion at submaximal intensities (75% of VO_{2max}) (Madsen et al., 1993). In contrast to this deteriorating effect of decreased training, Paper III and others (Rietjens et al., 2001, Neufer, 1989) show that maintaining 30-50% of the training volume also maintains VO_{2max} in trained and elite cyclists for short periods (3 wks). The addition of the more demanding HIT seems necessary to maintain VO_{2max} when facing short periods (4 wks) of substantial (-93%) decreases in training volume (Madsen et al., 1993) and during longer periods (8 wks) of decreased (-35%) training volume (Ronnestad et al., 2014). The importance of maintaining training load is further underlined by studies on training cessation which decreases VO_{2max} severely by up to 6-20% after 3-8 wks in cyclists (Maldonado-Martin et al., 2017, Martin et al., 1986). Decreases in blood volume and haemoglobin mass and red blood cell count are reported in concert with decreased VO_{2max} (Eastwood et al., 2012, Maldonado-Martin et al., 2017) and are regarded as main causes for preliminary changes in VO_{2max} during short periods of decreased training load (Coyle et al., 1986). The unchanged VO_{2max} in Paper III is supported by an unchanged blood volume and haemoglobin mass, although this measure was only performed on a sub-set of the participants. Recently, small decreases in blood volume (<200 mL) have also been shown not to alter VO_{2max} (Skattebo et al., 2020), which could have been the case in our short intervention study. However, the results in Paper III indicates that elite cyclists are able to reduce total training load by ~60% for short periods, without affecting VO_{2max}, while submaximal performance-related measures appear to be more affected by changes in training load.

Taken together, in Paper III, including an intense stimulus such as sprints during LIT-sessions during short periods of decreased training load, maintained 20-min performance after prolonged exercise in SPR. This was likely due to a maintained ability to work at a high $\%VO_{2max}$ and possibly an improved anaerobic capacity in SPR, while a decreased volume and intensity of training

deteriorated all of the above in CON. Although these small to large effects were not different between groups, maintaining competition-specific performance after prolonged exercise is, arguably, relevant for elite cyclists. However, sprints did not affect the power output at L_4 which decreased equally in SPR and CON in a volume-dependent fashion, while VO_{2max} is unaffected by short 3-wk transition periods.

In Paper IV, inclusion of sprints during LIT-sessions on a training camp of increased training load followed by a recovery period, induced a 4% larger increase in 5-min performance in SPR compared to CON, the effect being small to moderate. These improvements were accompanied by a tendency towards increased $\%VO_{2max}$ during the 5-min test after prolonged exercise in SPR. This notion is in line with the findings of Paper III and hints of a possible fatigue-resisting effect of sprints on endurance performance after prolonged exercise. While an improved $\%VO_{2max}$ indicates alterations in the aerobic capacity, an improved 30-s sprint performance, as previously discussed, indicates an effect on anaerobic capacities. The inclusion of 30-s sprints during LIT-sessions, therefore, seems to improve the ability to sustain work at short, high efforts more than LIT only (Gunnarsson et al., 2019). In addition, longer endurance performances such as 40-min and 45-min tests are also improved by ~3% to 4%, respectively, when adding sprint sessions to a habitual volume of LIT (Laursen et al., 2002) and including sprints during ~1-h LIT-sessions (Gunnarsson et al., 2019) in well-trained and trained cyclists. However, in the study by Gunnarsson et al. (2019), 45-min mean power was not different in the SPR-group compared to LIT only. The more pronounced beneficial effects of adding sprints to LIT-sessions in the current study compared to Gunnarsson et al. (2019) might relate to a few differences in study protocols. The longer duration of our LIT-sessions (>4 hrs), the cessation of habitual HIT or sprint training in our control group, and the more anaerobic nature of the 5-min performance compared to the much longer 45-min tests, may all have contributed to explain this discrepancy. The latter point possibly also explains the absence of difference in the longer 20-min performance between groups in Paper III despite improved anaerobic capacity.

The general practice of increasing training load for short periods (1 to 3 wks) to stimulate training adaptations in elite athletes has recently been questioned (Bellinger, 2020). Several studies have assessed the effects of substantial increases in training load (40 to 400%), mainly achieved by increasing volumes of HIT. Collectively, they have reported immediate decreases in endurance performance in elite cyclists, or at best, unchanged performance and performance-related measures (Jeukendrup et al., 1992, Hansen et al., 2016, Slivka et al., 2010, Svendsen et al., 2016, Halson et al., 2002). This immediate decrease in performance is likely due to a temporal state of overreaching and is sought compensated for by recovery periods. Therefore, to avoid a state of overreaching in Paper IV, the 14-d training camp of ~50% increased training load by increased LIT-volume, was followed by a subsequent 10-d recovery period of 56% reduced training load and did not include HIT. In both SPR and CON, this led to ~2% increased VO_2 at L_4 and ~3-4% increased RBCV, while only CON, concomitantly, tended to increase Hb-mass (~2%, ~21 g), but neither were different from SPR. However, the borderline increase in Hb-mass in CON was of the similar magnitude (~2%) as the typical error of measurement in our laboratory (Ronnestad et al., 2020). Further, the change was smaller compared to reports in elite cyclists (~5%) after a longer (~5 wks) exercise-heat acclimation interventions of regular training load (Ronnestad et al., 2020). If these minor changes in Hb-mass were of relevance for endurance performance after prolonged exercise, an improvement of 5-min performance was expected within CON. However, the unaltered performance renders this notion equivocal. The increases in RBCV support previous evidence of increased levels of reticulocytes (Mujika et al., 2000) and RBCV (Shepley et al., 1992) after periods of training overload and recovery in well-trained athletes. Unsurprisingly, the observed increases in RBCV in our elite cyclists were slightly lower than those seen after 2-4 wks of increased endurance training in untrained or moderately trained subjects (Montero and Lundby, 2018). The high fitness level of our participants

may have limited the adaptive capacity to habitual changes in training load and could also explain why we did not observe changes in VO_{2max}. In this context, the general lack of effects on other haematological measures, VO_{2max}, $\%VO_{2max}$ at L_4, and W_{max} may also be attributed to the lack of HIT during the intervention. Several studies on increased training load of HIT followed by a recovery period have shown increased VO_{2max} (Neary et al., 2003a, Neary et al., 2003b), W_{max} (Jeukendrup et al., 1992) or both (Ronnestad and Vikmoen, 2019) in elite cyclists, and consequently improved 4-km to 40-km performances (Jeukendrup et al., 1992, Neary et al., 2003a, Ronnestad and Vikmoen, 2019, Farhangimaleki et al., 2009, Vollaard et al., 2006, Woods et al., 2018, Neary et al., 2003b), however this is not seen in all studies (Shing et al., 2007). Although the repeated sprint exercises in the present study were performed at maximal effort, the time spent above 90% of VO_{2max} is relatively short (Buchheit et al., 2012). In healthy young people, sprint training has been reviewed to increase VO_{2max} by small to moderately degrees (Gist et al., 2014), but unsurprisingly, seems insufficient in elite cyclists to stimulate further increases in VO_{2max} in elite cyclists.

A reduction in training load consistently seems to decrease power output at L_4 in a volume-dependent fashion. Conversely, periods of increased training load does not lead to a correspondingly increase in L_4, when the increase applies to all exercise intensities (i.e., LIT, MIT, and HIT) (Le Meur et al., 2013, Le Meur et al., 2014, Rietjens et al., 2005, Costill et al., 1991, Dionne et al., 2018). When only increasing training load by volumes of LIT, as in the present study, VO_2 at L_4 increased independently of sprinting, but the power output and $\%VO_{2max}$ at L_4 remained unchanged in both groups. This discrepancy could relate to small changes in VO_{2max}, as both SPR and CON showed 0.5 to 1.8% insignificant increases in absolute VO_{2max} and GE was unchanged in both groups. Despite the current study lacked a negative control group (i.e., a group not changing their training load), the unchanged power output at L_4 in both SPR and CON indicates that short overload periods obtained by increasing volumes of LIT, do not improve performance-related measures such as L_4 in elite cyclists. Together with unchanged endurance performances (5-min to 40 km tests) and VO_{2max} (Le Meur et al., 2014, Le Meur et al., 2013, Rietjens et al., 2005), this probably underlines a necessity for applying rather high loads of intense training (e.g., HIT or sprints) to obtain performance improvements measurable in the laboratory after short training camps in already highly trained elite athletes.

Overall, inclusion of sprints during LIT-sessions on a training camp in the preparatory period improved 5-min performance more than LIT only and tended to improve $\%VO_{2max}$ during the 5-min test after prolonged exercise. Increasing training load by LIT only, followed by a subsequent recovery period, increased RBCV and VO_2 at L_4 irrespectively of inclusion of sprints. However, neither power output and $\%VO_{2max}$ at L_4, VO_{2max}, nor W_{max} were affected by substantial changes in training load or by the addition of sprints.

GE in the fresh and semi-fatigued state

The importance of GE for endurance performance has long been recognized (Joyner and Coyle, 2008, Bassett and Howley, 2000). GE is highly related to years of cycling (Coyle et al., 1991) and seems to improve over years of continuous training in a professional cyclist (Santalla et al., 2009), but has also been reported to change during the season (Hopker et al., 2009a). Improvement of efficiency of movement, (i.e., improved GE), could be related to a decreased antagonist-activity (Hautier et al., 2000). This reflects a refinement in the motor-unit program, as reported after high loads endurance training or intense training such as sprint and strength training (Skovgaard et al., 2018a, Hopker et al., 2009b, Loveless et al., 2005). In addition, maintaining a high volume and intensity of training throughout the season preserves GE in trained cyclists (Hopker et al., 2009a, Jobson, 2012). Hypothetically, inclusion of sprints during LIT-sessions might, therefore, serve to maintain GE in periods of reduced training volume (i.e., in transition period) or improve GE in periods of increased

training volume (i.e., during training camps). However, the addition of sprints during LIT-sessions did not alter GE compared to LIT only in Paper III or IV, neither in the fresh nor in the semi-fatigued state. Despite this, small alterations in GE were observed within SPR and CON, which might have affected endurance performance after prolonged exercise. Acutely, GE decreases during prolonged cycling from the fresh to the semi-fatigued state during both SPR and CON protocols (Paper I). Minimizing this decrement is, therefore, important to preserve endurance performance later in a cycling competition. Interestingly, before the transition period in Paper III (at Pre), GE decreased during the prolonged exercise test from the fresh to the semi-fatigued state in SPR only, whereas it was unchanged at Post. This might have contributed to maintain 20-min performance from Pre to Post in SPR, although not different from CON. In addition, in Paper IV, SPR maintained GE in the semi-fatigued state from Pre to Rec, whereas CON decreased GE. Therefore, including an intense training stimulus such as sprints during LIT-sessions might serve to maintain GE during prolonged exercise after periods of predominantly LIT. In accordance, improved GE exclusively after prolonged exercise, has also been reported in studies of combined strength and endurance training (Ronnestad et al., 2011, Vikmoen et al., 2017), with a concomitant improvement of 5-min performance, without affecting performance in the fresh state (W_{max}). Maintaining GE during prolonged exercise is arguably relevant during the up to 300 km long road races, and the relevance of measuring GE and performance after prolonged exercise has recently been pointed out (Noordhof et al., 2020). Future investigations should be directed towards the possible ways to preserve GE during prolonged exercise in order to further enhance competition-specific performance in elite cyclists.

Taken together, the addition of sprints during LIT-sessions did not improve GE in the fresh or semi-fatigued state compared to LIT only. Although not different from performing LIT only, the addition of sprint training for short periods may prevent reductions in GE during prolonged exercise, possibly affecting endurance performance in the semi-fatigued state in elite cyclists.

Skeletal muscle adaptations

In Paper IV, inclusion of sprints during LIT-sessions on a training camp of increased training load did not alter protein content of CS, HAD and PFK differently in SPR compared to CON, but maintained content of Na^+-$K^+\beta1$ in SPR, whereas it decreased more in CON compared to SPR. However, protein content of PFK was decreased in both groups after the training camp, while no changes occurred in enzyme activity of CS and PFK.

Unfortunately, changes in protein abundance in response to decreased training load cannot be concluded on, since we did not obtain muscle biopsies in Paper III. However, it is well documented that enzyme activity and protein content of oxidative enzymes decreases with training cessation in trained and well-trained athletes (Fournier et al., 1982, Madsen et al., 1993, Coyle et al., 1985, Coyle et al., 1984), while glycolytic enzyme activities are reviewed to change more non-systematically (Mujika and Padilla, 2001). However, the decreased power output at L_4 in both SPR and CON after a 60% decrease in total training load indicates a decreased oxidative enzyme activity, and the inclusion of sprints did not seem to affect this submaximal performance-related measure. Interestingly, the addition of sprint training during a 4-wk period of 65% decreased training volume maintained mitochondrial oxidative enzyme activity and 10-km performance in well-trained runners (Iaia et al., 2009). Recently, it was demonstrated that the mitochondrial affinity for oxygen increased after only 7 sessions of 4 to 6 x 30-s sprints in untrained subjects and that this was associated with improvement in pulmonary oxygen uptake (Larsen et al., 2020). While these results could explain the increased $\%VO_{2max}$ during the 20-min test in Paper III and the tendency to increased $\%VO_{2max}$ during the 5-min test in Paper IV, the unchanged VO_{2max} in Paper III and IV did not support this notion.

The observed improvements in 5-min performance in SPR compared to CON in Paper IV might relate to muscular adaptations such as increased enzyme activity and protein content of

mitochondrial and glycolytic enzymes as reported after sprint training (MacDougall et al., 1998, Gunnarsson et al., 2019). For example, enzyme activity of CS and HAD increased with increasing training load from spring to summer in four elite cyclists (Sjogaard, 1984). Additionally, administration of lactate for 3 wks in mice has increased enzyme activity of CS and HAD selectively in the glycolytic phenotype muscle (Takahashi et al., 2019) and might support the positive effect of including sprints during LIT-sessions (Gunnarsson et al., 2019). However, the relatively short intervention of the present study performed on a limited number of cyclists did not alter CS enzyme activity or protein content of HAD in either group. It is interesting to note that CON tended to decrease protein content of CS, while this remained unchanged in SPR, the difference being small to moderate (ES: 0.6). This might thus relate to the additional type II muscle fibre activation during sprints and the consequently higher [BLa^-] levels during exercise in SPR compared to CON.

Contradictory, we found decreased PFK protein content in SPR and CON, whereas PFK enzyme activity remained unchanged. Sprint training for longer periods (6 to 15 wks) has shown to lead to increased PFK enzyme activity in untrained subjects (MacDougall et al., 1998, Simoneau et al., 1987), indicating an improved glycolytic capacity, which correlates with improved aerobic capacity (i.e., power output at L_4) (Tesch et al., 1985). The discrepancies between our data and other studies might be due to a combination of differences (e.g., fitness-level and duration of the intervention). Indeed, 8 wks of including sprints during habitual LIT led to greater increase in PFK-activity in trained subjects compared to LIT only (Gunnarsson et al., 2019). However, despite increased PFK activity has been reported after periods of sprint training, several studies have not found a concomitant improved sprint performance (Parra et al., 2000, Jacobs et al., 1987). This leaves to role of PFK in sprint performance as questionable in already highly trained athletes.

Only SPR maintained protein content of Na^+-K^+β1, which decreased moderately more (8%) in CON, despite the increase in training volume of LIT. In previous studies, endurance training has increased protein content of Na^+-K^+-ATPases in muscle tissue of untrained subjects (Green et al., 2004), whereas in well-trained subjects, higher intensity of exercise seems to be necessary to increase abundance of Na^+-K^+-ATPases (Nordsborg et al., 2010). Sprint training in recreationally active subjects has shown to increase abundance of Na^+-K^+β1 selectively in type II muscle fibres (Wyckelsma et al., 2015, Christiansen et al., 2018). The greater decreases of Na^+-K^+β1 protein content in out elite cyclists in CON might, therefore, relate to a substantial decrease in high-intensity training stimulus compared to SPR. Ion-transportation capacities has been suggested to play a fatigue-postponing role during all-out performances (Hostrup and Bangsbo, 2017). Hence, the decreased Na^+-K^+β1 protein content might contribute to explain the reduction in 5-min performance in CON compared to SPR. Notably, muscle characteristics are scarcely investigated in elite cyclists and the present study gives one of the first insights into the muscular adaptation to a habitual training load alteration in the preparatory period of elite cyclists.

5.3.4. Stress, recovery, and motivation

Maximal sprints are physically strenuous and may, therefore, increase the time needed for recovery between daily exercises in elite cyclists.

Acutely, muscular strength is decreased after prolonged LIT-sessions and the addition of sprints did not decrease isokinetic knee extension torque more than LIT only (Paper II). Three hours after exercise, SPR was associated with reduced torque both at the lowest ($60°\cdot s^{-1}$) and the highest ($240°\cdot s^{-1}$) contraction velocity compared to CON, suggesting greater muscular fatigue in both slow and fast motor units during the initial phase of the recovery period (Coutts et al., 2007). However, as this reduction was temporary and not evident after 24 hrs, including 30-s sprints during a prolonged LIT-session does not lead to excessive need for recovery in elite athletes, a perspective that was

supported by the lack of marked endocrine stress responses. To the contrary, it seemed to be a feasible training strategy that did not reduce muscle performance on the following day.

After the 3-wk transition period of decreased training load in Paper III, one might expect a decrease in markers of mental burnout due to the great reduction in training load and absence of strenuous competitions, which previously have been argued a necessity for elite athletes (Mujika et al., 2018). However, the total burnout was unchanged from before to after the transition period and SPR did not affect total burnout differently than CON. The average score for all subscales were comparable to a recent study in a population of young elite-sportsmen (Gerber et al., 2018). The general low scores in the mental subscales indicates a state of relatively low burnout in the elite cyclists, possibly explaining why this did not change during a 3-wk transition period. In addition, only small changes were observed in the subscales, which indicates that changes in mental recovery might be difficult to measure during such short periods in a small group of elite cyclists. In any case, inclusion of sprints during one weekly LIT-session during the transition period does not seem to pose any effect on mental recovery compared to LIT only in elite cyclists with initially low levels of burnout scores.

It is well established that periods of drastically increased training loads of HIT, can hamper endurance performance (Jeukendrup et al., 1992, Hansen et al., 2016, Slivka et al., 2010, Svendsen et al., 2016, Halson et al., 2002). In addition, a 6-d overload of HIT seems to increase levels of mental fatigue in elite cyclists (Ronnestad and Vikmoen, 2019), while a general increase in training load applied to all endurance-intensities on a training camp, do not seem to affect levels of stress in elite athletes (Becker-Larsen et al., 2017, Slivka et al., 2010). In corroboration with the latter, total stress and recovery measures did not change during the training camp of increased training load in Paper IV and SPR did not affect these measures differently than CON. Short maximal-effort intervals have been reported to be of less strain compared to longer HIT-intervals (Valstad et al., 2018), and might indicate a greater physical and mental stress of HIT compared to sprints. However, five days of recovery reduced levels of stress in elite cyclists below the levels measured before an overload period of HIT (Ronnestad and Vikmoen, 2019), possibly indicating a rather fast recovery in elite athletes and a great capacity to cope with substantial changes in training load. Interestingly, a 2-wk training camp of 37% increased training load lowered total stress, general stress and sport specific stress in elite para-triathletes, while general recovery increased during the training camp compared to normal training (Stephenson et al., 2019). Therefore, habitual increases in training load performed on training camps do not seem to pose any greater mental stress in elite athletes and may instead be associated with reduced levels of stress. This possibly relates to minimization of external life stressors (Slivka et al., 2010) and a break from the monotony of habitual indoor training-routines in the preparatory period when shifting to outdoor training (Dionne et al., 2018). However, training camps may also introduce an aspect of monotony when solely focusing on LIT. Indeed, CON tended to reduce motivation for training towards the end of the camp, while inclusion of sprints in SPR seemed to prevent this staleness, as motivation was "good" throughout the entire training camp. Finally, inclusion of sprints during habitual LIT-sessions were initially experienced as more strenuous, but this experience decreased gradually throughout the training camp. Compared to LIT only, inclusion of sprints was, therefore, rated as harder in the first 3 sessions, despite similar cycling distances, but was not rated as harder in the last session. This indicates a familiarization effect and may be linked to the finding in Paper II, suggesting that inclusion of sprints during LIT does not affect recovery of muscular strength on the following day. The gradual psychological (and physiological) habituation to performing 30-s sprints during LIT-sessions compared to LIT only indicates that this regimen might be beneficial to implement in habitual LIT-sessions without affecting the time to recover.

In conclusion, inclusion of sprints during LIT-sessions during periods of both drastically reduced and increased training loads do not seem to affect markers of mental stress or recovery

negatively compared to LIT only. Therefore, it is suggested that sprints can be incorporated into habitual training routines of elite cyclists in other parts of the annual training.

5.4. Practical considerations and applicability of sprint training in elite cyclists

Despite performing enormous annual training loads, the performance improvements reported in elite cyclists from the start of the preparatory period to the end of the competition period are quite miniscule (e.g., ~0-5% in VO_{2max}) compared to improvements in untrained subjects after only 10 wks of HIT (up to 44% increased VO_{2max}) (Hickson et al., 1977). The small to large positive effects of including sprints during relative short intervention on for all-out performance and performance-related measures in Papers III and IV, are therefore, arguably, of relevance for elite cyclists. Especially considering the marginal differences by which cycling competitions are won in a sprint-finish.

As initially stated, adding specific sprint training sessions (i.e., not including sprints during LIT-sessions) to the massive training schedule of elite cyclists might not be time-efficient and cost-beneficial. This practise would likely take up time otherwise spent on higher load exercises such as HIT-sessions, probably reducing aerobic capacities. Indeed, inclusion of sprints during LIT-sessions appeared to provide too weak a stimulus for elite cyclists to further increase performance-related measures such as VO_{2max} and power output at L_4. However, the ~3-wk interventions in Papers III and IV might have been too short to induce changes in these performance-related measures. When facing periods with an inherent risk of mental burnout or physical overreaching, such as the transition period and during training camps of increased training volume, inclusion of sprints does not seem to increase the risk of burnout or increase mental and physical stress, compared to LIT. However, since none of the elite cyclists indicated a tendency to burnout at the end of the season in Paper III, the applicability of sprints compared to other high-load training regimes (i.e., HIT) remains speculative. In spite of this, our positive effects on competition-specific sprint and endurance performance when implementing sprints during habitual LIT-sessions confirm their applicability for elite cyclists compared to LIT, without increasing the need for recovery. We therefore suggest that this exercise modality is incorporable in regular training routines of elite cyclists, which may even break the monotony of the numerous, prolonged LIT-sessions. Furthermore, continuing sprint training for prolonged periods (17 wks) in combination with habitual LIT, has also led to continuous improvements in all-out endurance performance in trained athletes (Skovgaard et al., 2017). This makes inclusion of sprints to the training routines in other parts of the annual training cycle an intriguing assessment for elite cyclists. The effect of implementing sprints during LIT-sessions during prolonged training periods need further investigation.

We chose to perform sprints in sets of 3 x 30-s since our experience is that applying even more consecutive sprints within a short time frame, drastically lowers the sprint performance and correspondingly increases the experienced exertion. On a practical note, performing more than three 30-s sprints also tend to induce nausea, which has a rather negative effect on exercise capacities. We further advocated at least 15 min of active recovery between sprint-sets (Paper III), to allow recovery of sprint performance (Glaister et al., 2005). In Paper IV, for practical reasons, we performed one set every hour during the ≥4-h LIT-sessions. Although 30-s is a rather long sprint, the cyclists showed gradual familiarization towards these strenuous endeavours, which led to a gradual habituation, diminishing the difference in experienced strain compared to the habitual prolonged LIT-sessions.

The micro biopsy technique used in the present studies to collect muscle samples proved to be of little pain during sampling as cyclists from Paper IV on average scored the pain to be 2.6 ± 1.3 on a scale from 0 (felt nothing) to 10 (extremely painful). Compared to the perceived exertion of the prolonged exercise protocol, the average score was 8.7 ± 0.9 on the sRPE scale. On the following day, the pain from the biopsy was rated 1.6 ± 1.1 which underlines the practical application of this

method in elite cyclists, enabling them to continue their normal training routine. Utilization of the minimally invasive micro biopsy procedure should therefore open the avenue for more such studies in the future, minimizing risks for adverse events while simultaneously providing valid and reliable data.

5.5. Methodological considerations

Convincing elite athletes to change their habitual training routines is not easy, hence the duration and the impact of the present studies were rather short/small. The relatively short interventions of ~3 wks applied in Paper III and IV and the rather low number of participants, provide limited insight into the long-term effects of including sprints during LIT-sessions. On the other hand, the small effects of the SPR intervention were expected in these elite participants, and the observed benefits may well prove to be of relevance in cycling competitions. The lack of standardization of the training performed prior to the intervention might have affected the outcomes, despite our effort to match training groups based on total training load and fitness.

Based on the current methods of defining training intensity and volume, prescribing individual training load to maximize training adaptations in elite athletes is a complex endeavour. To quantify training load in Papers III and IV, we applied the iTRIMP method based on an individual's own HR vs [BLa^-] relationship. This calculation is based on [BLa^-] obtained during incremental tests to exhaustion and does not, per se, reflect the actual [BLa^-] obtained after three consecutive 30-s sprints. Hence, the homeostatic perturbations obtained during repeated sprinting might not be the same as after an incremental test to exhaustions and could therefore have underestimated our calculated training loads in SPR. However, our questionnaires on mental and physical recovery did not indicate a greater risk of burnout in or increased stress in SPR compared to CON in Paper III and IV. Inclusion of sprints during LIT-sessions do therefore not seem to pose any great stress on elite cyclists compared to only LIT. Indicatively, studies applying HIT during training camps to induce an overload seem to yield more potent adaptations than the ones induced by inclusion of sprints during LIT-sessions (see Table 10, appendix). However, a clear connection between available load-calculations and chronic adaptations to training of different intensities has recently been questioned (Jamnick et al., 2020). Thus, future studies verifying the adaptation/load-relationship to training at different relative, individual intensities, are certainly needed.

As outlined in Table 2, the performance and performance-related measures of elite cyclists are reported to be lowest after the transition period. The increases in both training volume and intensity during the preparatory period and competition period, likely lead to the improved performance, peaking in the competition period. After a short transition period, which typically lasts for 2 to 3 wks, elite cyclists often increase training load gradually. Whether the current small, positive effects observed in SPR compared to CON will translate into improved performance later in the preparatory period and competition period, however, needs further investigation. Indicatively, inclusion of a HIT-session once a week in an 8-wk long transition period revealed a long-lasting effect on performance in well-trained cyclists that extended well into the subsequent preparatory period (16 wks) (Ronnestad et al., 2014). Together with the continuous improvement of all-out performance after prolonged periods (17 wks) of sprint training (Skovgaard et al., 2017), this indicates a relevance for maintaining or adding an intense training stimulus, such as sprinting or HIT, through periods of reduced training load dominated by LIT.

Regretfully, we did not include an all-out 30-s Wingate sprint in Paper III and IV to examine the effects of including sprints during LIT-session on sprint performance the fresh state. However, training adaptations are specific to stimulus applied (Haugen et al., 2019), whereby the improved sprint performance in face of sprint training is unsurprising, even in elite cyclists (Fortes et al., 2019). Hence, the main aims of the present studies were to measure sprint in a more competition-relevant

state after prolonged exercise. In addition, inclusion of an all-out sprint would have increased the load of the already prolonged test protocol even further.

The inclusion of sprints in Paper III and IV was not associated with greater stress or prolonged need for recovery compared to habitual LIT-sessions during such short interventions. Surprisingly, neither stress nor recovery measures included in these studies were affected by the substantial changes in total training load, which poses uncertainty to the applicability of such questionnaires. However, the short interventions and relative low sample size in the present studies may have been inadequate to induce significant, measurable alterations in moods. Indicatively, continued monitoring of stress and recovery for longer periods (6 wks) has previously shown a close connection between increased levels of stress and reduced levels of recovery when increasing training load of 12 male rowers (Jurimae et al., 2004). Furthermore, stress and recovery monitoring on a much larger sample size of 473 participants has also shown a dose-response relationship with training load across sex, sport and fitness-level (Nicolas et al., 2019). This indicates a relevance for continued monitoring of mental stress/recovery and burnout in elite athletes (Gerber et al., 2018) in relation to changes in training load, underlining the notion that the elite cyclists in Paper III and IV, indeed coped well with substantial changes in training load.

Food consumption was not strictly controlled in Paper III or IV and might thus have introduced unaccounted noise in the outcomes. However, on a general basis, elite cyclists do seem to be able to control their body mass despite drastically changes in activity level. At least, our elite cyclists did not increase body mass despite reductions of 60% in total training load in Paper III, and when facing a 50% increased training load, the elite cyclists even tended to increase body mass. This suggests rather well-developed nutritional routines among elite athletes and the possible effects of this were thus regarded to be small.

6. Conclusion

Acutely (Paper I), including sprints during a habitual LIT-session (SPR), temporarily increases [BLa^-] and changes pedalling technique and muscle activity patterns during exercise but these alterations do not affect GE differently from a work-matched LIT-session (CON), which decreases as a function of time. Furthermore, repeated sprint ability is not reduced during a LIT-session and recovery of muscle strength in not affected 24 hrs after exercise when including sprints.

On the muscular level (Paper II), SPR acutely induced more pronounced changes in mRNA levels of several genes compared to CON. Overall hormonal responses were small and did not differ greatly between SPR and CON, indicating relatively low degrees of metabolic strain, with no evidence for a prolonged stress responses in the three hours following exercise. Collectively, inclusion of sprints hint of a potentially beneficial effect in skeletal muscles, without adversely affecting GE or hormonal responses or impairing recovery of muscle strength on the following day and could, therefore, be implemented in longer training interventions.

When implementing sprints during LIT-sessions during a 3-wk transition period of reduced training load (Paper III), sprint performance was more improved in SPR compared to CON. In addition, 20-min performance and $\%VO_{2max}$ were maintained in SPR, while they were reduced in CON, though not different from SPR. Inclusion of sprints did not affect the power output at L_4, which was equally reduced in both SPR and CON. However, neither VO_{2max}, W_{max}, nor total burnout seemed affected by the reduced training load and were not affected by sprinting.

Including 30-s sprints during five LIT-sessions during a training camp in the preparatory period (Paper IV) improved sprint and 5-min performance more in SPR than in CON without affecting total stress/recovery. In addition, SPR maintained protein abundance of Na^+-$K^+\beta1$ which was reduced more in CON. Together with a tendency towards increased $\%VO_{2max}$, this indicated that inclusion of sprints improves competition-specific performance after prolonged exercise. Furthermore, the increased training load during the training camp, followed by a subsequent recovery period, increased RBCV and VO_2 at L_4, irrespectively of inclusion of sprints. Finally, measures in the fresh state; power output and $\%VO_{2max}$ at L_4, together with VO_{2max} and W_{max}, were unaffected.

Overall, including sprints during LIT-sessions seem to yield positive effects on competition-specific performances and performance-related measures primarily in the semi-fatigued state (i.e., after prolonged exercise), while measures in the fresh state are unaffected. Specifically, inclusion of sprints during periods of decreased and increased training load, respectively, maintain and improve protein abundance of Na^+-$K^+\beta1$ and $\%VO_{2max}$ and prevent reductions in GE, exclusively in the semi-fatigued state, concomitant with improved sprint and endurance performances. Furthermore, inclusion of sprints during LIT-sessions do not seem to increase risk of burnout or affect markers of mental stress or recovery more than habitual LIT. Including sprints during LIT-sessions, therefore, yields an intriguing and highly competition-specific adaptation in elite cyclists compared to performing LIT only.

7. References

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8. Appendix

***Table 10**: Changes in performance and performance-related measures in response to reduced and increased training load in elite cyclists. Data are mean %-changes.*

Reference	Sport	n (m,f)	Age (Yrs)	VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	Design	Training load (%Δ)	VO_{2max}, W_{max} (%Δ)	Submaximal power output (%Δ)	Submaximal $\%VO_2$ (%-points Δ)	Performance tests (%Δ)	Haematological/muscular measures (%Δ)
Intervention studies conducted in the transition period resembling the design of Paper III (decreased training load)											
(Eastwood et al., 2012)	Triathletes, recreational	9	29-44	65±5	30-d detraining	-87 %	VO_{2max}: -5				BV: -9 Hbmass: -3 CD: NS,
(Madsen et al., 1993)	Cyclists, triathletes, runners	9	27±2	66±1	4 wks detraining, exchange 8 hrs LIT with 35 min HIT/wk	-93 %	VO_{2max}: 0NS			TTE at 75%VO_{2max}: -21	CS: NS HAD: -12% Na^+-K^+-ATPase: NS.
(Maldonado-Martin et al., 2017)	Cyclists	10	20±1	79±6	5 wks training cessation	-100%	VO_{2max}: -11 W_{max}: -7	LT_1: -13 L_4: -12			RBC: -7 Hbconc: -5
(Martin et al., 1986)	Cyclists and runners	6 (5,1)	26±1	63±4	3 wks and 8 wks detraining	-100%	VO_{2max}: 3wks: -6 8wks: -20				SV 3wks: -10 8wks: -17
Paper III	Cyclists,	7	23±3	73±5	3 wks transition, LIT and Sprint	-62%	VO_{2max}: -3NS W_{max}: 1NS	L_4: -4	L_4: 1NS 20min: 2NS	20-min: -1NS 30-s: 4	BV: 2NS Hbmass: -1NS
Paper III	Cyclists,	9	21±4	71±5	3 wks transition, LIT only	-64%	VO_{2max}: -1NS W_{max}: -1NS	L_4: -5	L_4: -4NS 20min: -3	20-min: -3 30-s: -4	BV: -2NS Hbmass: -1NS
(Ronnestad et al., 2014)	Cyclists,	7	32±8	69±6	8 wks transition, maintain HIT every 7-10 d	-40%	VO_{2max}: 0NS W_{max}: 0NS	L_4: 5	L_4: 1NS	40-min: 5	
(Ronnestad et al., 2014)	Cyclists,	6	30±7	68±5	8 wks transition period, only LIT	-39%	VO_{2max}: -3 W_{max}: -2NS	L_4: -5	L_4: -2NS	40-min: -6	
Intervention studies conducted in the preparatory period resembling the design of Paper IV (comprising an overload and a subsequent taper)											
(Farhangimaleki et al., 2009)	Cyclists	12	26±4	67±1	8 wks progressive endurance training, 3-wk taper	Not specified increase 50% decreased				40-min: 2	
(Fortes et al., 2019)	Cyclists	17	24±2	-	12 wks increasing load including 37 sprint sessions, 4-wk taper including 10 sprint sessions	~50% increased, 15 to 60 decreased					
(Jeukendrup et al., 1992)	Cyclists	7	25±7	~65	2 wks increased load, 2 wks recovery	40% increase mainly HIT, 60% decrease.	VO_{2max}: 0 W_{max}: 6	L_4: 3		8.5-km: 4	
(Le Meur et al., 2013)	Triathletes	16	30±5	62±5	3 wks intensified training, 2-wk rec	47% increased, 50% decreased				TTE incremental test: 4	
(Le Meur et al., 2014)	Triathletes	12	34±5	61±5	3 wks intensified training, 2-wk rec	31% increased, 59% decreased	VO_{2max}: 1NS PPO: 4				
(Le Meur et al., 2014)	Triathletes	12	34±5	61±5	3 wks intensified training, 2-wk rec	29% increased, 61% decreased	VO_{2max}: -2NS PPO: 2NS				
(McKenzie et al., 2016)	Cyclists	8 (6,2)	25±7	63±8	10-d overload, 10-d recovery	20% increased, 33% decreased					Satellite cells type I: 14-16 Type II: 13
(Neary et al., 2003a)	Cyclists	11	23±5	68±9	3 wks high-load high-intensity training,	Not specified increase	VO_{2max}: 5			20-km: 5	

					7-d taper	50% decreased					
(Neary et al., 2003b)	Cyclists	7	25±6	61±2	7 wks progressive overload, 7-d taper	Increase not compared but mainly HIT. 52% decreased volume	VO_{2max}: 3	VT_1: 12		40-km: 4	<u>Type I:</u> CSA: 7NS SDH: 12 <u>Type II:</u> CSA: 14 SDH: 16 HAD: 16 CYTOX: 16
(Neary et al., 2003b)	Cyclists	8	25±6	61±2	7 wks progressive overload, 7-d taper	Increase not compared but mainly HIT. Volume maintained, 45% decreased intensity	VO_{2max}: 1NS	VT_1: 8		40-km: -2NS	<u>Type I:</u> CSA: 7NS HAD: 17 CYTOX: 9 <u>Type II:</u> CSA: 11NS HAD: 18
Paper IV	Cyclists	9	21±1	75±5	14-d training camp, 10-d rec	50% increased, 53% decreased	VO_{2max}: 2NS W_{max}: 1NS	L_4: 1NS	L_4: 1NS 5 min: 2NS	5-min: 2NS 30-s: 3	RBCV: 3NS CS: 2NS HAD: -1NS PFK-14 NaKb: 2NS
Paper IV	Cyclists	9	21±2	75±5	14-d training camp, 10-d rec	47% increased, 59% decreased	VO_{2max}: 1NS W_{max}: 1NS	L_4: 2NS	L_4: 1NS 5 min: 2NS	5-min: -2NS 30-s: -1NS	RBCV: 4 CS: -9NS HAD: 5NS PFK: -17 Na^+-K^+βl: -6
(Rietjens et al., 2005)	Cyclists	7	25±5	61±7	2-wk overload, 1-wk recovery	107% increased and 15% increase in intensity, -50% decreased	VO_{2max}: 1NS W_{max}: 4NS			TTE at 75% of W_{max}: 1NS	Hb conc: -5 RBC: -3NS
(Ronnestad and Vikmoen, 2019)	Cyclists	9	21±7	77±5	6 d overload of HIT, 5-d step taper	9% increased, -57% decreased	VO_{2max}: 4 W_{max}: 5	L_4: 3			
(Shing et al., 2007)	Cyclists	15	27±2	69±1	5 d overload of HIT, 1 wk habitual trainng	256% increased HIT Habitual training				40-km: NS	
(Vollaard et al., 2006)	Triathletes	10	30±6	64±6	1 wk increased load, 1-wk recovery	40% increased, 60% decreased				15-min: 5	
(Woods et al., 2018)	Cyclists	13	35±8	61±6	2 wks increased load, 2-wk recovery	40-50% increased, 20% decreased				4-km: 5 15-s: -18	

When changes in performance or performance-related measures were not specified, the respective changes were calculated based on the change in average from before to after the respective intervention. Positive numbers represent improvements, negative numbers represent decreases. Changes in training load are compared to previous training period. VO_{2max}: Relative maximal oxygen uptake. HIT: High-intensity training. W_{max}: Maximal aerobic power, average power output of the last minute of incremental test t exhaustion. LT_1: Lactate threshold 1 (i.e., baseline [BLa^-] + 1 mmol·L^{-1}. L_4: Power output or VO_2 at a [BLa^-]of 4 mmol·L^{-1}. TTE: Time to exhaustion. CSA: Cross-sectional area. RBC: Red blood cell count. RBCV: Red blood cell volume. Hbconc: Haemoglobin concentration. SV: Stroke volume. BV: Blood volume. Hbmass: Haemoglobin mass. CD: Capillary density. CS: Citrate synthase HAD: β-hydroxyacyl. PFK: phosphofructokinase. Na^+-K^+βl: Sodium-potassium pump βl. SDH: Succinate dehydrogenase. CYTOX: Cytochhrome oxidase. NS denotes non-significant changes.

9. Papers

Paper I

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Paper II

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WILEY

ORIGINAL ARTICLE

Effects of including sprints during prolonged cycling on hormonal and muscular responses and recovery in elite cyclists

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This study investigated the acute effects of including 30-second sprints during prolonged low-intensity cycling on muscular and hormonal responses and recovery in elite cyclists. Twelve male cyclists (VO_{2max}, 73.4 ± 4.0 mL/kg/min) completed a randomized crossover protocol, wherein 4 hours of cycling at 50% of VO_{2max} were performed with and without inclusion of three sets of 3 × 30 seconds maximal sprints (*E&S* vs *E,* work-matched). Muscle biopsies (m. vastus lateralis) and blood were sampled at Pre, immediately after (Post) and 3 hours after (3 h) finalizing sessions. *E&S* led to greater increases in mRNA levels compared with *E* for markers of fat metabolism (PDK4, Δ-Log2 fold change between *E&S* and *E* ± 95%CI Post; 2.1 ± 0.9, Δ3h; 1.3 ± 0.7) and angiogenesis (VEGFA, Δ3h; 0.3 ± 0.3), and greater changes in markers of muscle protein turnover (myostatin, ΔPost; −1.4 ± 1.2, Δ3h; −1.3 ± 1.3; MuRF1, ΔPost; 1.5 ± 1.2, all $P < .05$). *E&S* showed decreased mRNA levels for markers of ion transport at 3h (Na^+-K^+ α1; −0.6 ± 0.6, CLC1; −1.0 ± 0.8 and NHE1; −0.3 ± 0.2, all $P < .05$) and blunted responses for a marker of mitochondrial biogenesis (PGC-1α, Post; −0.3 ± 0.3, 3h; −0.4 ± 0.3, $P < .05$) compared with *E E&S* and *E* showed similar endocrine responses, with exceptions of GH and SHBG, where *E&S* displayed lower responses at Post (GH; −4.1 ± 3.2 µg/L, SHBG; −2.2 ± 1.9 nmol/L, $P < .05$). Both *E&S* and *E* demonstrated complete recovery in isokinetic knee extension torque 24 hours after exercise. In conclusion, we demonstrate *E&S* to be an effective exercise protocol for elite cyclists, which potentially leads to beneficial adaptations in skeletal muscle without impairing muscle recovery 24 hours after exercise.

KEYWORDS

30-sec sprints, aerobic and anaerobic fitness, blood hormones, Elite athletes, mRNA, muscular responses, prolonged low-intensity cycling

1 | INTRODUCTION

Elite cycling involves prolonged and strenuous competitions with distances up to 250-300 km.[1,2] During such competitions, intensities vary from aerobic low-intensity to all-out sprinting, and preparations thus need to include development of both aerobic and anaerobic fitness.[3] Accordingly, elite cyclists typically cover annual distances of ~ 26-32 000 km and ~ 850 hours of training, wherein ~ 70%-80% is low-intensity training (LIT),[1,4] with singular sessions lasting up to 7 hours, interspersed by high-intensity training.[2] Given the extent of these training loads, there is no simple measure for further improving performance by altering training protocols. Simply increasing the duration of LIT does not seem to be sufficient.[5] Instead, it seems necessary to increase the training volume at higher exercise intensities.[6] This is achievable by implementation of sprint training, which improves both aerobic and anaerobic performances.[6] However, for elite cyclists, it is not time-efficient to dedicate singular sessions to sprint training, which also might increase the need for more restitution. A possible solution would be to include small volumes of sprint intervals during habitual LIT exercises.[7-10] This may improve performance in athletes that are already close to their genetic maximum.[11] Previous studies have exclusively studied this by adding sprints during relatively short LIT sessions (1-1.5 hours).[8-10] For such training to be advocated for elite cyclists, it is important to assess its effects and feasibility during LIT sessions of regular duration (>3-4 hours).[2,7] It seems expedient to start such an exploration by investigating its acute effects on muscular and hormonal adaptations, as well as the associated need for post-exercise restitution.

In skeletal muscle, inclusion of 30-second sprints during short-lasting LIT session leads to acute increases in markers of mitochondrial function and biogenesis in muscle of trained subjects compared with LIT-only.[8,10] This fits the notion that markers of mitochondrial biogenesis, such as peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), respond to acute exercises in an intensity-dependent fashion,[12] making it a potential determinant of these beneficial effects. Furthermore, sprint training seems to increase abundances of ion transport-proteins in muscle, thus augmenting ion transport capacity, which has been suggested to postpone fatigue development and improve high-intensity endurance performance.[13] To date, the effects of including sprints during prolonged LIT sessions on markers of ion transport in muscle remain unknown. Finally, sprint exercise may also affect protein turnover in skeletal muscle, as orchestrated through acute regulation of a range of muscular signaling factors such as PGC-1α4, insulin-like growth factor 1 (IGF1), and myostatin.[14] Together, this may facilitate the development of oxidative muscle fibers and may be beneficial for the ability to develop maximal aerobic power and fractional utilization of VO_{2max}.

Evidently, muscular responses to exercise largely reside on signaling events arising from increased muscular activation. However, such responses are also affected by systemic factors, including exercise-associated changes in hormone levels in blood.[15,16] This perspective remains scarcely investigated after combined sprint and LIT exercise,[8-10] though repeated maximal sprints alone lead to acute increases in testosterone, growth hormone (GH), and cortisol.[15,17] Testosterone has been suggested to increase muscle protein turnover, perhaps acting in concert with GH and insulin-like growth factor 1 (IGF1).[18] In line with this, testosterone may also be positive for recovery in endurance athletes[19] and may act to increase plasma volume and erythropoiesis,[20] subsequently leading to increased VO_{2max}.[20] Such growth and repair responses may also involve cortisol, which may be necessary for recycling proteins, resulting in a pool of free amino acids that may facilitate muscle protein synthesis during the restitution phase.[21] Overall, inclusion of sprints during prolonged, habitual LIT sessions of elite cyclists (~4 hours) may provide an effective stimulus for inducing muscular and systemic plasticity in elite cyclists, a perspective that remains unstudied.

The aim of the present study was to investigate the acute effects of including repeated 30-second sprints during a 4-hour LIT session on muscular and hormonal responses and the subsequent recovery (peak knee extension torque) in elite cyclists. We hypothesized that adding sprints to LIT (*E&S*) would lead to increased mRNA responses for genes involved in mitochondrial function and biogenesis, angiogenesis, ion transport, and protein turnover in m. vastus lateralis, as well as increased hormonal responses, compared to LIT alone (*E*).

2 | METHODS

2.1 | Participants

Twelve male cyclists (26.2 ± 6.3 years of age) volunteered for the study. Average endurance training recorded the last 30 days preceding study inclusion amounted to 13 ± 8 hour/week of which 0.6 ± 0.4 hour/week was high-intensity training. The participants did not perform sprint training on a regular basis. All participants were defined as elite cyclists, with nine participants being performance level 5 athletes and the remaining three participants being level 4 athletes, as classified using data on VO_{2max}, maximal aerobic power produced during the last minute of an incremental test to exhaustion (W_{max}) and training volume[22] (Table 1). Prior to inclusion, participants were informed of the possible risks and discomforts associated with the study and provided written, informed consents to participate. The study was approved by the Norwegian Center for Research Data (NSD) and the local

ethical committee at Inland Norway University of Applied Sciences and was performed according to the Declaration of Helsinki (except pre-registration in public databases).

2.2 | Experimental design and procedures

Data on acute physiological responses to the exercise protocol are published elsewhere,[7] but for clarification, the design of this randomized, work-matched, crossover study is briefly outlined here (Figure 1). Participants visited the laboratory on four occasions to perform (1) screening, (2) familiarization, and (3 + 4) experimental protocols. The screening session consisted of a 30-second all-out sprint (*Wingate*), a blood lactate profile and an incremental test to exhaustion to determine VO_{2max}. Familiarization to the experimental protocol consisted of a 4 hours bout of low-intensity cycling including three 30-second maximal sprint efforts toward the end of the first, second, and third hour (*E&S*). On experimental days, the cyclists performed in a randomized manner *E&S* or 4 hours of low-intensity cycling without sprinting (*E*). Prior to all visits at the laboratory, participants were instructed to refrain from intense exercise, caffeine, beta-alanine, and bicarbonate for at least 24 hours. Prior to the first experimental session, participants were also instructed to register food intake during the 24 hours leading up to the session, including time of consumption, and were instructed to repeat this prior to the second experimental session 24 hours. Each visit to the laboratory was separated by 4-9 days and started at the same time of day (~8.00 AM), in a controlled environmental condition (16-21°C and 20%-35% relative humidity), with a fan ensuring air circulation.

TABLE 1 Subject characteristics and physiological parameters at baseline

Body mass (kg)	**76 ± 3**
Height (cm)	183 ± 5
Power output at 4 mmol/L [Bla^-] (W/kg)	4.3 ± 0.6
VO_{2max} (mL/kg/min)	73.4 ± 4.0
W_{max} (W/kg)	6.3 ± 0.3
Wingate mean power output (W)	851 ± 64

Note: Maximal oxygen consumption (VO_{2max}), maximal power produced the last minute during incremental test to exhaustion (W_{max}), mean power output on a 30-s all-out sprint (Wingate). Values are mean ± standard deviation (SD), n = 12.

2.3 | Experimental protocols

2.3.1 | Blood and muscle sampling

Upon arrival, participants rested in the supine position for 30 minutes prior to venous blood sampling from the antecubital vein and muscle biopsy sampling from the m. Vastus Lateralis of a randomized leg, performed under local anesthesia (~2 mL Lidokain, Mylan Dublin, Ireland) using the micro-biopsy

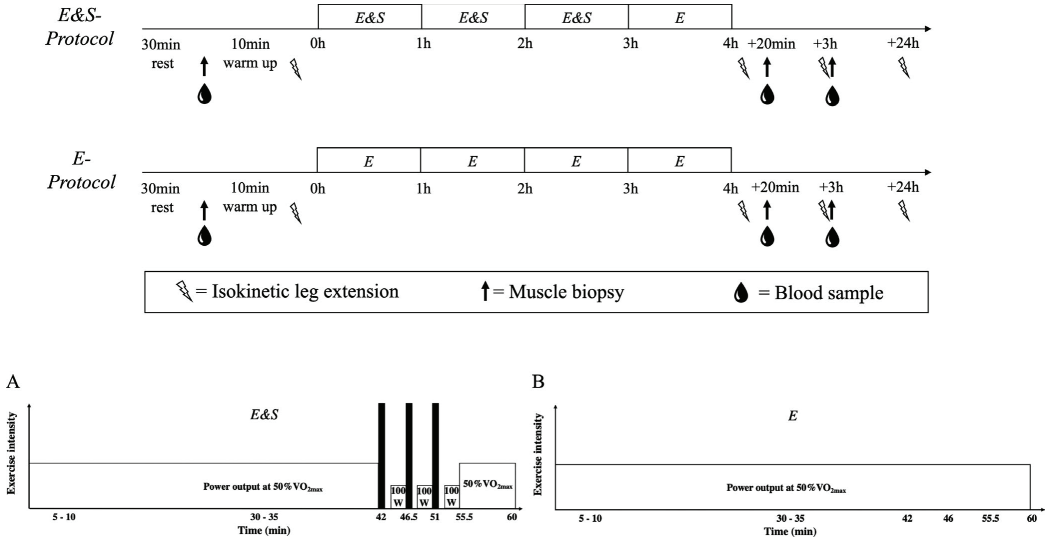

FIGURE 1 Overview of experimental protocols (two top panels) endurance exercise including sprints (E&S) and work-matched endurance exercise (E) and detailed description of every hour in E&S (panel A) and E protocols (panel B). Lightnings symbolize isokinetic knee extension, black arrows symbolize muscle biopsy, and blood drops indicate blood sample

technique (Bard Magnum, Bard Nordic, Helsingør, Denmark) with a 14-gauge needle (Medax medical devices, Poggio Rusco, Italy). Blood and muscle samples were collected before (Pre), 20-minute after (Post), and 3 hours after (3h) each experimental protocol. The first muscle biopsy was sampled one third of the distance from the patella to anterior superior iliac spine, with subsequent biopsies being sampled approximately 2 cm proximal to the previous sample. Blood was collected in 9-mL vacuettes containing Z Serum Clot Activator for serum (Greiner bio-one GmbH, Austria). After 30 minutes, collected blood was spun at 2600 rpm for 15 minutes on a KUBOTA 2420 (JZ4725-M000, Tokyo, Japan) and serum was separated from red blood cells, frozen, and stored at − 80°C until further analysis.

2.3.2 | Four-hour exercise protocols with and without sprinting

The *E&S* protocol consisted of 4-hour cycling at a power output equivalent to 50% of VO_{2max}, with 3 × 30-second maximal sprints interspersed by 4-minute recovery (1 minutes completely rest and 3-minute cycling at 100 W) 41 minutes into every hour during the first 3 hours. No sprinting was performed during the last hour (Figure 1). The *E* protocol consisted of 4-hour cycling at power output equivalent to 50% of power output at VO_{2max}. Overall, the average power output was 182 ± 4 W (mean ± SD) and 182 ± 4 W in *E&S* and *E*, respectively. Power output at 50% of VO_{2max} was calculated using submaximal values from the blood lactate profile test together with data from the VO_{2max} test. However, to ensure work-matched protocols, *E&S* involved slightly higher power output during steady-state periods (*E&S*: 186 ± 5 W vs *E*: 182 ± 4 W), as caused by the 4-minute recovery periods between sprints. As reported in a previous paper,[7] mean power output during each of the three sets of 30-second sprints in E&S was 787 ± 66 W (1. Set), 782 ± 59 W (2. Set), and 772 ± 60 W (3. Set), equivalent to 93 ± 1%, 92 ± 1%, and 91% ± 1% of values obtained during the all-out Wingate test. Metabolic cost of cycling increased by 5 ± 1% and 6 ± 1% from the first hour to the last hour of cycling in *E&S* and *E*, respectively, with no difference between protocols, coinciding with oxygen consumption increasing from 50 ± 1% to 53 ± 1% of VO_{2max} in *E&S* and from 48 ± 1% to 51 ± 1% of VO_{2max} in *E*. The two experimental protocols were separated by 6 ± 2 d and were performed in a randomized order.

2.3.3 | Isokinetic knee extension for evaluation of recovery

To evaluate recovery of muscle strength after *E&S* and *E*,[23] one-legged isokinetic knee extension was performed Pre, Post, 3 hours, and 24 hours after (24h) experimental protocols, using the leg that was not exposed to biopsy sampling. Isokinetic torque was evaluated at 60, 180, and 240°seconds^{-1} using an isokinetic dynamometer (Humac Norm, Computer sports Medicine Inc USA). Subjects performed 3 familiarization trials on both legs prior to experimental protocols. Peak torque of the best repetition was analyzed using the software (HUMAC 2015 v.15, Computer Sports Medicine Inc).

2.3.4 | Food and liquid consumption

During familiarization trials, participants consumed water, energy drink, and gels without caffeine (Squeezy Sports Nutrition GmbH, Germany) ad libitum to prevent dehydration and glycogen depletion. Consumption was recorded and duplicated during subsequent experimental sessions. Participants consumed 3.2 ± 0.1 L and 3.2 ± 0.1 L of energy drink and water, during *E&S* and *E*, amounting to 277 ± 17 g and 274 ± 15 g of carbohydrate, respectively. After completion of experimental tests (and after the first post-exercise muscle biopsy), participants received a body mass standardized meal 30 minutes after exercise, consisting of > 500 mL Chocolate Milk (Sjokomelk, Tine, Norway) and Fruit Müsli (Fruktmüsli, First Price, Germany), amounting to 0.36 g/kg protein, 1.17 g/kg carbohydrate, and 0.16 g/kg fat. Participants otherwise rested at the location and were only allowed to consume water during the preceding 3 hours.

2.4 | Blood and muscle analyses

Serum concentrations of total testosterone, cortisol, GH, sex hormone-binding globulin (SHBG), and IGF1 were measured using an Immulite 1000 ExUs edition (Siemens, USA) using kits from the Immulite Immunoassay System Menu (Siemens Medical Solutions Diagnostics, NY, USA). The ratio between free testosterone and SHBG was calculated from this.

Total RNA was extracted from muscle tissue using a combination of phase separation and silica-column clean-up. Muscle tissue (~30 mg) was homogenized in 200 μL of TRIzol® Reagent (Invitrogen, Life technologies AS, Oslo, Norway), using 0.5 mm RNAse-free Zirconium Oxide beads and a bead homogenizer (Bullet Blender, Next Advanced, Averill Park, NY, USA), according to manufacturer's instructions, as previously described.[24] Following homogenization, TRIzol® Reagent was added to a total volume of 1 mL and the homogenate was vortexed and incubated at room temperature for 5 minutes, after which 200 μL of chloroform was added, followed by 3 minutes of incubation and

subsequent phase separation by centrifugation (12 000 g, 10 minutes, 4°C). Four hundred microliters of the aqueous phase mixed with an equal volume of 100% ethanol and incubated 10 minutes at room temperature on a silica spin-column (Zymo-Spin™ IIC, Zymo Research, Irvin, USA). Following brief centrifugation, the flow-through was discarded and the column was washed by centrifugation once with RWT buffer and twice with RPE buffer (Qiagen Nordic, Oslo, Norway). RNA was eluted from the column in TE buffer heated to 60°C by centrifugation. RNA purity and quantity were assessed by evaluation of absorbance at 230, 260, and 280 nm using a micro-volume spectrophotometer (NanoDrop 2000, Thermo Scientific, USA).

Samples were reverse transcribed in duplicates (500 ng total RNA) using SuperScript® IV Reverse Transcriptase (Invitrogen, Life technologies AS, Oslo, Norway), using anchored Oligo-dT and random hexamer primers (Thermo Scientific, Life technologies AS, Oslo, Norway), according to manufacturer's instructions, as previously described.[24] Real-time RT-PCR was performed on 2 μL cDNA (1:50 dilution) in a 10 μL reaction volume, using 2X SYBR® Select Master Mix (Applied Biosystems, Life technologies AS, Oslo, Norway) and specific primers added at a 0.5 μmol/L final concentration, using a fast-cycling real-time detection system using Applied Biosystems™ QuantStudio 5 Real-Time PCR System (Thermo Fischer Scientific). Cycling consisted of 40 cycles; three seconds at 95°C followed by 30 seconds 60°C. Melt-curve analysis was performed for all reactions to verify single product amplification. Real-time RT-PCR parameters are presented in Table 2.

Quantification cycles (Cq) were determined using the second derivate method from raw fluorescence data, exported from the Applied Biosystems software and analyzed using the qpcR-library.[25] Primer efficiency values were estimated from single reactions and averaged for each primer pair.[25] Gene abundance data were efficiency corrected, and analysis was done using log-transformed values. In order to control for RNA quantity in cDNA synthesis and subsequent dilution, suitable reference genes were determined from systematic evaluation of 12 transcripts (sequences available on request) with modification for the present repeated-measures study design, as previously described.[24] Beta-2-microbulin (B2m), TATA-box-binding protein (TBP) and peptidyl-prolyl cis-trans isomerase A (PPIA), as their abundance did not change with sampling time-points or exercise condition. Normalization of target genes was thus performed using the geometric average of these three internal reference genes as described previously.[24]

TABLE 2 Sequences of qRT-PCR primers utilized for analysis of mRNA abundance

	Primers for qRT-PCR		Efficiency	Cq
Gene	**Forward primer**	**Reverse primer**	**Mean ± SD**	**Mean ± SD**
β2-m	TGACTTTGTCACAGCCCAAGA	CGGCATCTTCAAACCTCCATGA	1.98 ± 0.01	23.2 ± 0.3
CLCN1	TTCAGCGCCTTTGTGTTTCG	AATCCCGATGGCAGCAAAAG	1.82 ± 0.01	29.7 ± 1.2
IGF1	ATGTATTGCGCACCCCTCAA	GTACTTCCTTCTGGGTCTTGGG	1.86 ± 0.01	28.9 ± 0.6
MSTN	AGGAGAAGATGGGCTGAATCC	CCCTTCTGGATCTTTTTGGTGTG	1.91 ± 0.01	32.4 ± 0.9
ATP1A1	ATCCTTGAGTACACCTGGCTTG	TTTCCTTGCCATGCGTTTGG	1.62 ± 0.01	32.6 ± 0.8
ATP1B1	ATTTTGGACTGGGCAACTCC	ATTTGGGCTGCAGGAGTTTG	2.09 ± 0.01	23.6 ± 0.4
ATP1A2	TTCCTCGGGGCTTCAAATTC	ATGAGCCCCACAAAGCAAAG	2.16 ± 0.01	23.1 ± 0.6
SLC9A1	TCCATGCAAGTGCTGTTTGG	TTCTTCTGTACAGGCAGCAGAG	1.87 ± 0.01	31.0 ± 0.6
PDK4	CCAGACCAACCAATTCACATCG	TTCAACTGTTGCCCGCATTG	2.06 ± 0.01	25.2 ± 2.0
PGC1αs1	TATGGAGTGACATCGAGTGTGC	ACCCAGAAAGCTGTCTGTATCC	1.94 ± 0.01	26.2 ± 0.6
PGC1αs4	TGTGCCATATCTTCCAGTGACC	TGCAGTTCCAGAGAGTTCCAC	2.06 ± 0.01	27.5 ± 1.3
PPIA	AAGGGTTCCTGCTTTCACAG	TGTGAAGTCACCACCCTGAC	1.66 ± 0.01	25.6 ± 0.4
TBP	AACAGGTGCTAAAGTCAGAGCAG	ACGTCGTCTTCCTGAATCC	1.87 ± 0.01	30.8 ± 0.4
TFAM	AAAGCTCAGAACCCAGATGC	AATCAGGAAGTTCCCTCCAACG	2.06 ± 0.01	27.0 ± 0.3
THBS1	AACAAACAGGTGTGCAAGCC	ACTTGGCGTTCTTGTTGCAG	1.93 ± 0.01	29.5 ± 2.0
VEGFA	CCTGCAAAAACACAGACTCG	CTCGGCTTGTCACATCTGC	1.99 ± 0.01	26.2 ± 0.9

Note: Abbreviations: ATP1A1, Na^+-K^+ α1; ATP1A2, Na^+-K^+ α2; ATP1B1, Na^+-K^+ β1; CLC-1, chloride voltage-gated channel 1; IGF1, insulin-like growth factor 1; PDK4, pyruvate dehydrogenase kinase 4; PGC-1αs1, peroxisome proliferator-activated receptor gamma coactivator-1α splice 1; PGC-1αs4, peroxisome proliferator-activated receptor gamma coactivator-1α splice 4; RPL32, ribosomal protein L32; SLC9A1/NHE1, sodium-hydrogen exchanger 1; TBP, TATA-box binding protein; TFAM, mitochondrial transcription factor A; THSB1, thrombospondin; VEGFA, vascular endothelial growth factor A; β2-m, β2 microglobulin.

Average primer efficiencies and Cq-values for all reactions are given. Values are mean ± SD.

2.5 | Statistics

For Log2 fold changes in mRNA abundance and blood hormone levels, a marginal model was applied, wherein effects of time (Pre, Post, 3h) and exercise condition (*E&S*, *E*) and their interaction were assessed using SPSS-software version 23. Time and condition were specified as fixed effects. Repeated measures were specified by subject. To compare changes between conditions, a marginal model with baseline values as a co-variate was used. A significant main effect or interaction was further evaluated by a multiple-comparison approach with Sidak adjustment. A significance level of 0.05 was applied, and data were expressed as mean ± 95% confidence interval (CI). Gene abundance data are presented as Log2 fold change from pre-exercise ± 95% CI.

3 | RESULTS

3.1 | Gene abundance in m. Vastus lateralis

E&S led to different changes in abundance of mRNA on markers related to mitochondrial function, angiogenesis, ion transport, and protein synthesis compared with *E*. For markers of mitochondrial function, *E&S* was not associated with changes in PGC-1αs1 mRNA (Figure 2A), while *E* led

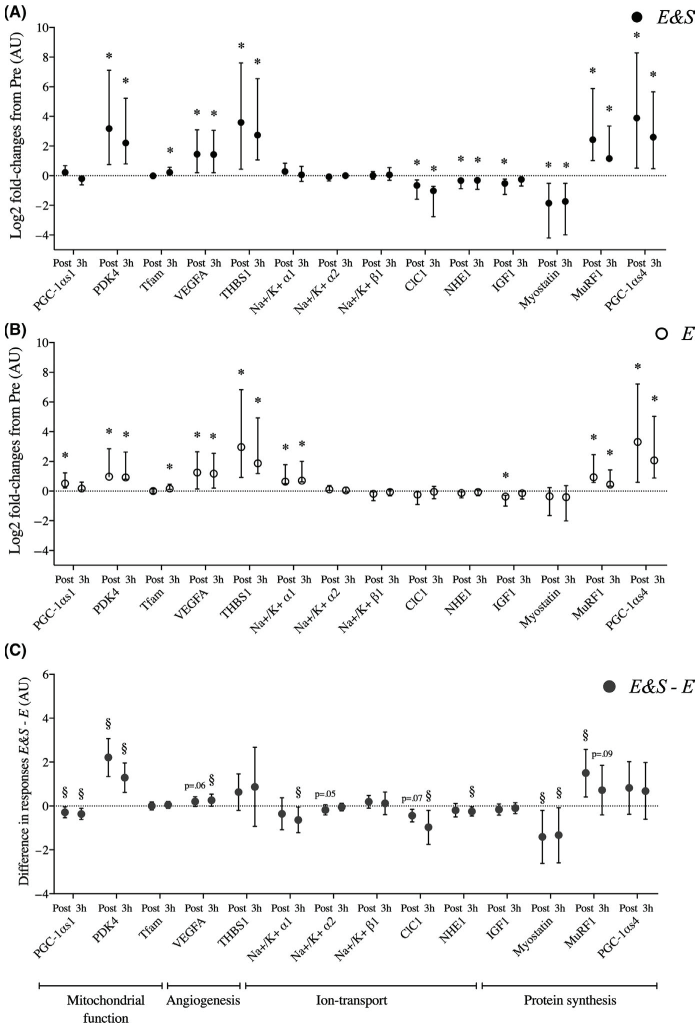

FIGURE 2 A and B) Effects of 4 h low-intensity cycling with (E&S, panel A) or without sprint intervals (E, panel B) on mRNA abundances of markers of mitochondrial function, angiogenesis, ion transport, and protein turnover in m. vastus lateralis of elite cyclists, measured directly after (Post) and 3 h exercise (3 h). Values are log2-fold changes with 95% CI. C, Differences in responses between E&S and E. Markers of mitochondrial function: peroxisome proliferator-activated receptor gamma coactivator-1α splice 1 (PGC-1αs1), pyruvate dehydrogenase kinase 4 (PDK4), mitochondrial transcription factor A (TFAM), angiogenesis: vascular endothelial growth factor A (VEGFA) and thrombospondin (THBS1). Markers of ion transport: Na^+-K^+ α1 (ATP1A1), Na^+-K^+ α2 (ATP1A2), and Na^+-K^+ β1 (ATP1B1), chloride voltage-gated channel 1 (CLC-1), sodium-hydrogen exchanger 1 (SLC9A1/NHE1). Markers of protein synthesis regulation: insulin-like growth factor 1 (IGF1), myostatin, muscle ring finger 1 (MuRF1), peroxisome proliferator-activated receptor gamma coactivator-1α splice 4 (PGC-1αs4). * indicates significant ($P < .05$) difference from pre-exercise, § indicates significant ($P < .05$) difference in response between conditions, tendencies to difference in responses are indicated by *P*-values. n = 12

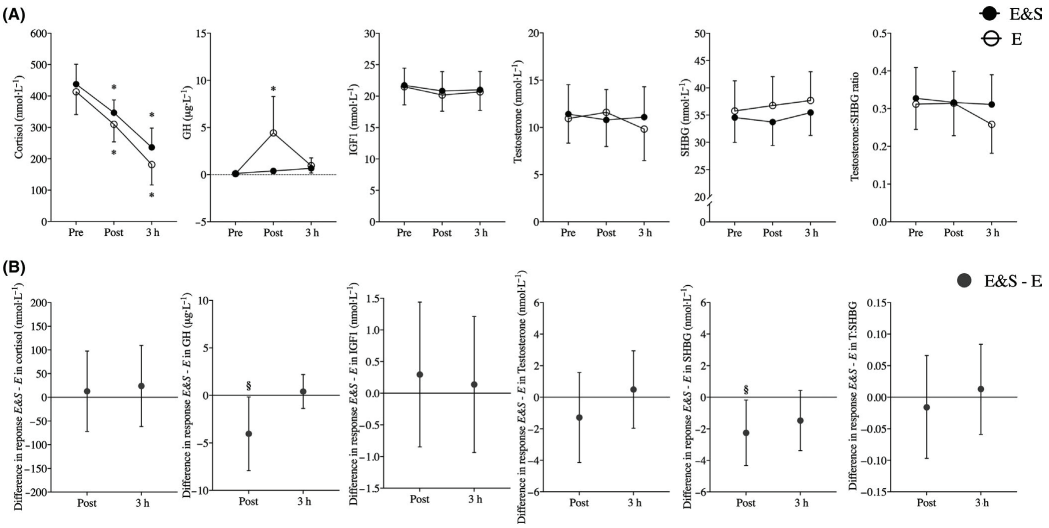

FIGURE 3 Blood hormone responses to 4-h low-intensity exercise with (E&S) or without sprints (E). A, Hormone concentrations in blood measured before (Pre), 20 min after (Post) and 3 h after exercise (3h). B, Differences in absolute changes in blood hormone concentrations between Pre and Post, and Pre and 3h (values are E&S – E). Cortisol, growth hormone (GH), insulin-like growth factor 1 (IGF1), testosterone, sex hormone-binding globulin (SHBG), and testosterone:SHBG ratio. * indicates significant ($P < .05$) difference from pre-exercise, § indicates significant ($P < .05$) difference in response between conditions, mean ± 95% CI, n = 12

to increased abundances at Post ($P < .05$; Figure 2B), but not at 3h. In effect, *E&S* was associated with reduced PGC-1αs1 mRNA levels compared with *E* (Figure 2C), measured as changes from Pre to Post (*E&S:* 1.2 ± 0.2 fold vs *E:* 1.5 ± 0.3 fold) and from Pre to 3h (*E&S:* 0.9 ± 0.2 fold vs *E:* 1.2 ± 0.3 fold). For PDK4, *E&S* and *E* led to increased mRNA abundances at both Post and 3h (Figure 2A+B), with responses being more pronounced in *E&S* at both Post (*E&S:* 11.9 ± 8.7-fold vs *E:* 2.8 ± 2.6-fold, $P < .05$) and 3h (*E&S:* 6.1 ± 3.9-fold vs *E:* 2.6 ± 2.4-fold, $P < .05$; Figure 2C). For both *E&S* and *E*, TFAM was only elevated 3h (both $P < .05$; Figure 2A+B) without differences between conditions (Figure 2C).

For markers of angiogenesis, *E&S* and *E* led to increased abundances of VEGFA and THBS1 mRNA at both Post and 3h (all $P < .05$; Figure 2A+B), with VEGFA responses being more pronounced in *E&S* at 3h (*E&S:* 2.8 ± 0.6-fold *vs E:* 2.3 ± 0.5-fold, $P < .05$; Figure 2C).

For markers of ion transportation, *E&S* was associated with suppression of mRNA levels for several components involved in Na^+, K^+, and H^+ transport compared with *E* (Figure 2A+B). While *E&S* had no effect on Na^+-K^+ α1 mRNA levels, *E* led to increased levels at both Post and 3h, resulting in differential responses between the two exercise modalities at 3h (*E&S:* 1.2 ± 0.4-fold vs *E:* 2.1 ± 1.2-fold $P < .05$; Figure 2C). *E&S* led to decreased levels of CLC1 mRNA at Post and 3h, with *E* having no effect (Figure 2A+B), resulting in negative changes in *E&S* compared with *E* at 3h (*E&S:* 0.6 ± 0.4-fold *vs E:* 1.1 ± 0.6-fold $P < .05$; Figure 2C). Neither of the two exercise modalities affected Na^+-K^+ α2 or Na^+-K^+ β1 mRNA abundances, and there were no differences between conditions.

For markers of protein synthesis, *E&S* and *E* led to reduced IGF1 mRNA abundance at Post ($P < .05$), and increased MuRF1 and PGC-1αs4 abundances at Post and 3h ($P < .05$; Figure 2A+B). *E&S* only led to decreased myostatin mRNA abundances ($P < .05$; Figure 2A). Overall, *E&S* was associated with reduced myostatin mRNA abundances compared with *E* at Post (*E&S:* 0.3 ± 0.2-fold vs *E:* 1.3 ± 0.8-fold, $P < .05$) and 3h (*E&S:* 0.3 ± 0.2-fold vs *E:* 2.1 ± 2.6-fold, $P < .05$; Figure 2C), and increased MuRF1 mRNA abundances at Post (*E&S:* 4.5 ± 1.2-fold vs *E:* 2.3 ± 0.9-fold, $P < .05$; Figure 2C), measured as changes from Pre.

3.2 | Blood hormone responses

Overall, *E&S* and *E* had little impact on hormonal concentrations in blood, with only cortisol showing clear-cut time-dependent changes (Figure 3A). For GH, *E&S* led to blunted responses at Post, contrasting the 4.3 ± 4.3 µg/L increase observed in *E*, resulting in negative changes in *E&S* compared with *E* ($P < .05$; Figure 3B). For SHBG, no effect was seen of either condition on blood concentrations (Figure 3A),

Paper III

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ORIGINAL RESEARCH
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Effects of Including Sprints in One Weekly Low-Intensity Training Session During the Transition Period of Elite Cyclists

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The purpose of this study was to investigate the effects of including 30-s sprints in one weekly low-intensity training (LIT) session during a 3-week transition period in elite cyclists. Sixteen male elite cyclists (maximal oxygen uptake, VO_{2max}: 72 ± 5 ml·kg^{-1}·min^{-1}) reduced their training load by ~60% for 3 weeks from the end of competitive season and performed only LIT or included 30-s sprints (SPR) in one weekly LIT-session. Performance and physiological capacities were evaluated during a prolonged (~2.5 h) test-session, including a strength test, a submaximal blood lactate profile test, an incremental test to exhaustion to determine VO_{2max}, 1 h continuous cycling including four maximal 30-s sprints, and a 20-min all-out test. In addition, mental recovery was evaluated using the Athlete Burnout Questionnaire (ARQ). The only significant between-group change during the transition period was an 8 ± 11% larger improvement in 30-s sprint performance in SPR compared to control (CON; SPR: 4 ± 5%, CON: −4 ± 5%, p = 0.01). Although not different from CON, SPR maintained 20-min all-out performance (−1 ± 5%, p = 0.37) and fractional utilization of VO_{2max} (1.9 ± 6.1%-points, p = 0.18) during the 20-min all-out test, whereas corresponding declines were observed in CON (−3 ± 5%, p = 0.04, and −2.5 ± 2.9%-points, p = 0.02, respectively). Power output at 4 mmol·L^{-1} blood lactate concentration decreased similarly in SPR (−4 ± 4%, p = 0.02) and CON (−5 ± 5%, p = 0.01), while VO_{2max}, maximal aerobic power (W_{max}), and total burnout score were unaffected in both groups. Including sprints in one weekly LIT-session in the transition period improves sprint performance and maintains 20-min all-out power and fractional utilization of VO_{2max} without compromising mental recovery. Inclusion of sprints in LIT-sessions may therefore be a plausible, time-efficient strategy during short periods of reduced training.

Keywords: periodization strategies, off-season, sprint training, elite athletes, athlete burnout questionnaire

INTRODUCTION

The annual training season for an elite cyclist can be broken into three distinct periods, the preparatory, competition, and transition period (Mujika et al., 2018). Elite cyclists typically spend up to 100 days in competition (Lucia et al., 2001), which is both a high physical and psychological exertion, with an inherent risk of burnout toward the end of the season (Silva, 1990; Lemyre et al., 2006). Although the need for a subsequent period of physical and mental recovery is regarded as necessary for elite athletes (Mujika et al., 2018), the manipulation of training in these transition periods is scarcely investigated (Garcia-Pallares et al., 2009; Ronnestad et al., 2014). To recover from the strenuous competition period, cyclists' training load is often drastically reduced for 2–3 weeks in the subsequent transition period (Lucia et al., 2000; Sassi et al., 2008). However, too long periods (>4 weeks) of training cessation might lead to deterioration of performance (Mujika and Padilla, 2000; Decroix et al., 2016; Maldonado-Martin et al., 2017).

Maintaining a minimum of training load in periods of decreased training volume seems necessary to avoid performance decrements (Mujika, 1998; Bosquet et al., 2007), with high-intensity training (HIT) playing a key role for maintenance of endurance performance (Neufer, 1989; Garcia-Pallares et al., 2009; Ronnestad et al., 2014). Maintenance of fitness in the transition period might also be crucial for continuous improvement in the following seasons of elite athletes (Mujika et al., 1995). Indeed, a study by Ronnestad et al. (2014) on well-trained cyclists showed that performing a HIT session every 7–10 days during an 8-week period following the competition period maintained power output at 4 mmol·L^{-1} [BLa^{-}], maximal oxygen uptake (VO_{2max}), and 40-min all-out performance better than low-intensity training (LIT; Ronnestad et al., 2014). However, performing HIT-sessions during the transition period where physical and mental recovery is needed might be too strenuous, leading to overreaching and burnout. Therefore, including sprint (SPR) training instead might be a beneficial, low-load alternative for elite cyclists.

Short maximal-effort intervals have been reported to be of less strain compared to longer HIT-intervals (Valstad et al., 2018) and might serve as an intensive stimulus, sufficient for maintaining endurance performance in shorter periods of reduced training volume. For example, the addition of sprint training in periods with 25–65% reductions in training volume has shown to maintain endurance performance-determining factors in moderately trained athletes (VO_{2max}, muscle oxidative capacity, and capillarization; Joyner and Coyle, 2008) and improved performance at or above intensities eliciting VO_{2max} (Bangsbo et al., 2009; Iaia et al., 2009; Skovgaard et al., 2018). Furthermore, including 30-s sprints every 10 min in 60-min LIT-sessions during an 8-week intervention has recently shown improved performance in trained cyclists (Gunnarsson et al., 2019). Therefore, implementing 30-s sprints in habitual LIT-sessions for short transition periods (3 weeks) might be a time-efficient strategy of relatively low strain for maintaining endurance performance.

Therefore, the main aim of this study was to investigate the effect of including 30-s sprints in one weekly LIT-session during a 3-week transition period on measures of sprint and endurance performance in elite cyclists, as well as the associated changes in physiological capacities and mental recovery. We hypothesized that inclusion of sprints during the transition period would improve sprint performance and maintain endurance performance-related measures compared to LIT only.

MATERIALS AND METHODS

Participants and Ethics Statement

Twenty-one cyclists volunteered for the study. Two participants withdrew due to circumstances unrelated to the study and three participants were excluded due to sickness or lack of adherence to the intervention, leaving a total of 16 participants. Physiological parameters, participants' characteristics, and training volume are presented in **Table 1**. All participants were informed of the possible risks and discomforts associated with the study and all gave their written informed consent to participate before commencing the study. The study was approved by the Local Ethical Committee at Inland Norway University of Applied Sciences and performed according to the Declaration of Helsinki, 1975. The study was a multi-center study conducted at four Norwegian universities with identical laboratory equipment using the same standardized testing procedures supervised by the same physician. To categorize the cyclists, the physiological characteristics suggested by De Pauw et al. (2013) was used.

TABLE 1 | Participants' characteristics measured 3–5 days after each cyclists' last competition and weekly training volume in the last 4 weeks of the competition period.

	SPR n = 7	CON n = 9	Group diff.
Age (years)	22.9 ± 3.0	21.1 ± 3.9	p = 0.32
Body mass (kg)	73.6 ± 9.0	73.1 ± 4.8	p = 0.89
VO_{2max} (L·min^{-1})	5.4 ± 0.7	5.2 ± 0.5	p = 0.57
VO_{2max} (ml·kg^{-1}·min^{-1})	73.4 ± 4.9	71.3 ± 4.5	p = 0.40
W_{max} (W)	439 ± 58	442 ± 48	p = 0.93
Power output at 4 mmol·L^{-1} [BLa^{-}] (W)	328 ± 66	321 ± 41	p = 0.80
Training volume 30 days prior to inclusion (h·wk.$^{-1}$)	14 ± 4	12 ± 3	p = 0.33
Reduction in iTRIMP training load (%)	−62 ± 9	−64 ± 11	p = 0.72

VO_{2max}, maximal oxygen uptake; W_{max}, maximal minute power output (W), reduction in training load per week from 4 week of competition period to 3 week of transition period quantified using individualized TRIMP, mean ± SD and matching of groups.

Eleven participants were regarded as performance level 5 athletes (VO_{2max}: >71 ml·kg^{-1}·min^{-1}, W_{max}: >5.5 W·kg^{-1}) and five participants were regarded as level 4 athletes (VO_{2max}: 65–71 ml·kg^{-1}·min^{-1}, W_{max}: 4.9–6.4 W·kg^{-1}), hence referred to as elite cyclists.

Experimental Design

The intervention was initiated 3–5 days after each cyclist's last competition of the season and was carried out over 21.2 ± 0.4 days. The participants were randomly assigned to either a SPR group or a control (CON) group. During the 4 weeks prior to the intervention, the cyclists performed on average the same number of training sessions per week (SPR: 6.4 ± 0.7 vs. CON: 6.2 ± 1.1 sessions, p = 0.80) of which an equal amount was characterized as HIT-sessions (SPR: 15 ± 10% vs. CON: 15 ± 9%, p = 0.95) and the training load from HIT was not different between groups (p = 0.24). SPR and CON reduced training load from the competition period to the transition period equally (**Table 1**), and only LIT was performed during the intervention (SPR: 13 ± 4 vs. CON: 12 ± 3 sessions, p = 0.58). However, once a week SPR performed a supervised 90-min LIT-session, riding at a power output equivalent to 60% of VO_{2max}, including three sets of 3 × 30-s maximal sprints, interspersed by 4-min of active recovery (100 W) and 15 min between sets. CON performed a time-matched supervised session at a power output equivalent to 60% of VO_{2max}.

Testing Procedures

The participants were instructed to refrain from caffeine, beta-alanine, and bicarbonate 24 h prior to testing. Participants were also instructed to register and duplicate food intake and time of consumption 24 h prior to both tests, but food diaries were not collected. All testing was performed on the same time of the day (±1 h) in a controlled environmental condition (16–18°C and 20–35% relative humidity) with a fan ensuring air circulation around the rider. A schematic presentation of the prolonged test protocol is outlined in **Figure 1**.

Strength Test

After a 10-min cycling warm-up at self-selected power output (150–200 W) a predetermined, standardized, 10-repetition incremental leg press test set to 250 kg for all participants on a Keiser AIR300 horizontal leg-press dynamometer (Keiser Sport health equipment INC., Fresno, CA) was initiated. Changes in strength parameters might affect the sprint ability and was therefore included (Ronnestad et al., 2017). The Keiser AIR300 uses pneumatic resistance to measure force and velocity in each repetition. The incremental test was performed in the seated position with a 90° knee-joint angle, starting at 41 kg and increasing to 250 kg at the tenth repetition with increased and standardized increments and rest-periods between repetitions. If the participant exceeded 250 kg, the test continued with 60-s rest between attempts until failure. The participants were instructed to push as explosively as possible until failure. The theoretical, maximal velocity (V_{max}), maximal force (F_{max}), and maximal power (P_{max}) was then calculated based on the second-order polynomial relationship between force and power (Colyer et al., 2018).

Blood Lactate Profile

After a 5-min break, a blood lactate [BLa$^-$] profile test to determine the relationship between power output, and [BLa$^-$] concentration during a submaximal continuous incremental test was initiated. This test has previously been described in detail (Ronnestad et al., 2010). Briefly, participants cycled for 5 min at 175 W, followed by 50-W increments every 5 min until a [BLa$^-$] of 3 mmol·L^{-1}, after which increments were 25 W. The test was terminated at a [BLa$^-$] of 4 mmol·L^{-1} or higher.

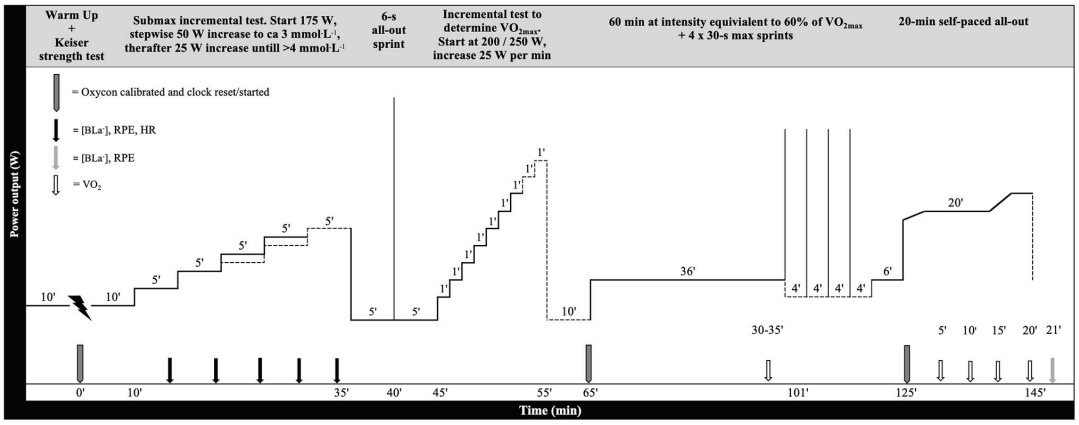

FIGURE 1 | Schematic illustration of the test protocol, including strength test, blood lactate [BLa$^-$] profile, 6-s all-out sprint, incremental test to exhaustion, and 60 min continuous cycling including 4 × 30-s maximal sprints and 20-min all-out.

All cycling tests were performed on an electromagnetic braked cycle ergometer (Lode Excalibur Sport, Lode B. V., Groningen, The Netherlands), which was adjusted to each cyclists' individual preferences and replicated throughout all testing. The fixed modus was used during continuous cycling, allowing the cyclists to freely choose frequency with a fixed resistance. VO_2 measurements started from 2 min into every bout and VO_2 was calculated as an average from 2.5 to 4.5 min. VO_2 was measured using a computerized metabolic system with mixing chamber (Oxycon Pro, Erich Jaeger, Hoechberg, Germany), which was calibrated every hour. Blood was sampled from the fingertip on completion of each 5-min bout and analyzed for whole blood [BLa^-] using a lactate analyzer (Biosen C line, EKF Diagnostic, Germany). Heart rate (HR) was recorded at the end of each steady-state increment using the participants' own HR-monitor and rate of perceived exertion (RPE) was recorded according to Borg Scale 6–20. Based on these measures, the power output at 4 mmol·L^{-1} [BLa^-] was calculated by interpolation and was used as a submaximal performance measure to compare each participant from Pre to Post.

6-s All-Out Sprint

After 5 min of active recovery, a 6-s all-out sprint was performed in the seated position with a stationary start and a resistance of 0.8 Nm•kg^{-1} body mass. Peak power output was defined as the highest value achieved during the 6-s all-out with recordings at 6 Hz.

VO_{2max} Test

After an additional 5 min of active recovery at ~150 W, an incremental test to exhaustion to determine VO_{2max} was initiated at 200 or 250 W depending on previous individual results. Power output increased by 25 W every minute until the RPM decreased below 60 min^{-1} despite audible encouragement from the test leader. VO_{2max} was calculated as the highest average of a 1-min moving average using 5-s VO_2-measurements and peak heart rate (HR_{peak}) was registered. W_{max} was calculated as the mean power output during the last minute of the incremental test.

60 min Continuous Cycling With 4 × 30-s Maximal Sprints and Subsequently 20-min All-Out

Ten minutes after the incremental test to exhaustion, the participants proceeded with a 60-min continuous cycling session at an intensity equivalent to 60% of VO_{2max}, which was calculated from the [BLa-] profile and VO_{2max} using interpolation. Four repeated 30-s maximal sprints separated by 4 min active recovery (100 W) were undertaken between 36–50 min and the test was concluded by a self-paced 20-min all-out without rest-periods in between (**Figure 1**). The chosen intensity of 60% of VO_{2max} corroborates well with reported intensities of competitions (van Erp and Sanders, 2020), making the repeated sprints and 20-min all-out competition-relevant performance measures. At Post, the participants rode at the same power output as Pre during the 60-min continuous cycling. The start power output on the 20-min all-out was self-selected at Pre and power and cadence was self-administered throughout the Pre and Post tests, however, the participants were blinded to the average power output. The start power output was replicated at Post to ensure the same pacing conditions. VO_2, HR, RPE, and [BLa^-] were measured during the test, according to **Figure 1**. During sprints, the resistance was set to 0.8 Nm·kg^{-1} in the Wingate modus and started at 80 RPM. Mean power output was presented as the 30-s average power output sustained throughout each maximal 30-s sprint. Fractional utilization of VO_{2max} during the 20-min all-out was calculated from an average of respiratory VO_2-measurements obtained in the periods 4–5, 9–10, 14–15, and 19–20 min, expressed relatively to VO_{2max} obtained at the respective time-point. VO_2-measurements started 30-s prior to each period to ensure steady measures of VO_2. Water, energy-drink in standard mixture according to manufacturer's description (HIGH-5, UK), and gels (SIS Isotonic Energy Gel, UK) without caffeine were provided ad libitum after the incremental test to exhaustion and throughout the test. All participants but one ingested energy-drink and gels during the experimental tests. The amount was recorded and repeated at Post to ensure the same relative hydration-level. On average, 745 ± 369 ml energy-drink and 44 ± 21 ml gel were consumed at Pre and 811 ± 454 ml (p = 0.37) energy-drink and 38 ± 24 ml gel (p = 0.17) were consumed at Post.

Gross efficiency (GE) was defined as the ratio between the mechanical power output (PO) and the metabolic power input (PI) calculated using VO_2 measurements and the energetic equivalent (Peronnet and Massicotte, 1991) PI = VO_2 L·s^{-1} x (4,840 J·L^{-1} × RER + 16,890 J·L^{-1}). GE was calculated from the [BLa-] profile test in the fresh state using the power output closest to that each participant rode at in the 60-min continuous cycling test. Equivalently, the GE in the semi-fatigued state was calculated using the steady-state period before sprinting (30–35 min) in the 60-min continuous cycling test (**Figure 1**). The power output was not different in fresh and semi-fatigued state in SPR (fresh: 227 ± 39 vs. semi-fatigued: 225 ± 41 W, p = 0.71) or CON (fresh: 215 ± 28 W vs. semi-fatigued: 219 ± 30 W, p = 0.35).

Training Load and Administration

Training load was quantified using the individualized training impulse (iTRIMP) as described elsewhere (Manzi et al., 2009), by weighing exercise intensity according to an individual's own HR vs. [BLa^-] relationship, calculated by line of best fit from the lactate profile and VO_{2max} test. iTRIMP uses the weighting factor y_i, which increases exponentially based on the HR vs. [La^-] relationship to weight every HR. An accumulated iTRIMP score was calculated by the following equation:

$$\text{iTRIMP}\left(\text{arbitrary units}\left(\text{AU}\right)\right) = \text{D}\left(\text{min}\right) \text{x}\ \Delta HR_{\text{ratio}}\ \text{x}\ y_i$$

where ΔHR_{ratio} is calculated from (HR_{work}−HR_{rest})/(HR_{max}−HR_{rest}), and D is time spent exercising. Design and training load administration is specified in **Figure 2**.

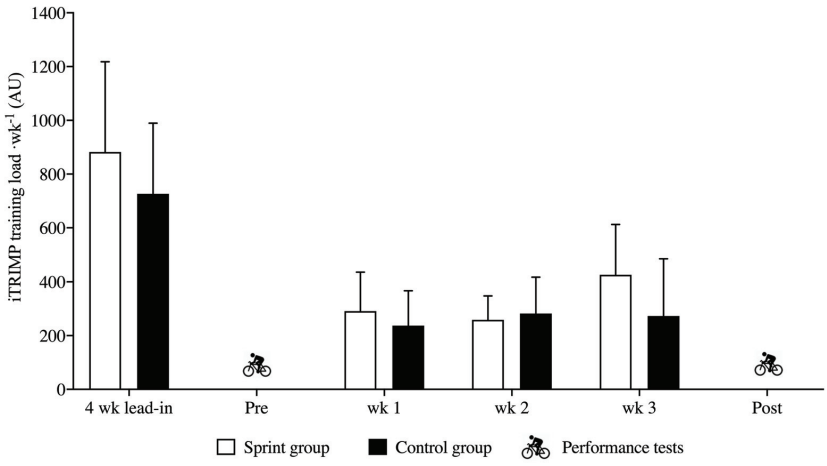

FIGURE 2 | Training load during lead-in and 3 week intervention using the individualized TRIMP method. Mean ± SD.

Athlete Burnout Questionnaire

To evaluate mental recovery, the 15-item sport-specific Athlete Burnout Questionnaire (ABQ) was used (Raedeke and Smith, 2001). Athletes were asked to rate "How often do you feel this way?" in 15 different statements to evaluate their participation motives in their sport on a 5-point Likert-scale from 1 = almost never to 5 = almost always. The ABQ has three 5-item subscales assessing three key dimensions of burnout: (1) reduced sense of accomplishment (e.g., "It seems that no matter what I do, I don't perform as well as I should"), (2) emotional and physical exhaustion (e.g., "I feel so tired from my training that I have trouble finding energy to do other things"), and (3) devaluation of sport participation (e.g., "The effort I spend participating in my sport would be better spent doing other things"). A total summarized score for the ABQ is achieved by averaging all three subscale scores. The questionnaires were completed at Pre and Post.

Statistics

Variables were tested for normal distribution using Shapiro-Wilk test. Based on a study on amateur road cyclists performing sprint training (Fortes et al., 2019), power calculations were made to determine the minimum of participants to include in the present study to detect changes in sprint performance. Based on the estimated effect size (ES) of 0.60 in changes in sprint performance when reducing training load for 3 weeks (Fortes et al., 2019) together with an alpha-level of 0.05, a power of 0.80, and a correlation between repeated measures of 0.50, the minimum sample size needed to determine significant differences in sprint performance was calculated to be eight in each group. A mixed linear model was applied to compare relative changes between groups in physiological, performance, and strength measures with group (and sprint) defined as fixed effects and corrected using Pre-values as a covariate using the software SPSS v.25. To compare main effects of time, a mixed linear model was applied with fixed effects defined by group, and time and random effects were defined by subject. Data are presented as mean ± SD. To evaluate the relationship between percentage changes in 20-min all-out performance and other performance measures, a stepwise, multiple linear regression was applied. The percentage changes in power output at 4 mmol·L^{-1} [BLa$^-$], absolute VO_{2max}, W_{max}, [BLa$^-$], and RPE at the end of 20-min all-out and fractional utilization during 20-min all-out, 30-s sprint performance, and GE in the semi-fatigued state were included in the model. For values expressed in %, the changes were calculated as percentage-points (%-points) by subtracting Post-values from Pre-values. All variables included in the final model had a variance inflation factor between 1.2–1.6 and $p < 0.05$. Whenever a significant main effect was obtained, a Sidak *post hoc* analysis was performed with an alpha-level of 0.05. Values of $p > 0.05$ and $p < 0.1$ were described as approaching significance. Hopkins' ES using pooled SD ± 95% confidence interval (CI) was calculated to highlight the practical significance of differences in performance changes between groups. Interpretations of the magnitude of ES were as follows: <0.2 trivial, 0.2–0.6 small, 0.6–1.2 moderate, 1.2–2.0 large, and 2.0–4.0 very large difference (Hopkins et al., 2009).

RESULTS

Sprint Performance

After the 3-week transition period, SPR had a larger increase in 30-s sprint performance than CON from Pre to Post (8 ± 11%, $p = 0.01$) with ES on changes between groups being moderate to large (ES: 0.6–1.7, **Figure 3B**).

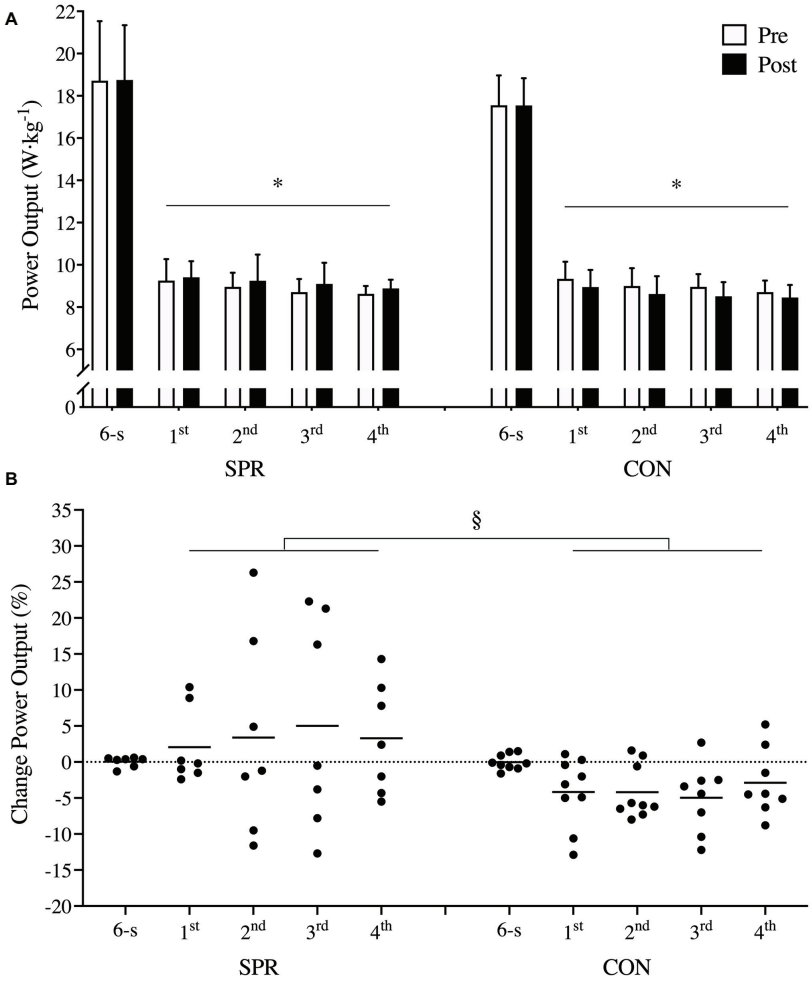

FIGURE 3 | Peak power output (W·kg^{-1}) on maximal 6-s sprint and mean power output on 4 × 30-s sprints (**A**, mean ± SD) before (Pre) and after (Post) a 3-week transition period of reduced training load in elite cyclists, including sprints in a low-intensity training session once a week [sprint group (SPR); $n = 7$] or only performing low-intensity training [control group (CON); $n = 8$] and individual percentage changes from Pre to Post **(B)**. *indicates main effect of time ($p < 0.05$). §indicates main effect of group on changes from Pre to Post ($p < 0.05$). Mean ± SD.

An overall, positive effect of time was observed in 30-s sprint performance in SPR ($p = 0.04$) and a negative effect of time was observed in CON ($p = 0.01$, **Figure 3A**). ES were considered small to moderate for all Post sprints in SPR (first sprint: 0.2 ± 0.3, second sprint: 0.4 ± 0.9, third sprint: 0.9 ± 1.1, fourth sprint: 0.7 ± 0.5) and small to moderate effects for CON (first sprint: −0.5 ± 0.3, second sprint: −0.5 ± 0.2, third sprint: −0.7 ± 0.3, fourth sprint: −0.5 ± 0.3) in relation to Pre. Peak power output during 6-s sprint did not change differently between groups ($p = 0.59$, **Figure 3B**) and did not change from Pre to Post in either group (**Figure 3A**).

20-min All-Out

Twenty-minutes all-out performance did not change differently between groups ($p = 0.63$, ES: 0.1, **Figure 4C**). However, 20-min all-out performance was maintained from Pre to Post in SPR (−1 ± 5%, $p = 0.37$, ES: −0.2 ± 0.4), whereas a small decline of −3 ± 5% was observed in CON ($p = 0.04$, ES: −0.4 ± 0.3, **Figure 4A**). Fractional utilization of VO_{2max} during 20-min all-out did not change differently between groups but the difference in change was considered moderate ($p = 0.19$, ES: 0.8, **Figure 4D**). Specifically, SPR maintained utilization from Pre to Post (1.9 ± 6.1%-points, $p = 0.18$,

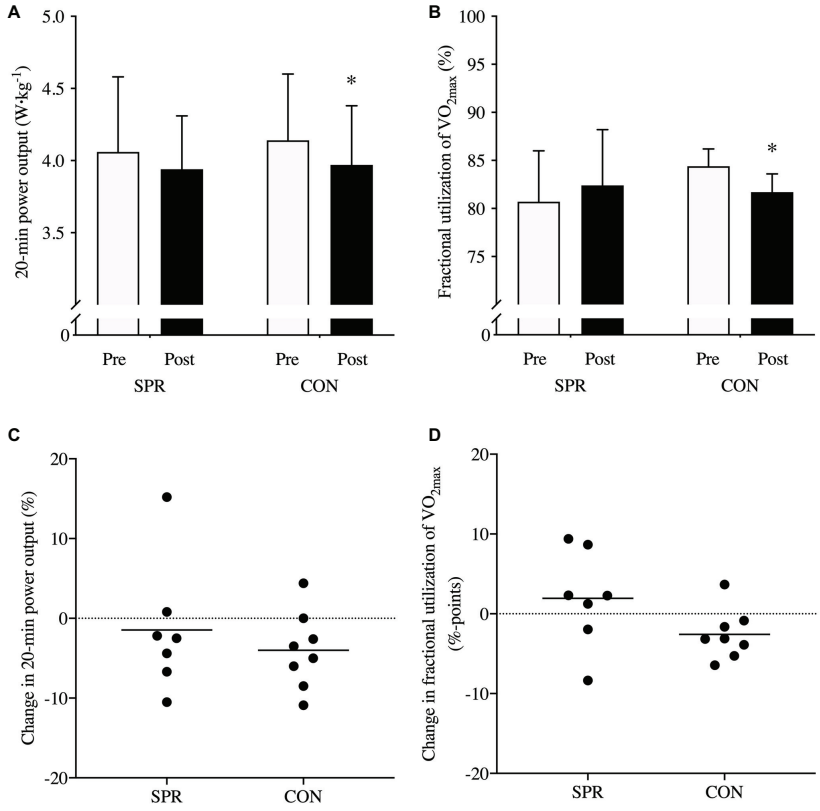

FIGURE 4 | 20-min all-out performance expressed in relative power output (W·kg^{-1}; **A**) and percentage change **(C)** from before (Pre) to after (Post) a 3-week transition period of reduced training load in elite cyclists including sprints in a low-intensity training session once a week (SPR group; $n = 7$) or only performing low-intensity training (CON group; $n = 8$). Fractional utilization of VO_{2max} during 20-min all-out (%; **B**) and changes in %-points from Pre to Post **(D)**. *indicates main effect of time ($p < 0.05$). Mean ± SD.

ES: 0.2 ± 4.5), whereas CON decreased moderately (−2.5 ± 2.9%-points, $p = 0.02$, ES: −0.6 ± 2.3, **Figure 4B**). [BLa$^-$] and RPE after 20-min all-out did not change differently between groups ($p = 0.54$ and $p = 0.26$, respectively) and was unaltered from Pre to Post in SPR and CON (**Table 2**). Likewise, the change in $\%HR_{peak}$ during 20-min all-out was not different between groups ($p = 0.18$) and was unaltered from Pre to Post in SPR and CON (**Table 2**). Stepwise multiple linear regression revealed that changes in fractional utilization of VO_{2max} during 20-min all-out ($p < 0.01$), VO_{2max} ($p < 0.01$), and GE in the semi-fatigued state ($p = 0.05$) explained the changes observed in 20-min all-out ($p < 0.01$, adjusted $R^2 = 0.89$).

Performance-Related Measures and Body Mass

Power output at 4 mmol·L^{-1} [BLa$^-$] decreased similarly from Pre to Post ($p = 0.83$, ES: 0.1, **Figure 5C**) in SPR (−4 ± 4%, $p = 0.02$, ES: −0.4 ± 0.2) and CON (−5 ± 5%, $p = 0.01$, ES: −0.6 ± 0.4, **Figure 5A**). Fractional utilization of VO_{2max} at 4 mmol·L^{-1} [BLa$^-$] did not change differently between groups but the ES was considered moderate ($p = 0.16$, ES: −1.0, **Figure 5D**). Specifically, SPR maintained fractional utilization of VO_{2max} at 4 mmol·L^{-1} [BLa$^-$] ($p = 0.69$, ES: 0.2 ± 1.1) while CON approached significance to decrease moderately ($p = 0.09$, ES: −1.0 ± 0.7, **Figure 5B**). GE did not change differently between groups from Pre to Post in fresh ($p = 0.18$) or semi-fatigued state ($p = 0.63$; **Table 3**). The change in GE from fresh to semi-fatigued state was not different between groups at Pre ($p = 0.13$) or Post ($p = 0.26$); however, GE decreased from the fresh state to the semi-fatigued state in SPR at Pre ($p = 0.02$). The increase in body mass did not differ between groups from Pre to Post ($p = 0.93$, ES: 0.0, **Table 2**). Specifically, body mass tended to increase in SPR ($p = 0.07$, ES: 0.1 ± 0.1) and increased in CON from Pre to Post ($p = 0.05$, ES: 0.1 ± 0.1, **Table 2**).

TABLE 2 | Changes (Δ) in performance-related measures and body mass from before (Pre) to after (Post) a 3-week transition period of reduced training load in elite cyclists including sprints in a low-intensity training session once a week [sprint group (SPR); *n* = 7] or only performing low-intensity training [control group (CON); *n* = 9]. Mean ± SD.

	SPR			CON		
	Pre	**Post**	**Δ**	**Pre**	**Post**	**Δ**
[BLa$^-$] 20-min all-out (mmol·L^{-1})	4.7 ± 3.3	5.6 ± 3.0	0.7 ± 2.2	7.0 ± 2.2	6.2 ± 1.6	−0.7 ± 2.2
RPE 20-min all-out	18.1 ± 2.9	18.1 ± 1.6	0.0 ± 2.8	18.9 ± 0.6	19.3 ± 1.1	0.4 ± 1.4
HR (% of HR_{peak})	91.8 ± 4.4	93.4 ± 1.3	1.6 ± 3.8	91.3 ± 0.9	92.3 ± 1.6	1.1 ± 1.6
GE fresh (%)	19.9 ± 1.0	19.5 ± 1.0	−0.4 ± 1.0	19.1 ± 1.0	19.2 ± 1.0	0.1 ± 1.0
GE semi-fatigued (%)	18.9 ± 1.0	18.9 ± 1.0	0.1 ± 1.0	19.1 ± 1.0	19.3 ± 1.0	−0.2 ± 1.1
ΔGE fresh vs. semi-fatigued	−1.0 ± 1.2†	−0.6 ± 1.1	−0.4 ± 1.2	0.0 ± 1.3	0.0 ± 1.1	0.0 ± 1.2
Body mass (kg)	73.6 ± 9.0	74.2 ± 9.4	0.7 ± 1.0	73.1 ± 4.8	73.7 ± 4.9*	0.8 ± 1.0
VO_{2max} (ml·min^{-1}·kg^{-1})	73.4 ± 4.9	71.4 ± 4.0	−2.5 ± 5.7	71.3 ± 4.5	71.0 ± 4.8	−0.5 ± 4.0
W_{max} (W·kg^{-1})	6.0 ± 0.3	6.0 ± 0.3	1.1 ± 6.5	6.0 ± 0.5	6.0 ± 0.4	−0.9 ± 4.9

%HR_{peak}, percent of peak heart rate during 20-min all-out; [BLa$^-$], blood lactate concentration measured 1 min after conclusion of 20-min all-out; RPE, rate of perceived exertion immediately after 20-min all-out; GE, gross efficiency measured in steady-state periods in the fresh and the semi-fatigued state during the ~2.5 h long test protocol; ΔGE, change in gross efficiency from the fresh state to the semi-fatigued state (%-points); VO_{2max}, maximal oxygen uptake; W_{max}, maximal minute power output.

**indicates main effect of time ($p < 0.05$).*

†significant difference between fresh and semi-fatigued state ($p < 0.05$).

There was no difference in changes between groups in VO_{2max} or W_{max} and both groups remained unchanged from Pre to Post (**Table 2**).

Strength Parameters

Maximal velocity, F_{max} and $P_{max,}$ did not change differently between groups (p = 0.13 p = 0.65, p = 0.36, respectively) and F_{max} and $P_{max,}$ did not change within the group from Pre to Post (**Table 3**). However, V_{max} was increased by 14 ± 19% from Pre to Post in CON (p = 0.02).

Burnout Symptoms

Total burnout did not change differently (p = 0.49) between SPR and CON and both groups were unchanged from Pre to Post. However, for the subscale "reduced sense of accomplishment," the difference in development between groups approached significance (p = 0.09). In the change from "rarely" toward "sometimes, SPR did not change (Pre: 2.3 ± 0.5 vs. Post: 2.2 ± 0.5, p = 0.62) whereas CON approached significance" (Pre: 2.5 ± 0.7 vs. Post: 2.8 ± 0.5, p = 0.04). For "devaluation," the difference in development between groups approached significance (p = 0.09) but neither SPR (Pre: 1.6 ± 0.6 vs. Post: 1.8 ± 0.7, p = 0.26) nor CON (Pre: 1.7 ± 0.4 vs. Post: 1.5 ± 0.4, p = 0.15) changed from Pre to Post. No group-differences or within-group changes were observed for "emotional and physical exhaustion."

DISCUSSION

The present study investigated the effects of including 30-s sprints in a LIT-session once a week during a 3-week transition period of reduced training load in elite cyclists. The main finding was that inclusion of sprints in SPR improved sprint performance compared to LIT only in CON who had a deterioration hereof. Although no group differences occurred, 20-min all-out performance and fractional utilization of VO_{2max} during the 20-min test were maintained in SPR, whereas small to moderate declines were observed in CON. Power output at 4 mmol·L^{-1} [BLa$^-$] was equally reduced in both groups, while VO_{2max}, W_{max} and total burnout were unaffected in both groups.

Sprint Performance

As expected, SPR improved 30-s sprint performance in the present study, whereas absence of sprinting led to deterioration of 30-s sprint performance in CON. Although sprint training has proven to be a potent training modality for both untrained and trained participants (Gist et al., 2014), this study is the first to show the potency of improving sprint performance in elite athletes even by inclusion of a relatively small amount of sprints (27 × 30-s) during the transition period. We also expected that an improved anaerobic capacity, indicated by the improved 30-s sprints, should improve short high-intensity endurance performance, such as W_{max} determined here. However, this was not the case in the present study, which is in contrast to previous studies where sprint training is added to the habitual volume of LIT (Laursen et al., 2002) or when sprints are implemented in LIT-sessions (Gunnarsson et al., 2019). This discrepancy could be related to the ~60% decrease in training load, the relatively short intervention of the present study, compared to previous studies (3 vs. 7–8 weeks) and smaller amounts of sprint training (27 vs. 96–144 × 30-s sprints; Laursen et al., 2002; Gunnarsson et al., 2019). In our approach, neither peak power during 6-s sprint nor V_{max} changed differently between groups but V_{max} was improved in CON only. This is in contrast to previous findings where improved peak power output (Fortes et al., 2019) and muscle strength (Martin et al., 1994) were found from short periods (2–4 weeks) of reduced training volume and maintained intensity-distribution in well-trained cyclists and runners.

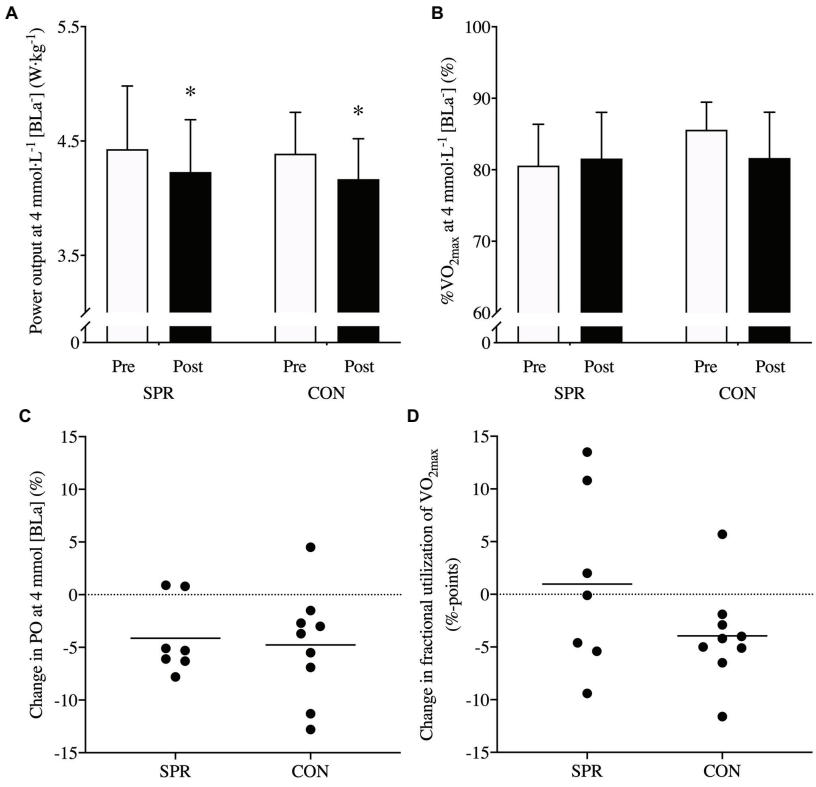

FIGURE 5 | Relative power output at 4 mmol·L^{-1} [BLa^{-}] W·kg^{-1} **(A)**, fractional utilization of VO_{2max} at 4 mmol·L^{-1} [BLa^{-}] **(B)** and individual changes in percentage and %-points **(C,D)** from before (Pre) to after (Post) a 3-week transition period of reduced training load in elite cyclists including sprints in a low-intensity training session once a week (SPR group; n = 7) or only performing low-intensity training (CON group; n = 9). *indicates main effect of time ($p < 0.05$). Mean ± SD.

TABLE 3 | Strength parameters from before (Pre) to after (Post) a 3-week transition period of reduced training load in elite cyclists including sprints in a low-intensity training session once a week [sprint group (SPR); n = 7] or only performing low-intensity training [control group (CON); n = 9].

	SPR			CON		
	Pre	**Post**	**Time**	**Pre**	**Post**	**Time**
V_{max} (M·S^{-1})	4.0 ± 0.8	4.1 ± 0.9	p = 0.87	3.8 ± 08	4.2 ± 0.5	p = 0.02
F_{max} (N)	3,030 ± 441	2,971 ± 528	p = 0.74	3,400 ± 902	3,095 ± 725	p = 0.11
P_{max} (W)	1,516 ± 332	1,524 ± 460	p = 0.88	1,553 ± 251	1,611 ± 310	p = 0.25

Effects of time are defined by p. Mean ± SD. V_{max}, maximal velocity; F_{max}, maximal force; P_{max}, maximal power.

Inactivity has previously been reported to change fiber-type distribution toward type IIX phenotype (Coyle et al., 1985; Andersen and Aagaard, 2000). Hypothetically, an absence of type II muscle fiber activation as might be assumed during 3 weeks of LIT only and an absence of muscular activation might therefore favor a switch in fiber-specific characteristics, toward a more fast-twitch phenotype, possibly explaining an improved V_{max} in CON.

20-min All-Out Performance

Although changes in 20-min all-out performance did not differ between groups, performance was unaltered in SPR, whereas a small decline of ~3% was observed in CON. However, the relevance of avoiding a decrease in 20-min all-out performance after prolonged cycling would arguably be of importance in cycling competitions (van Erp et al., 2019). Endurance performance, such as 20-min all-out test, is mainly determined

by fractional utilization of VO_{2max}, VO_{2max}, and efficiency and to a lesser fraction anaerobic capacity (Jeukendrup et al., 2000; Joyner and Coyle, 2008). In the current study, the maintained 20-min performance in SPR was coincided by maintained fractional utilization of VO_{2max} during the test, whereas it decreased by ~3%-points in CON. However, VO_{2max}, W_{max}, or GE in fresh state or semi-fatigued state did not change in any group, and both SPR and CON showed similar decreases in power output at 4 mmol·L^{-1} [BLa^-]. Thus, the different development pattern in fractional utilization of VO_{2max} within groups is probably the main explanation for the changes in 20-min all-out performance. This was further confirmed by a stepwise multiple linear regression analysis, where changes in fractional utilization of VO_{2max} during 20-min all-out, together with changes in VO_{2max} and GE explained 89% of the variance in 20-min all-out changes, supporting the importance of these variables for high-intensity endurance performances (Jeukendrup et al., 2000; Joyner and Coyle, 2008).

The reductions in submaximal exercise measures in CON, such as fractional utilization of VO_{2max} are possibly related to a decreased oxidative capacity (Coyle et al., 1984), which has been reviewed to decline with training reduction in a volume-dependent fashion (Neufer, 1989). Maintaining or increasing intensity of exercise during such reduced training volumes, however, seems of importance to maintain submaximal endurance performance (Neufer, 1989; Ronnestad et al., 2014), probably explaining the unchanged fractional utilizations of VO_{2max} in SPR. This is supported by a previous study of inclusion of sprint training in a 4-week period of 65% decreased training volume, where mitochondrial oxidative enzyme activity was maintained (Iaia et al., 2009). Furthermore, the present study and others (Neufer, 1989; Rietjens et al., 2001) show that maintaining 30–50% of the training volume maintains VO_{2max} in trained and elite cyclists for short periods (3 weeks). The importance of maintaining a minimum of endurance training is showed in studies where training cessation decreases VO_{2max} by 7–11% after 3–5 weeks in trained athletes (Coyle et al., 1984; Maldonado-Martin et al., 2017). Changes in blood volume and hemoglobin mass are regarded as main causes for changes in VO_{2max} (Coyle et al., 1986), and the unchanged VO_{2max} in the present study is supported by an unchanged blood volume and hemoglobin mass, although this measure was only performed on a sub-set of the participants (see **Appendix**). However, small decreases <200 ml in blood volume has recently been shown not to alter VO_{2max} (Skattebo et al., 2020), which could have been the case in our short intervention study. Overall, our study indicates that elite cyclists are able to reduce training load by ~60% for short periods without affecting the maximal aerobic power.

Mental Recovery

One might expect a decrease in the burnout markers during the transition period due to the great reduction in training load and absence of strenuous competitions, which has earlier been argued a necessity for elite athletes (Mujika et al., 2018). However, in our study, total burnout was unchanged from Pre to Post within both groups. The average score for all subscales were comparable to a recent study in a population of young elite-sportsmen (Gerber et al., 2018), and the general low scores in the mental subscales indicates a state of relatively low burnout in the elite cyclists, possibly explaining why this does not change during a 3-week transition period. In addition, only small changes were observed in the subscales, which indicates that changes in mental recovery might be difficult to measure during such short periods in a small group of elite cyclists. In any case, inclusion of sprints in one weekly LIT-session during the transition period does not seem to pose any effect on mental recovery compared to LIT only in elite cyclists with initially low levels of burnout scores.

Limitations

The relatively short intervention applied in the current study yields limited insight into the effects of including sprints in LIT-sessions on performance and mental recovery in elite cyclists. The lack of control of the training and competitions performed prior to the intervention might affect the outcomes, despite our effort for matching the groups according to training load and fitness. In addition, food consumption was not strictly controlled for in the present study and might have introduced unaccounted noise in the outcomes. However, with the unchanged body composition (see **Appendix**) and well-developed nutritional routines among elite athletes, we regard this possible effect to be small. After the short transition periods of typically 2–3 weeks, elite cyclists often increase training load gradually. Whether the current small, positive effects observed in SPR compared to CON translate into improved performance later in the preparatory period and competition period, however, needs further investigation.

In conclusion, including series of 30-s sprints in a LIT-session once a week during a 3-week transition period improves sprint performance compared to LIT only. In addition, 20-min all-out performance and fractional utilization of VO_{2max} was maintained in SPR while LIT only reduced these variables. Inclusion of sprints does not affect the power output at 4 mmol·L^{-1} [BLa^-], which was equally reduced in both groups. However, neither VO_{2max} and W_{max} nor total burnout seem affected by a 3-week transition period with severely reduced training load independent of sprinting. Inclusion of sprints in LIT-sessions may therefore be a plausible, time-efficient strategy to maintain performance for elite cyclist during short periods of reduced training load without affecting mental recovery.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Local Ethical Committee at Inland Norway

University of Applied Sciences. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NA, MS, MK, KS, ØS, and BR contributed to conception and design of the study. NA, IL, PB, MK, and KS executed the study and collected data. NA performed the statistical analysis. NA wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.01000/full#supplementary-material

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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.

Paper IV

Inclusion of 30-s sprints during low-intensity sessions during a high-load training camp improves performance in elite cyclists

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Conflict of interest

None

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Abstract

This study investigated the effects of including sprints during low-intensity training (LIT)-sessions during a 14-d training camp focusing on LIT, followed by 10 days recovery (REC), on performance and performance-related measures in elite cyclists. During the camp, a sprint training group (SPR, n=9) included 12x30-s maximal sprints during five LIT-sessions, whereas a control group (CON, n=9) performed distance-matched LIT only. Training load was equally increased in both groups by 48±27% and subsequently decreased by -56±23%, respectively, compared to habitual training. Performance tests were conducted before the training camp (Pre) and after REC. Muscle biopsies, haematological measures and stress/recovery questionnaires were collected Pre and after the camp (Post). SPR improved 30-s sprint (4±4%, $p<0.01$) and 5-min mean power (4±8%, $p=0.04$) more than CON. In muscle, Na^+-K^+β1 protein content changed differently between groups, decreasing in CON compared to SPR (-8±14%, $p=0.04$), while other proteins showed similar changes. SPR and CON displayed similar increases in red blood cell volume (SPR: 2.6±4.7%, $p=0.07$, CON: 3.9±4.5%, $p=0.02$) and VO_2 at 4 $mmol \cdot L^{-1}$ [BLa^-] (SPR: 2.5±3.3%, $p=0.03$, CON: 2.2±3.0%, $p=0.04$). No changes were seen in VO_{2max}, W_{max}, haematological measures, enzyme activity and stress/recovery measures. Inclusion of 30-s sprints during LIT-sessions during a training camp, improved competition-relevant performances more than LIT only without affecting sport-specific stress/recovery in elite cyclists.

Introduction

Road cycling competitions involve daily distances up to 300 km, with intensities varying from low-intensity to all-out sprinting[1,2] and competitions lasting from 1 to 22 days (i.e., the Vuelta a España). Elite cyclists therefore manipulate exercise stimuli throughout the annual training cycle to maximize training adaptations and meet the physiological requirements of these prolonged, strenuous competitions. The main performance-determining factors in cycling are maximal oxygen uptake (VO_{2max}), fractional utilization of VO_{2max} (%VO_{2max}) and gross efficiency[3,4] whereas body mass affects uphill performance i.e., in mountain stages[5]. The high VO_{2max} levels of elite cyclists

(70-80 mL·kg^{-1}·min^{-1}) and their high %VO_{2max} during prolonged exercise[1,6] are obtained through an immense volume of endurance training. Annual training volumes are reported to amount to 30-35,000 km and 900-1000 hours, with the majority (70-80%) of time spent at low intensity[7,8]. During the preparatory period, high-intensity exercise makes up only ~2-5% of the total training time, a proportion that is usually increased to ~8-18% during the competition period[7-9].

In elite cyclists, a common strategy to manipulate training stimulus is to increase training volume (hours and km) for 1-3 wk periods, often organized as training camps, followed by periods of less volume to avoid overreaching[10,11]. However, this increase in training volume is often not accompanied by increases in training of high intensity, and might thus provide a too low-intense and monotonous stimulus to lead to improvements in endurance performance[12]. Conversely, maintaining training intensity distribution during periods of increased volume will drastically increase the total training load, thus increasing the risk of overreaching[13]. In fact, several studies have shown that periods of concomitant increases in training volume and intensity result in decreased time-trial performance in trained cyclists and triathletes, as well as decline in performance indices such as VO_{2max} and maximal heart rate suggesting a state of overreaching[14-18].

A plausible strategy to maintain high-intensity stimulus during periods of increased LIT volume i.e., during training camps could be to add sprint training. Indeed, short maximal-effort intervals have been reported to be of less strain compared to longer HIT-intervals[19], and adding sprint training to a habitual volume of LIT has been shown to improve sprint performance as well as performance during 40-min tests in trained cyclists[20,21]. These benefits of sprint-related exercise likely result from peripheral adaptations in skeletal muscle such as increased enzyme activity[22,23] and improved ion-transportation[24], leading to improved aerobic and anaerobic metabolism and postponement of fatigue. Whereas these muscular adaptations can be measured rather rapidly after training sessions, improvements in performance are expected to appear after a subsequent recovery period[25].

However, dedicating singular sessions to sprint training might not be a time-efficient approach for elite cyclists during training camps. Therefore, including 30-s sprints during habitual LIT-sessions is an intriguing alternative that does not affect day-to-day muscular recovery[26]. Acutely, including 30-s sprints within a LIT-session amplifies exercise responses of markers relating to fat oxidation and angiogenesis compared to LIT only in muscle of elite cyclists[26], as well as mitochondrial function in well-trained cyclists[27,28]. After 8 weeks of training, this translates into increased citrate synthase (CS) protein content and phosphofructokinase (PFK) activity in trained subjects[23], while responses to such prolonged training remain scarcely investigated in elite cyclists[29,30]. The possible benefits of including sprints during habitual LIT-sessions of prolonged duration (>4 h) during a training camp with increased overall training load has not yet been investigated.

The primary aim of this study was to investigate the effects of including 30-s maximal sprints during five LIT-sessions during a 14-d training camp on 30-s sprint and 5-min performance in elite cyclists, measured after a 10-d recovery period, as well as muscular adaptations, haematological variables and stress/recovery measured immediately after the training camp.

Methods

Subjects

Nineteen male professional and amateur-elite cyclists were included in the study. Of these, 18 participants completed the intervention, with one drop-out due to reasons not related to the intervention. To categorize the cyclists, the physiological characteristics suggest by De Pauw et al. (2013) were used (Table 1)[31]. Sixteen participants were regarded as level 5 athletes (relative VO_{2max}: >71 mL·kg^{-1}·min^{-1}, relative W_{max}: >5.5 W·kg^{-1}) and two were regarded as performance

level 4 athletes (relative VO_{2max}: 65-71 $mL \cdot kg^{-1} \cdot min^{-1}$, relative W_{max}: 4.9-6.4 $W \cdot kg^{-1}$). The population is hence referred to as elite cyclists. All participants were regularly tested in the lab as part of the collaboration between the test laboratory and the national cycling clubs and pro-continental teams and were therefore accustomed to the testing procedures, maximal sprinting and self-paced performance tests. Before inclusion in the study, participant received and gave written informed consent to participate and were made fully aware of the possible risks and discomforts associated with the participation. The study was approved by the local ethics committee at Inland Norway University of Applied Sciences and was conducted in accordance with the Declaration of Helsinki, and was pre-registered in a public Norwegian database (Norwegian Center for Research data, 14/08/2017, project number 55322). The study was subsequently registered in Clinical Trials ref number: .

Insert Table 1 around here

Design

The study consisted of a 14-d training camp, followed by a 10-d recovery period (Figure 1), and was preceded by a 14-d lead-in period. During the lead-in period (prior to pre-testing, Pre), the habitual, individual training was recorded using the participants' own bicycle computer and heart rate monitors, which was uploaded to the online program (TrainingPeaks, Colorado, USA) for further analysis. To create as equal groups as possible, participants were pair-matched based on their total training load, VO_{2max} and sporting discipline/specification (mountain biking or road cycling/sprinter or climber) and assigned to a Sprint-group (SPR) or Control group (CON). A self-administered familiarization trial to combined sprint and LIT-session, consisting of 1-hr low-intensity endurance cycling and 4 x 30-s sprints, was performed on the day preceding Pre- and Rec-testing. Testing on Pre and Rec included 1) Dual-energy X-ray absorptiometry (DXA) scan, 2) muscle biopsy sampling, 3) performance testing and 4) haemoglobin-mass measurement (Hb-mass). The training camp started 5 ± 1 days after Pre-testing, and the daily training load, using the individualized training impulse method (iTRIMP), was increased equally between groups by 48 ± 27% compared to lead-in (Table 1). The two groups rode together but on five occasions during the 14-d training camp, SPR included four series of 3 x 30-s maximal sprints interspersed by 4 min of active recovery every hour during the LIT-session of at least 4 h in duration. In average 51 ± 12 sprints were completed during the camp in SPR. CON rode the same route without sprinting and were thereby matched on distance. All other sessions were individualized to reach the personal increase in training load ~50% compared to lead-in but were instructed to keep intensity low. Immediately after returning from the training camp, a DXA scan, a resting muscle biopsy and Hb-mass measurement were conducted (Post), followed by a recovery period of 10 ± 1 days where daily training load was equally reduced between groups by 56 ± 23% compared to lead-in, although frequency-distribution of training and intensity was maintained. Performance tests, DXA and Hb-mass measurement were performed after the recovery period (Rec). There was no difference in training load between SPR and CON in any part of the study and changes in load during the intervention were equal. To clarify training intensity distribution, training logs were analysed and categorized based on the 3-zone model presented by Sylta et al. (2014) into sessions of LIT (60-82% of peak heart rate), moderate-intensity training (83-87% of peak heart rate) and high-intensity training (88-100% peak heart rate)[32] and individual load was calculated for each session (Figure 1). A further categorization of the combined sprint and LIT-sessions (Sprint ex) and distance-matched LIT-sessions (Control ex) were also included.

Training load

Training load was quantified using the iTRIMP as described elsewhere[33], by weighting exercise intensity according to an individual's own HR vs [BLa^-] relationship, calculated by line of best fit from the lactate profile and VO_{2max} test. iTRIMP uses the weighting factor y_i, which increases exponentially based on the HR vs [La^-] relationship to weight every HR. An accumulated iTRIMP score was calculated by the following equation:

$$\text{iTRIMP (arbitrary units (AU))} = D\ (\text{min}) \times \Delta HR_{ratio} \times y_i$$

where ΔHR_{ratio} is calculated from $(HR_{work}-HR_{rest})/(HR_{max}-HR_{rest})$, and D is time spent exercising.

Insert Figure 1 around here

Testing procedures

The participants were instructed to refrain from caffeine, beta-alanine and bicarbonate 24 h prior to testing. Participants were also instructed to register and repeat food intake and time of consumption for the last 24 h leading up to both tests. All testing was performed on the same time of the day in a controlled environmental condition (16-18°C and 20-35% relative humidity) with a fan ensuring air circulation around the rider.

Body composition.

After an overnight fast, a DXA scan on a Lunar Prodigy (GE Healthcare, Chicago, Illinois, USA) was performed to determine body composition using the encore software (GE Healthcare v.17). All DXA-scans were performed by the same technician using standardized procedures and the technician was blinded for Pre and Post measures when analysing the images.

Muscle and blood sampling

After at least 2 h of fasting and resting for 30 min in a supine position, a blood sample was collected from the antecubital vein and manually analysed for haematocrit (Hct) in quadruplicate after a 5-min spin (14,800 RPM, Thermo Scientific Heraeus Pico 21) and haemoglobin concentration was determined on ABL800 (Radiometer, Copenhagen, Denmark). Subsequently, a muscle sample was collected from m. Vastus Lateralis of a randomized leg using the micro biopsy technique (Bard Magnum, Bard Nordic, Helsingør, Denmark), using 14-gauge needles (Medax medical devices, Poggio Rusco, Italy) under local anaesthesia (2-3 mL Lidokain, Mylan Dublin, Ireland) as described elsewhere[26]. The first biopsy was sampled at one third of the distance from the patella to anterior superior iliac spine with subsequent biopsies sampled approximately 2 cm proximal to the previous sample from the same leg on the day of return from training camp. Muscle samples were freeze dried in a Christ Alpha 1-2 LDplus freeze dryer, (Vakuum-Service A.S, Norway) and dissected free from blood and connective tissue before homogenization of ~1.0-4 mg d.w, for western blotting and enzyme activity assays.

Blood lactate profile test and VO_{2max} test

Following biopsy sampling at Pre and on Rec, participants performed a blood lactate profile test as described elsewhere[34]. Briefly, participants cycled for 5 min at 175 W, followed by 50-W increments every 5 min until a blood lactate concentration ([BLa^-]) of 3 $mmol \cdot L^{-1}$, after which increments were 25 W. The test was terminated at a [BLa^-] of 4 $mmol \cdot L^{-1}$ or higher. All cycling tests were performed on an electromagnetic braked cycle ergometer (Lode Excalibur Sport, Lode B. V., Groningen, The Netherlands) which was adjusted to the cyclist and replicated throughout all testing. VO_2 measurements started from 2 min into every bout and VO_2 was calculated as an average from 2.5 to 4.5 min. VO_2 was measured using a computerized metabolic system with mixing chamber (Oxycon Pro, Erich Jaeger, Hoechberg, Germany) which was calibrated every

hour. Blood was sampled from the fingertip at the end of each 5-min bout and analysed for whole blood [BLa^-] using a lactate analyser (Biosen C_line, EKF Diagnostic, Germany). Based on these measures, the power output at 4 $mmol \cdot L^{-1}$ [BLa^-] was calculated using interpolation and was used as a submaximal performance measure to compare each participant from Pre to Rec.

After 10-min of active recovery, an incremental test to exhaustion was initiated to determine VO_{2max} with 1-min increments, starting at 200 W. Power output increased by 25 W every minute until the RPM dropped below $60 \cdot min^{-1}$ despite audible encouragement from test leader. VO_2 was measured using a computerized metabolic system with mixing chamber (Oxycon Pro, Erich Jaeger, Hoechberg, Germany) with standard calibration procedures. VO_{2max} was calculated as the highest average of a 1-min moving average using 5-s VO_2-measurements. W_{max} was calculated as the mean power output during the last minute of the incremental test. Gross efficiency, defined as the ratio between the mechanical power output, and the metabolic power input was calculated in the fresh state during the blood lactate profile test when riding at 225 W and in the following period of 60 min continuous cycling in the semi-fatigued state at an average power output of 234 ± 32 W and 235 ± 23 W in SPR and CON, respectively. Gross efficiency was calculated using the oxygen equivalent and respiratory exchange ratio (RER) as described previously[35]: Power input = $VO_2\ L \cdot s^{-1} \cdot (4840\ J \cdot L^{-1} \cdot RER + 16{,}890\ J \cdot L^{-1})$. Participants were asked to maintain the same pedalling frequency throughout periods of oxygen uptake measures.

60 min continuous cycling including 4 x 30-s maximal sprints and subsequent 5-min test

Ten min after the incremental test, a 60-min continuous cycling test was performed using a similar design from our lab[21]. Briefly, the test was conducted at a power output corresponding to 60% of VO_{2max}, calculated from blood lactate profile and VO_{2max} tests using interpolation, and included four repeated 30-s maximal sprints, performed between 36-50 min, and separated by 4 min active rest (100 W). The test was concluded by a self-paced 5-min test. During sprints, the resistance was set to 0.8 $nm \cdot kg^{-1}$ using the Wingate-modus, and the test started at 80 RPM and was performed in a seated position. Participants were blinded to the average power output during the 5-min test, but the resistance was self-administered. The start power output on the 5-min test was replicated from Pre on Rec to ensure similar pacing conditions. Gels (Enervit Sport Gel, Sweden) and energy-drink (Squeezy, Norway) without caffeine was provided ad libitum after the incremental test to exhaustion and throughout the remainder of the test protocol. The amount of nutritional intake was recorded at Pre and repeated at Rec. Mean power output during 30-s sprints were recorded as the 30-s average power output obtained during each sprint. VO_2 was recorded from 34-36 min and during 5-min and recording started 30-s prior to every VO_2-measure. $\%VO_{2max}$ was calculated from VO_2-measurements obtained during the blood lactate profile test and throughout the 5-min test and expressed relatively to VO_{2max} ($\%VO_{2max}$).

Haematological measures

After a cool-down from performance testing, participants rested for 20 min in a semi-recumbent position and Hb-mass was determined using a modified version of the carbon monoxide (CO) rebreathing technique, as described elsewhere[36], using OpCO (WGT, Austria). Briefly, the participant breathed 100% O_2 for 3.5 min before a blood sample was drawn from the fingertip (125 µL) and immediately analysed in quadruplicate for carboxy-Hb (%HbCO) on a hemoximeter (ABL800, Radiometer, Copenhagen, Denmark). Subsequently, the participants rebreathed a bolus of chemically pure CO (Multigas SA, Domdidier, Switzerland), corresponding to 1.5 $mL \cdot kg^{-1}$, mixed with O_2 for 9 min 25 s. A sensor registered and regulated the O_2-level during the rebreathing. After rebreathing, blood was sampled from the finger, analysed for %HbCO in quadruplicate. The change in %HbCO between first and second measurement was used to calculate Hb-mass with a

standard correction of 2.2 % of CO remaining in the system[37]. Hct measured from the blood sample collected prior to performance testing was used together with Hb-mass to calculate total red blood cell volume (RBCV), total blood volume and plasma volume using the following calculations as described earlier[38]:

$$Hb_{mass} = 644 \times nCO_{abs} \times 25/\Delta HbCO$$

where $\Delta HbCO$ is the change in %HbCO between the blood sample before and after administration of CO-dose.

$$RBCV\ (mL) = Hb_{mass} \times Hct/[Hb]$$
$$BV\ (mL) = RBCV \times 100\ /Hct$$
$$PV\ (mL) = BV - RBCV$$

Stress-Recovery state

The short version of the Recovery-Stress Questionnaire for Athletes (RESTQ-36-R-Sport) was used[39] to map the stress-recovery state of participants at several time points during the intervention: at Pre, three times during the training camp and at Rec. The 36 questions are divided in 12 subscales with 3 items for each subscale. The participants filled out the questionnaire in the morning, based on the past 3 days/nights, using a 7-point scale ranging from 0 (never) to 6 (always).

In addition, on days following sprint and control sessions during the training camp, participants were asked after breakfast to evaluate the intensity of yesterday's session using rate of perceived exertion (session RPE)[40] evaluated on a modified version (1-10) of the original Borg-scale[41] and their motivation to exercise by answering "how motivated are you to exercise today?" on a 9-point scale going from very, very motivated to very, very demotivated.

Muscle analyses

Western blotting

Preparation and analyses of muscle tissue was conducted using the same protocol as previously described[26]. Samples were homogenized for ~120 s using a plastic pestle in 80 µL·mg^{-1} fresh lysis buffer [2mM HEPES, pH 7.4; 1mM EDTA, pH 7.0; 5mM EGTA, pH 7.5; 10mM $MgCl_2$; 1% Triton-X-100; phosphatase, and protease inhibitors]. Subsequent to homogenization the samples were rotated end-over-end for 1 h and centrifuged for 10 min at 10000 g to separate undissolved tissue from the supernatant. Afterwards, the supernatant was carefully separated from the pellet and stored at -80°C until further analysis. Protein concentration was determined using the Pierce Detergent Compatible Bradfor Assay Kit #23246. Briefly, 5 µL samples were diluted 1:10 in ddH_2O and loaded in triplicates onto a 96-well micro titer plate, mixed with 250 µL Pierce Detergent Compatible Bradford Assay Reagent, and measured spectrophotometrically at 595 nm using a Multiscan FC microplate reader (Thermo Fisher Scientific), using the SkanIt software 2.5.1 for Multiscan (Thermo Scientific). Pierce Serum Albumin standards with protein concentrations ranging from 0.025 to 2.0 mg·mL^{-1} was used to create a standard curve. Protein concentrations were calculated from the standard curve after correction for the absorbance of the ddH_2O.

The lysates were normalized to a protein concentration of 2.0 µg·µL^{-1} in fresh HEPES. The lysates were prepared with a 4 x Laemmli sample buffer (Bio-Rad Laboratories AB, Oslo, Norway) containing 10% 2-Mercaptoethanol and heated for 5 min at 95°C. Proteins samples (15 µg of total protein) were separated at 300 V for 60 min using an Invitrogen gel (Novex™ 4-20% Tris-Glycine Plus Midi), followed by wet transfer to a PVDF membrane (0.2 µm Immun-Blot, Bio-Rad) at 400

mA for 60 min. For each participant, all samples were loaded on the same gel in technical duplicates. Membranes were then stained using a reversible total protein stain (Pierce Reversible Protein Stain, Thermo Fischer Scientific) to ensure appropriate protein transfer and to control for loading. Membranes were then blocked using 3% Bovine Serum Albumin in Tris-buffered Saline including 0.1% Tween-20 (TBST) for 60 min at room temperature, before overnight incubation in primary antibody on a rocking table at 4°C. Membranes were then washed 2 x 5 min in TBST, followed by incubation in a TBST solution containing 5% skimmed milk and horseradish-peroxidase-conjugated secondary antibody for 60 min at room temperature. The membranes were then washed 4 x 5 min in TBS-T, and bands were visualized using chemiluminescent detection (SuperSignal, West Femto Maximum Sensitivity Substrate, Thermo Fischer Scientific) and recorded with a digital camera (G:BOX, Syngene) with the software GENESys, Chemi-XR5. Band intensities were quantified using Image Lab 6.0.1 (Bio-Rad, Laboratories), adjusted for background intensity. Samples were expressed relative to total protein stain and normalized to a human pool (HP) containing equal amounts of all Pre-samples, which was loaded onto each gel in duplicates. Primary antibodies were purchased from Abcam; Anti-Citrate synthase, 1:2000 (ab96600), anti-HADH, 1:8000 (ab154088), Santa Cruz Biotechnology; PFK-1, 1:500 (sc166722), and Thermo Fischer Scientific; Na^+-K^+ β1, 1:1000 (MA3-930).

Enzyme activity

CS and phosphofructokinase (PFK) activity were assayed in muscle lysates using commercially available kits (CS: CS0720, PFK: MAK093, St. Louis, MO, Sigma-Aldrich) according to the manufacturer's instructions as described previously[42]. All activities were normalized to protein concentration as described above and expressed in international $mU \cdot mg^{-1}$ protein.

Statistics

All variables were tested for normal distribution using Shapiro-Wilk test and were log-transformed to obtain normality if not. To compare relative changes in physiological, performance, muscular and haematological measures from Pre to Post between groups, a mixed linear model was applied with group (and sprint) defined as fixed effects and corrected using Pre-values as a covariate using the software SPSS v.25. To compare main effects of time and group a mixed linear model was applied with fixed effects defined by group and time and random effects were defined by subject. Stress-recovery measures were tested for normal distribution by a Shapiro-Wilk test and main effects of time, group and interaction was tested using a 2-way ANOVA for repeated, dependent measures with an alpha-level of 0.05. Data are presented as mean ± SD unless otherwise stated. Whenever a significant main effect was obtained a Sidak post hoc analysis was performed with an alpha-level of .05 and p-values >0.05 and <0.1 were described as tendencies. Hopkins' effect sizes (ES) using pooled SD was calculated to compare the practical significance of differences in changes between conditions[43]. Interpretations of the magnitude of ES were as follows: <0.2 trivial, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large and 2.0-4.0 very large difference.

Results

Sprints

Mean power output of the four repeated 30-s sprints changed differently between groups (4 ± 4%, $p<0.01$, figure 2B). On average, SPR led to 3 ± 2% improvements in mean power during the four sprints from Pre to Rec ($p<0.01$), whereas CON remained unchanged (-1 ± 2%, $p=0.12$, figure 2A). Effect sizes on changes were small to moderate in favour of SPR (ES: 0.4, 0.3, 1.0 and 1.0, respectively).

Insert figure 2 around here

5-min test

5-min mean power output changed differently between groups from Pre to Rec (4 ± 8%, p=0.04, ES: 0.5, figure 3C), though neither SPR (2 ± 4%, p=0.14) nor CON (-2 ± 4%, p=0.14) led to changes in power output (figure 3A). $\%VO_{2max}$ did not change differently between groups from Pre to Rec (p=0.81, ES: 0.5, Figure 3D), but tended to increase in SPR (p=0.07), whereas it remained unchanged in CON (p=0.35, Figure 3B). [BLa^-] measured 1 min after the 5-min test increased more in SPR compared to CON from Pre to Rec (p<0.01). Specifically, SPR tended to increase [BLa^-] (Pre: 12.2 ± 2.7 vs Rec: 14.2 ± 2.8, p=0.05) and CON tended to decrease (Pre: 12.6 ± 2.7 vs Rec: 10.7 ± 2.7, p=0.06). RPE did not change differently (p=0.16), with SPR remaining unchanged (Pre: 19.2 ± 1.0 vs Rec: 19.6 ± 0.5, p=0.51) and CON tending to decrease (Pre: 19.6 ± 0.5 vs Rec: 18.7 ± 1.7, p=0.09).

Insert figure 3 around here

Blood lactate profile test, VO_{2max} test and gross efficiency in fresh and semi-fatigued state

There were no differential changes between groups for any measures from the blood lactate profile test or the VO_{2max} test from Pre to Rec. For most variables, neither SPR nor CON led to changes from Pre to Rec (Table 2). However, VO_2 at 4 $mmol \cdot L^{-1}$ [BLa^-] increased in both SPR (p=0.03) and CON (p=0.04) without affecting $\%VO_{2max}$. Gross efficiency did not change differently between groups from Pre to Rec, neither in the fresh or semi-fatigued state (Table 2). Specifically, gross efficiency was unchanged in SPR in both fresh and semi-fatigued state, whereas it tended to decrease and decreased, respectively in CON from Pre to Rec (fresh: -0.4 ± 0.6%-point, p=0.08, semi-fatigued: -0.7 ± 0.6%-point, p<0.01). No differences were observed in RER between states, groups or time.

Insert table 2 around here

Body composition and haematological measures

Body mass, lean body mass and body fat did not change differently between groups and was unaltered throughout the intervention in both SPR and CON (Table 3). BV, PV and MCV did not change differently between groups and was unaltered from Pre to Post and Rec (table 3). Hb-mass did not change differently between groups and was unaltered in SPR whereas it tended to increase in CON from Pre to REC (2.3 ± 3.1%, p=0.07). RBCV increased equally between groups (p=0.38, ES: 0.1) and tended to increase in SPR (2.6 ± 4.7%, p=0.07) and increased in CON (3.9 ± 4.5%, p=0.02) from Pre to Rec.

Insert table 3 around here

Muscle protein quantity and enzyme activity

Protein contents of CS (p=0.12, ES: 0.6), HAD (p=0.95, ES: 0.3) and PFK (p=0.70, ES: 0.4) did not change differently between groups (Figure 4A-C). Specifically, for CS, protein content was unchanged in SPR from Pre to Post (2 ± 18%, p=0.96), whereas it tended to decrease in CON (-9 ± 8%, p=0.06, figure 4A). For HAD, protein content was unchanged in both SPR (-1 ± 33%, p=0.58) and CON from Pre to Post (5 ± 38%, p= .97, figure 4B). For PFK, protein content was reduced in both SPR (-14 ± 13%, p=0.02) and CON from Pre to Post (-17 ± 12%, p<0.01, figure 4C). In contrast, protein content of Na^+-K^+ β1 changed differently between groups from Pre to Post (-8 ±

14%, p=0.04, ES: 0.6). Specifically, Na^+-K^+ β1 content was maintained in SPR (2 ± 7%, p=0.53), whereas it decreased by -6 ± 7% in CON (p=0.02, Figure 4D). Enzyme activities of CS (p=0.16, ES: 0.6) and PFK (p= 0.96, ES: 0.6) did not change differently between groups and were not changed from Pre to Post in either SPR (CS: 20 ± 40%, PFK: 7 ± 30%) or CON (CS: -2 ± 17%, PFK: -6 ± 10%, Figure 4E+F).

Insert figure 4 around here

REST-Q, session RPE and motivation to exercise

There was no difference between groups in total stress or total recovery during the intervention and both groups remained unchanged throughout (Figure 5A+B).When asked for the session RPE of yesterday's sprint- or control-workout, SPR rated the first three workouts as heavier compared to CON (Figure 5C), while the 4th and the 5th workout was rated equally exhaustive As such, session RPE decreased from the first to the fourth and fifth workout in SPR. Motivation to exercise was not different between SPR and CON on the morning after sprint- or control-workouts, but decreased in CON from the first to the last workout whereas it did not change during the training camp in SPR (Figure 5D).

Insert figure 5 around here

Discussion

The present study investigated the effects of including 30-s sprints during five LIT-sessions during a 14-d high-load training camp, followed by a 10-d recovery period, on sprint and endurance performance, performance-related variables and stress/recovery markers in elite cyclists. SPR displayed ~4% larger improvements in 30-s sprint and 5-min mean power from Pre to REC compared to CON. Protein content of Na^+-K^+ β1 was maintained in SPR, while it decreased by -8% in CON compared to SPR from Pre to Post, with no other differences in protein abundance and enzyme activity being evident between groups. The increased training load during the camp led to similar increases in RBCV and VO_2 at 4 $mmol \cdot L^{-1}$ [BLa^-] in SPR and CON from Pre to Rec, with no changes being evident in VO_{2max}, W_{max} or haematological measures. Stress and recovery measures were not affected by the intervention in any of the groups.

The small to moderate positive increases in 30-s sprint and 5-min mean power in SPR compared to CON, were accompanied by larger increases in [BLa^-] and a tendency towards increased $\%VO_{2max}$ during the 5-min test and higher RPE in SPR. This supports the notion that inclusion of sprints during prolonged LIT-sessions has a positive effect on anaerobic characteristics as well as on the ability to sustain work at high effort[23,25]. While this is the first study to examine inclusion of sprints in LIT-sessions during a high-load training camp, the findings confirm data from a recent study on elite cyclists from our laboratory[21]. In that study, inclusion of sprints during a LIT-session once a week during a 3-wk transition period with reduced training load led to larger increases in $\%VO_{2max}$ during a 20-min test compared to LIT only in elite cyclists[21]. In contrast to this, inclusion of sprints during ~1 h LIT-sessions in the study by Gunnarsson et al. (2019), did not lead to greater improvements in 45-min mean power compared to LIT only in trained subjects[23]. However, 45-min mean power did improve in the sprint group by 4% in the study by Gunnarsson et al. (2019) and similar improvements in 40-min mean power (~3%) have been reported when adding sprint training to a habitual LIT-based training program in well-trained cyclists[20]. The more pronounced benefits of adding sprints to LIT-sessions for endurance performance in the current study might be explained by the longer duration of LIT-sessions (>4 h), the cessation of HIT or sprint training in

our control group, and the more anaerobic nature of the 5-min test compared to the much longer ~40-45-min tests in previous studies[20,23].

In the present study, inclusion of sprints during LIT-sessions during a training camp of marked increases in training load was associated with maintained gross efficiency in the fresh and semi-fatigued state in SPR, whereas it decreased in the semi-fatigued state in CON. Although not different from CON, the maintained gross efficiency in the semi-fatigued state in SPR might be related to a decline in muscle antagonist activity, as has been reported after a period of sprinting[44], which may have affected gross efficiency. Improved gross efficiency exclusively in the semi-fatigued state has previously been reported in studies of combined strength and endurance training[45,46], consequently improving 5-min performance in the semi-fatigued state after prolonged exercise, without affecting performance in the fresh state (W_{max}). This would arguably translate into maintained gross efficiency during prolonged exercise, which is of relevance during the up to 300 km long cycling competitions, maintaining or improving competition-relevant performance, and might therefore also partly explain the improved 5-min mean power in SPR compared to CON. It thus seems necessary to sustain conduction of high-intense training (i.e., HIT or sprinting) during periods dominated by LIT to maintain high levels of gross efficiency as previously suggested[44,47], especially in the semi-fatigued state.

The observed performance improvements in SPR might also be related to muscular adaptations such as increased enzyme activity and protein content of mitochondrial enzymes[22,23]. For example, enzyme activity of CS and HAD have been reported to increase with increased training load from spring to summer in 4 elite cyclists[29]. However, after the relatively short intervention performed in the present study, involving a limited number of elite cyclists, protein content of CS and HAD together with CS enzyme activity remained unchanged in both groups. Furthermore, PFK protein content decreased in both groups, whereas PFK enzyme activity remained unchanged. These discrepancies between our data and other studies might be due to a combination of difference in fitness-level and duration of the intervention. A study on trained subjects including sprints during ~1 h LIT-sessions for 8 wks has been shown to lead to greater increases in CS protein content and PFK-activity compared to LIT only[23]. Interestingly, in the present study, CS content showed a numerical decrease in CON-only during the training camp, with the effect size being small to moderate compared to SPR (ES: 0.6), reiterating on the potential importance of maintaining high-intense exercise stimulus such as sprinting during periods of predominantly LIT in elite cyclists. Furthermore, whereas Na^+-K^+ β1 protein content was maintained in SPR, it decreased in CON, despite the overall increase in LIT-based training volume, with the response being different between groups. As such, previous studies have highlighted that there is an association between training intensity and changes in Na^+-K^+-ATPase expression in muscle in well-trained individuals, with increased training intensity being necessary to increase expression[48], contrasting the more readily occurring changes seen in untrained individuals[49]. Maintaining ion-transportation capacities might play a role during all-out performances[24], whereas a decrease in ion-transport might accelerate obscuring of skeletal muscle homeostasis during high-intensity exercises such as the 5-min test. This could therefore contribute to explain the difference in 5-min mean power between SPR and CON. Notably, muscle characteristics are scarcely investigated in elite cyclists and the present study gives one of the first insights into the muscular adaptation to a habitual alteration in training load in the preparatory period of elite cyclists.

Total stress and recovery measures did not change during the intervention in either SPR or CON and did not differ between groups, suggesting that implementing sprints during LIT during a period of augmented training volume was well-tolerated by the elite participants. Notably, the total stress was low, with corresponding high levels of total recovery, corroborating well with levels previously reported for elite athletes during a training camp[50]. This emphasizes that elite cyclists in

general cope well with habitual increases in training loads during training camps lasting 2 to 3 wks, and this does not negatively affect their mental state[14]. Interestingly, the relative monotony of LIT in CON tended to reduce motivation for training towards the end of the training camp, whereas inclusion of sprints during five LIT-sessions seemed to prevent this staleness, since motivation was rated as "good" throughout the entire camp. In a previous study of similar changes in training load, trained cyclists indicated a state of overreaching with increased stress scores, decreased sprint and 40-min mean power and decreased levels of VO_{2max}[15], which might relate to a negative energy balance[51]. In the current study, the participants maintained body composition throughout the training camp and the subsequent recovery period and did none indicated a state of overreaching since both mental state and performance measures were unaltered or improved. This might suggest that elite cyclists are better to match energy intake and output compared to merely trained cyclists and probably also tolerate habitual increases in training load during training camps. Interestingly, inclusion of sprints during habitual LIT-sessions were initially experienced as more strenuous, but this experience decreased gradually throughout the training camp. Compared to LIT only, inclusion of sprints was therefore rated as harder in the first three sessions, despite similar cycling distances, but was not rated as harder in the last session. This indicates a familiarization effect and supports the notion that inclusion of sprints does not affect recovery of muscular strength between daily exercises[35]. The gradual psychological (and physiological) habituation to performing 30-s sprints during LIT-sessions compared to LIT only indicates that this regimen might be beneficial to implement in the preparatory period without affecting the time to recover.

In general, the 14-d training camp was associated with 48% increases in training load, followed by a subsequent 10-d recovery period of -56% reduced training loads compared to habitual training. In both SPR and CON, this led to increased RBCV and VO_2 at 4 $mmol \cdot L^{-1}$ $[BLa^-]$, though without affecting BV, $\%VO_{2max}$ and power output at 4 $mmol \cdot L^{-1}$ $[BLa^-]$ or VO_{2max}. Only CON tended to increase Hb-mass (~2%), but was not different from SPR. This borderline increase was small compared to reports in elite cyclists (~5%) after a 5-wk exercise-heat acclimation intervention and was of similar magnitude to the typical error of measurement reported in our lab[52]. If this minor change in Hb-mass was of relevance for endurance performance, it should arguably have affected 5-min mean power, which was not observed. The increases in RBCV in both SPR and CON support previous evidence of increased levels of reticulocytes[53] and RBCV[54] after periods of training overload and recovery in well-trained athletes. Unsurprisingly, the observed increases in RBCV in our highly trained elite cyclists were slightly lower than those seen after 2-4 wks of increased endurance training in untrained or moderately trained subjects[55]. The high fitness level of our participants may have limited the adaptive capacity to habitual changes in training load and could also explain why we did not observe changes in VO_{2max}. In this context, the general lack of effects of the intervention on BV, $\%VO_{2max}$ and power output at 4 $mmol \cdot L^{-1}$ $[BLa^-]$, VO_{2max} and W_{max} may also be attributed to the lack of more demanding HIT in both groups during the intervention period. Although the repeated sprint exercises were performed at maximal effort, time spent above 90% of VO_{2max} is minimal[56] and was likely insufficient to lead to increases in VO_{2max} in elite cyclists. Regretfully, the current study did not include a negative control group i.e. elite cyclists that did not change their total training load. However, the present results still indicate that habitual training camps focusing on LIT-only do not improve competition-relevant cycling performance in elite cyclists.

In conclusion, including 30-s sprints during five LIT-sessions during a training camp in the preparatory period improved repeated sprint-ability and 5-min performance more than LIT-only, without affecting total stress/recovery in elite cyclists. In addition, SPR was associated with maintained Na^+-K^+ β1 protein content in muscle compared to CON. Together with a tendency towards increased $\%VO_{2max}$ on the 5-min test, this suggests that inclusion of sprints during LIT

improves competition-relevant performances and performance-related indices in elite cyclists. Finally, the 14-day training camp with an overall increase in training load in both SPR and CON, followed by a subsequent recovery period led to increased RBCV and VO_2 at 4 $mmol \cdot L^{-1}$ [BLa^-] in both groups but had no effect on BV, $\%VO_{2max}$ and power output at 4 $mmol \cdot L^{-1}$ [BLa^-], VO_{2max} or W_{max}. This suggests that training camps focusing on LIT-only, do not improve competition-relevant cycling performance (as measured in the present study), contrasting the beneficial effects of including sprints during LIT-sessions, which thus constitutes an intriguing addition to habitual training of elite athletes.

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Author contributions statement

NA, SE, ØS, and BR contributed to conception and design of the study. NA and MW executed the study and collected data. NA performed the statistical analysis. NA wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Additional information

The authors have no competing financial or non-financial interests to declare.

Tables

Table 1: Subject characteristics, physiological parameters and training load based on iTRIMP of 18 elite male cyclists determined during an incremental lactate profile and incremental maximal exercise test. Values are mean ± SD and matching of groups.

	SPR	CON	Matching
Age (Years)	20.9 ± 1.4	21.0 ± 1.7	p=0.96
Height (cm)	185 ± 5	183 ± 6	p=0.45
Body mass (kg)	73.6 ± 8.4	74.3 ± 5.0	p=0.85
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	75.4 ± 5.2	75.1 ± 5.4	p=0.91
W_{max} ($W \cdot kg^{-1}$)	6.5 ± 0.4	6.4 ± 0.3	p=0.97
Power output at 4 $mmol \cdot L^{-1}$ [BLa^-] ($W \cdot kg^{-1}$)	4.5 ± 0.3	4.4 ± 0.4	p=0.85
Increase in TL during camp (%)	50 ± 32	47 ± 23	p=0.68
Decrease in TL during recovery (%)	-53 ± 32	-59 ± 10	p=0.59

SPR: Sprint-group, CON: Control-group, VO_{2max}: Maximal oxygen consumption, W_{max}: Maximal aerobic power produced the last minute during incremental test to exhaustion, TL: daily training load calculated using the iTRIMP calculation (AU) relative to Lead-in load.

Table 2: Blood lactate profile test and VO_{2max} test. Data are mean ± SD.

	SPR			CON		
	Pre	Rec	Δ (%)	Pre	Rec	Δ (%)
			Absolute values			
VO_2 at 4 mmol·L^{-1} [BLa$^-$] (mL·min^{-1})	4394 ± 555	4494 ± 50*	2.5 ± 3.3	4423 ± 385	4520 ± 399*	2.2 ± 3.0
Power output at 4 mmol·L^{-1} [BLa$^-$] (W)	329 ± 47	332 ± 35	1.3 ± 4.1	330 ± 42	336 ± 32	2.3 ± 4.1
VO_{2max} (mL·min^{-1})	5538 ± 631	5629 ± 639	1.8 ± 5.4	5564 ± 370	5594 ± 495	0.5 ± 5.4
W_{max} (W)	476 ± 49	479 ± 43	1.0 ± 3.2	475 ± 41	477 ± 39	0.5 ± 3.2
			Relative values			
Power output at 4 mmol·L^{-1} [BLa$^-$] (W·kg^{-1})	4.5 ± 0.3	4.5 ± 0.2	0.4 ± 3.7	4.4 ± 0.4	4.5 ± 0.3	1.5 ± 3.7
%VO_{2max} at 4 mmol·L^{-1} [BLa$^-$] (%)	79.3 ± 3.2	79.9 ± 4.5	0.7 ± 3.7	79.5 ± 5.4	80.9 ± 4.9	1.4 ± 3.7
W_{max} (W·kg^{-1})	6.5 ± 0.5	6.5 ± 0.4	-0.2 ± 2.8	6.4 ± 0.3	6.4 ± 0.4	-0.1 ± 2.8
VO_{2max} (mL·kg^{-1}·min^{-1})	75.4 ± 5.2	75.9 ± 6.7	0.6 ± 6.7	75.1 ± 5.4	75.0 ± 6.7	-0.1 ± 6.7
Gross efficiency fresh (%)	19.7 ± 0.7	19.5 ± 0.8	-0.2 ± 0.6	19.8 ± 0.5	19.4 ± 0.6#	-0.4 ± 0.6
Gross efficiency semi-fatigued (%)	19.1 ± 0.8	18.9 ± 0.7	-0.4 ± 0.6	19.4 ± 0.7	18.8 ± 0.7*	-0.7 ± 0.6

Absolute and relative measures of power output at 4 mmol·L^{-1} [BLa^{-1}], maximal oxygen uptake (VO_{2max}), maximal aerobic power output (W_{max}), fractional utilization of VO_{2max} at 4 mmol·L^{-1} [BLa$^-$] (%VO_{2max} at 4 mmol·L^{-1} [BLa$^-$]) and gross efficiency in the fresh state during the blood lactate profile at 225 W and in the semi-fatigued state during the 60 min continuous cycling at an average power output of 234 ± 32 W and 235 ± 23 W in S and C, respectively. Before (Pre) and after 14-d training camp and 10-d recovery period (Rec) in Sprint-group (SPR, n=9) and Control-group (CON, n=9), and percentage or percentage-point changes. * indicates main effect of time ($p<0.05$). # indicates tendency to effect of time ($p<0.1$).

Table 3: Body composition and haematological measures. Data are mean ± SD.

	SPR			CON		
	Pre n=9	Post n=7	Rec n=9	Pre n=9	Post n=6	Rec n=9
BM (kg)	74.1 ± 7.5	74.1 ± 6.5	74.2 ± 8.1	74.4 ± 4.9	75.0 ± 6.5	74.5 ± 4.7
LBM (kg)	62.0 ± 6.2	62.4 ± 5.4	62.1 ± 5.1	60.8 ± 4.1	61.5 ± 5.4	60.6 ± 3.8
Body fat (%)	12.6 ± 1.5	12.1 ± 2.5	12.6 ± 2.1	14.6 ± 2.6	14.5 ± 2.5	15.0 ± 3.1
Hb-mass (g)	959 ± 133	950 ± 128	959 ± 116	963 ± 86	976 ± 83	984 ± 75#
BV (mL)	6769 ± 783	6668 ± 861	6739 ± 769	6596 ± 734	6728 ± 482	6604 ± 612
PV (mL)	3878 ± 400	3782 ± 606	3752 ± 489	3747 ± 481	3817 ± 295	3654 ± 405
MCV (fL)	90.5 ± 7.4	90.4 ± 4.3	91.0 ± 4.7	87.4 ± 2.7	88.3 ± 3.3	88.1 ± 3.4
RBCV (mL)	2891 ± 406	2886 ± 416	2981 ± 368#	2849 ± 305	2911 ± 265	2951 ± 227*

Body mass (BM), lean body mass (LBM), body fat (%) measured by DXA-scan and haemoglobin mass (Hb-mass), blood volume (BV), plasma volume (PV) red blood cell volume (RBCV) and mean corpuscular volume (MCV) before (Pre), after 14-d training camp (Post) and after 10-d recovery period (Rec) in Sprint-group (SPR) and Control-group (CON). * indicates significant ($p<0.05$) different from Pre. # indicates tendency to effect of time ($p<0.1$).

Figure legends

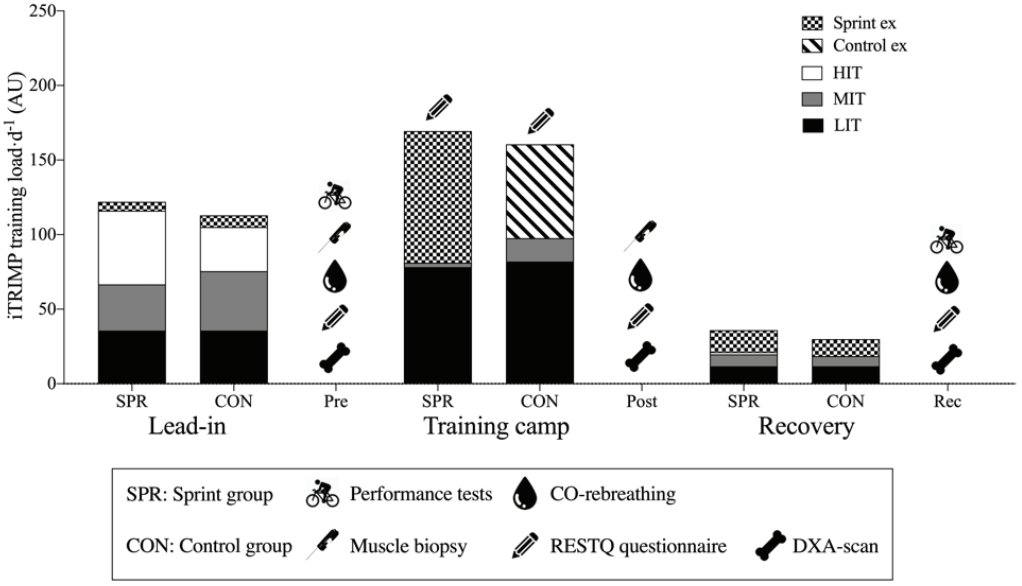

Figure 1: Study design showing the training load per day during the 14-d lead-in period, 14-d training camp and 10-d recovery period. Training loads are divided into low-intensity (LIT), moderate-intensity (MIT), high-intensity (HIT), LIT-sessions with sprints (Sprint ex) and distance-matched LIT-sessions without sprints (Control ex). Outcome measures include muscle biopsy from m. Vastus Lateralis, body composition by Dual-energy X-ray absorptiometry (DXA) scan, haemoglobin mass by CO-rebreathing method, performance test including; lactate profile test, incremental test until exhaustion (VO_{2max}), 60 min cycling at 60% of VO_{2max} including four 30-s maximal sprints, concluding with a 5-min test, Recovery-Stress Questionnaire for Athletes (RESTQ-36-R-Sport) to evaluate recovery and stress during the intervention. Bars symbolize average daily training load (AU), n = 18.

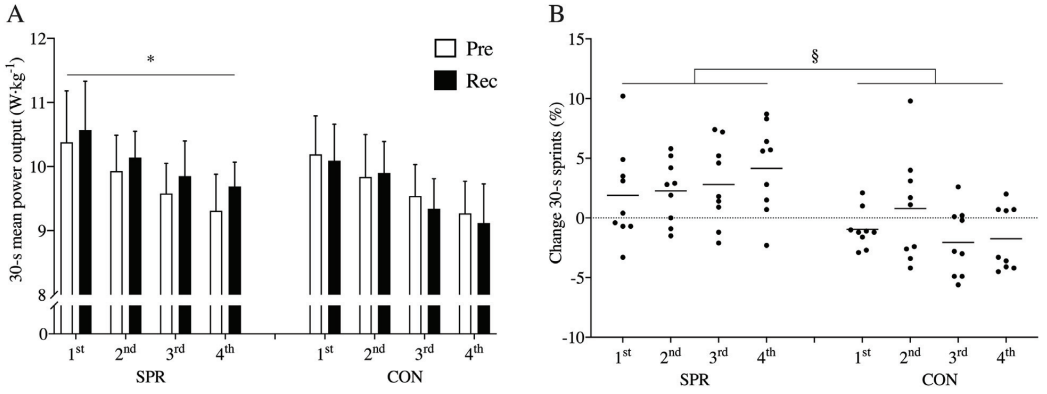

Figure 2: Mean power output of repeated 30-s sprints before the 14-d training camp (Pre) and after the 10-d recovery period (Rec) in Sprint group (SPR) and Control group (CON, n=9). Data are presented as mean ± SD (A) and individual changes (B). * indicates main effect of time ($p<0.05$). § indicates main effect of group on changes from Pre to Rec ($p<0.05$).

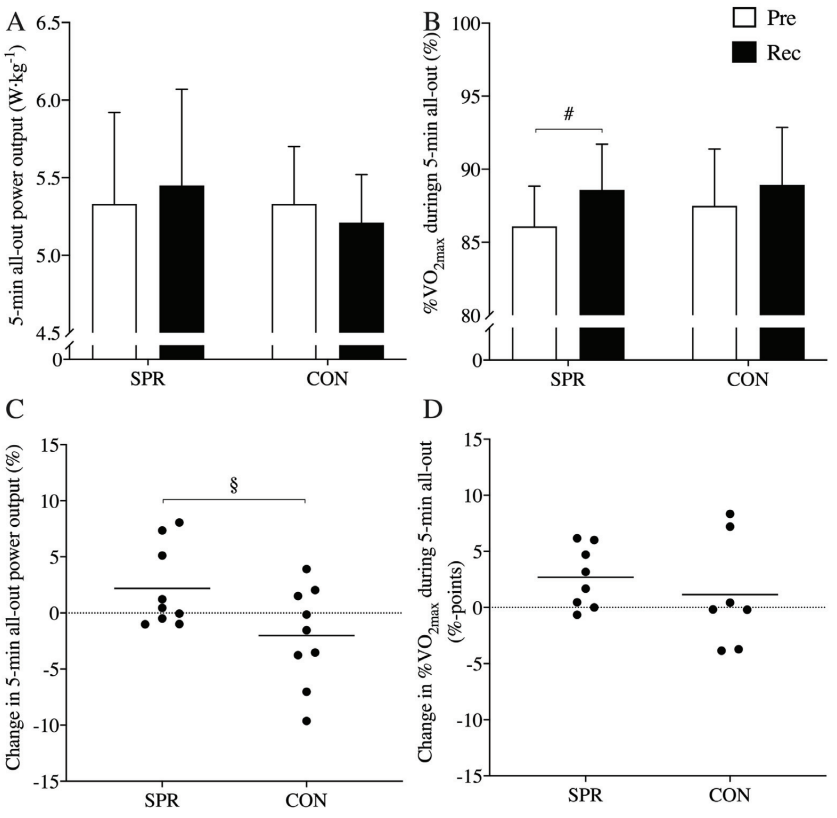

Figure 3: 5 min mean power (panel A) and mean oxygen uptake during 5-min test (panel B) and individual percentage changes (Pre vs Rec, panel C and D) in the semi-fatigued state before (Pre) and after 14-d training camp and 10-d recovery period (Rec) in Sprint-group (SPR, n=9) and Control-group (CON, n=9). * indicates main effect of time ($p<0.05$). # indicates tendency to effect of time ($p<0.1$). § indicates main effect of group on changes from Pre to Rec ($p<0.05$).

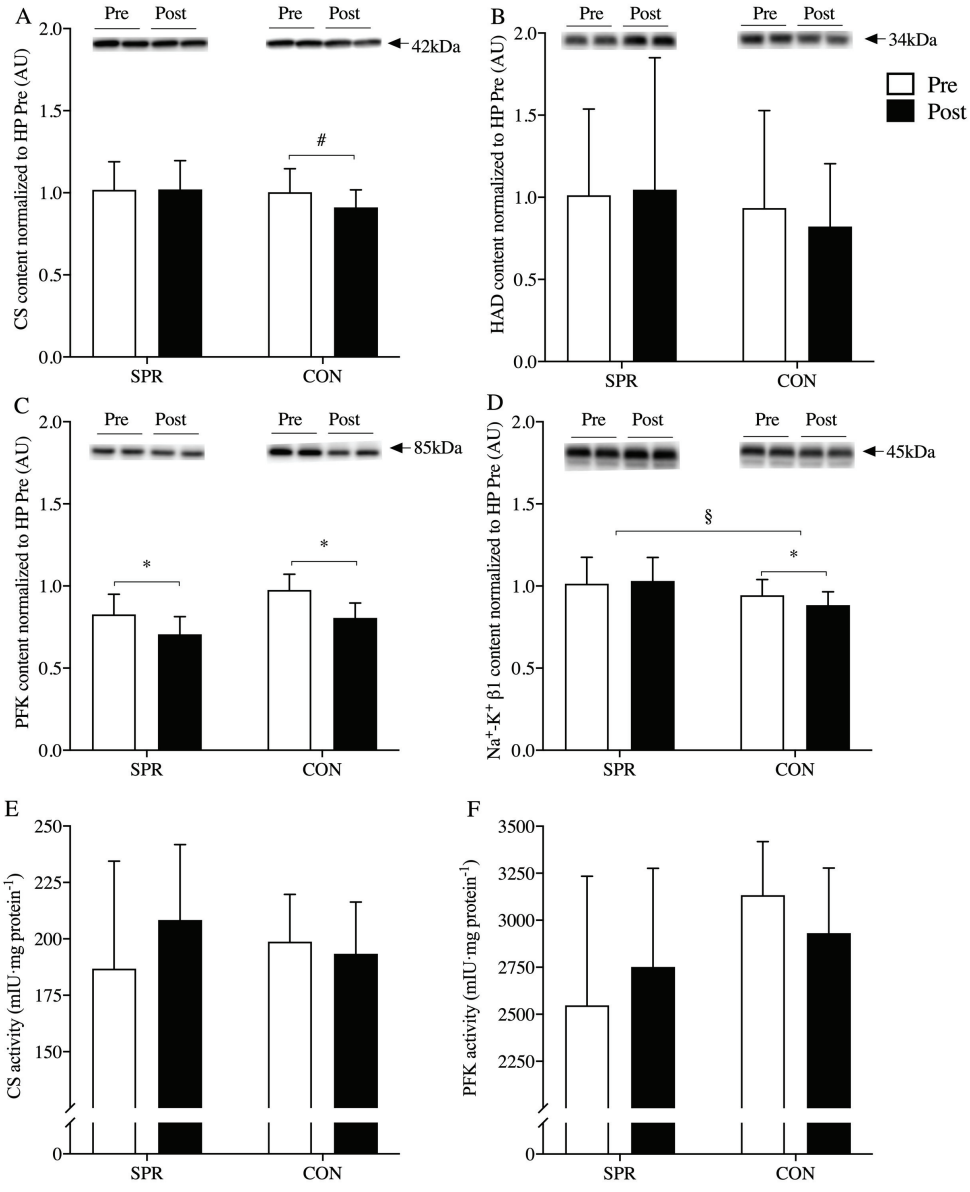

Figure 4: Muscle protein quantities (panels A-D) and activities (panels E-F) in m. vastus lateralis before and after a 14-d training camp in Sprint-group (SPR, n=8) and Control-group (CON, n=8). Panel A, Citrate synthase; panel B, β-hydroxyacyl (HAD); panel C, phosphofructokinase (PFK); panel D, Sodium-potassium pump b1 (Na^+-K^+ β1); panel E, enzyme activity of Citrate synthase; panel F, enzyme activity of Phosphofructokinase. Individual band-intensities were expressed relative to total protein stain and normalized to a human pool (HP) containing equal amounts of all Pre-samples. Mean ± 95% CI. * indicates main effect of time ($p<0.05$). # indicates tendency to effect of time ($p<0.1$). § indicates main effect of group on changes from Pre to Post ($p<0.05$).

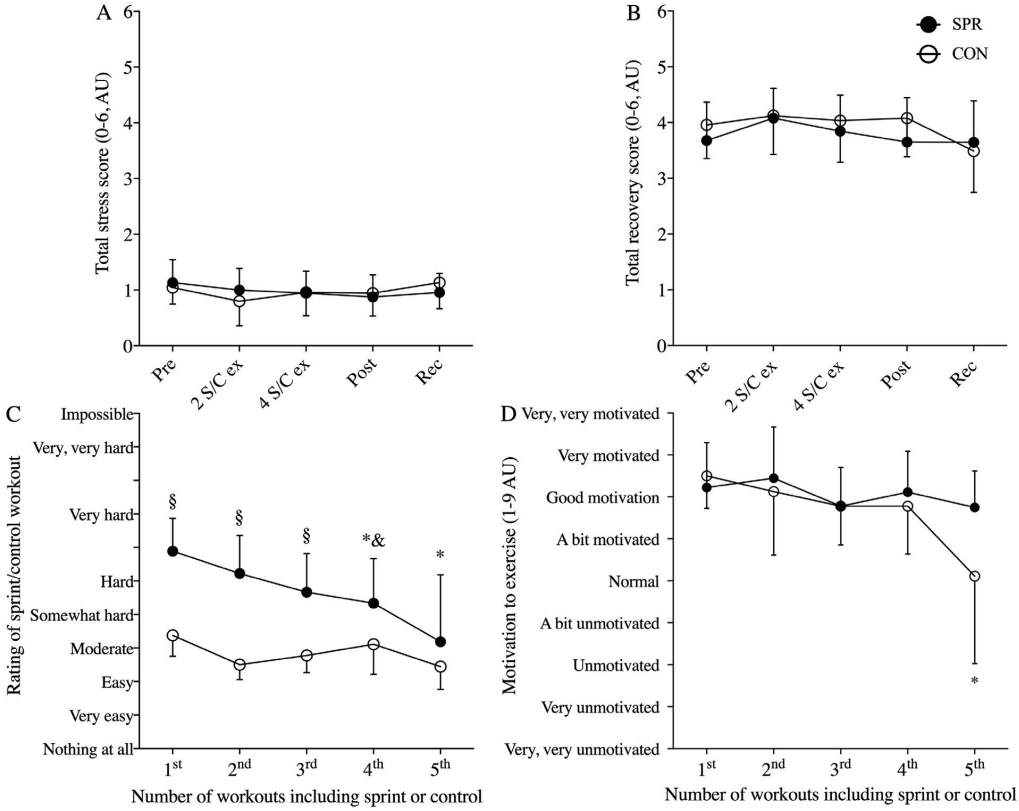

Figure 5: Total stress score (panel A) and total recovery score (panel B) before (Pre), after 2 (2 S/C ex) and 4 sprint/control exercises (4 S/C ex), after 14-d training camp (Post) and after 10-d recovery period (Rec) in Sprint-group (SPR, n=9) and Control-group (CON, n=9). Rating of perceived exertion of yesterday's workout (session RPE) the morning after the sprint or control exercises (panel C) and motivation to exercise (panel D). * indicates main effect of time ($p<0.05$). § indicates main effect of group ($p<0.05$). & indicates tendency to effect of group ($p<0.1$).

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