Effects of including sprints in LIT-sessions during a 14-d camp on muscle biology and performance measures in elite cyclists

Nicki Winfield Almquist[1,2], Malene Wilhelmsen[1], Stian Ellefsen[1], Øyvind Sandbakk[2], Bent R. Rønnestad[1].

[1]Section for Health and Exercise Physiology, Inland Norway University of Applied Sciences, Lillehammer, Norway.

[2]Centre for Elite Sports Research, Department of Neuromedicine and Movement Science, Norwegian University of Science and Technology, Trondheim, Norway

Corresponding author

Nicki Winfield Almquist

Nicki.almquist@inn.no

+4796911917

Conflict of interest

The authors have no professional relationships with companies or manufacturers who will benefit from the results of the present study. The results of the present study do not constitute endorsement by ACSM and the authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Abstract

Purpose: This study investigated the effects of including sprints within 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.

Methods: 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% during the training camp and subsequently decreased by -56±23% during the recovery period 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).

Results: 30-s sprint (SPR vs CON: 4±4%, $p<0.01$) and 5-min mean power (SPR vs CON: 4±8%, $p=0.04$) changed differently between groups. 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 for VO_{2max}, W_{max}, haematological measures, muscle enzyme activity and stress/recovery measures.

Conclusion: Inclusion of 30-s sprints within LIT-sessions during a high-volume training camp affected competition-relevant performance-measures and Na^+-K^+β1 protein content differently than LIT only, without affecting sport-specific stress/recovery or any other physiological measure in elite cyclists.

Keywords: Periodization, Sprint training, Muscular adaptations, Elite athletes, RESTQ

Introduction

Road cycling competitions involve daily distances up to 300 km, with intensities varying from low-intensity to all-out sprinting(1-3) 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(4, 5) whereas body mass affects uphill performance i.e., in mountain stages(6). The high VO_{2max} levels of elite cyclists (70-80 $mL \cdot kg^{-1} \cdot min^{-1}$) and their high %$VO_{2max}$ during prolonged exercise(1, 7) 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, and based on both heart rate (HR) and power output data is the majority of time spent at low- to moderate-intensities i.e., below the second ventilatory threshold(8-10). During the preparatory period, high-intensity exercise makes up only a small fraction of the total training time, a proportion that is usually increased during the competition period(8-12).

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(13), preferably followed by periods of reduced volume to avoid overreaching(14). However, this increase in training volume is often not accompanied by increases in training intensity, and might thus provide a too low-intense and monotonous stimulus to lead to improvements in endurance performance(15). Conversely, maintaining training intensity distribution during periods of increased volume will drastically increase the total training load, thus increasing the risk of overreaching(16). 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, decline in performance

indices such as VO_{2max} and maximal heart rate suggesting a state of overreaching(17-22) and impairment of mitochondrial function(23).

A plausible strategy to maintain high-intensity stimulus during periods of increased LIT volume during training camps could be to add sprint training. Indeed, short maximal-effort intervals have been reported to be of less perceived exertion compared to longer HIT-intervals(24), 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(25, 26). These benefits of sprint-related exercise likely result from peripheral adaptations in skeletal muscle such as increased metabolic enzyme activity (27, 28) and improved ion-transportation(29), leading to improved aerobic and anaerobic metabolism and postponement of fatigue in trained individuals. Whereas muscular adaptations can be measured rather rapidly after a demanding training period, improvements in performance may first appear after a subsequent recovery period(30).

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(31). 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(31), as well as markers of mitochondrial content in well-trained cyclists(32, 33). Indicatively, 8 weeks of sprint training increased citrate synthase (CS) protein content and phosphofructokinase (PFK) activity in trained subjects(28), however, responses to such prolonged training remain scarcely investigated in elite cyclists(34, 35). 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)(36). Sixteen participants were regarded as level 5 athletes (relative VO_{2max}: >71 $mL \cdot kg^{-1} \cdot min^{-1}$, relative W_{max}: >5.5 $W \cdot kg^{-1}$) 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 entire sample 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, participants were made fully aware of the possible risks and discomforts associated with participation and gave their written informed consent to participate before entering the study. 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, 23/11/2020, ref number: NCT04640883. This study was funded by the Norwegian Cycling Federation and Olympiatoppen.

Insert Table 1 around here

Design

The study consisted of a 14-d training camp, followed by a 10-d recovery period (Rec, 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 an 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-h 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) performance testing, and 3) haemoglobin-mass measurement (Hb-mass), while muscle samples were collected at Pre and immediately after the training camp (Post). The training camp started 5 ± 1 days after Pre-testing, and the daily training load, as measured by 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. On average 51 ± 12 sprints were completed during the camp in SPR. CON rode the same route without sprinting and were thereby matched on distance. A similar 4-h LIT-session protocol with and without inclusion of sprints was recently described by our research group(37), with the two protocols showing similar levels of total external

power output when sprints were interspersed by 4 min of recovery. Likewise, in the present study, the two training protocols were performed as distance-matched sessions and showed similar loads, calculated using iTRIMP (SPR: 88 ± 26 vs CON: 63 ± 27 AU, p=0.057). 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 (Post), a DXA scan, a resting muscle biopsy and Hb-mass measurement were conducted, followed by a recovery period of 10 ± 1 days where daily training load was equally reduced in both groups by 56 ± 23% compared to lead-in (SPR: -53 ± 32% vs CON: -59±10%, p=0.579), 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)(38) 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(39), 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. The iTRIMP methods have shown strong relationships between training load and endurance training adaptations in competitive cyclists (40) and was therefore employed in the present study. The 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

192 determined on ABL800 (Radiometer, Copenhagen, Denmark). Subsequently, a muscle sample was
193 collected from m. Vastus Lateralis of a randomized leg using the micro biopsy technique (Bard
194 Magnum, Bard Nordic, Helsingør, Denmark), using 14-gauge needles (Medax medical devices,
195 Poggio Rusco, Italy) under local anaesthesia (2-3 mL Lidocaine, Mylan Dublin, Ireland) as
196 described elsewhere(31). The first biopsy was sampled at one third of the distance from the patella
197 to anterior superior iliac spine with subsequent biopsies sampled approximately 2 cm proximal to
198 the previous sample from the same leg on the day of return from training camp. Muscle samples
199 were freeze dried in a Christ Alpha 1-2 LDplus freeze dryer, (Vakuum-Service A.S, Norway) and
200 dissected free from blood and connective tissue before homogenization of ~1.0-4 mg d.w, for
201 western blotting and enzyme activity assays.
202
203 *Blood lactate profile test and VO_{2max} test*
204 Following biopsy sampling at Pre and on Rec, participants performed a blood lactate profile test as
205 described elsewhere(41). Briefly, participants cycled for 5 min at 175 W, followed by 50-W
206 increments every 5 min until a blood lactate concentration ($[BLa^-]$) of 3 $mmol \cdot L^{-1}$, after which
207 increments were 25 W. The test was terminated at a $[BLa^-]$ of 4 $mmol \cdot L^{-1}$ or higher. All cycling
208 tests were performed on an electromagnetic braked cycle ergometer (Lode Excalibur Sport, Lode B.
209 V., Groningen, The Netherlands). The bike was adjusted to each cyclist and this adjustment was
210 replicated throughout all testing. VO_2 measurements started from 2 min into every bout and VO_2
211 was calculated as an average from 2.5 to 4.5 min. VO_2 was measured using a computerized
212 metabolic system with mixing chamber (Oxycon Pro, Erich Jaeger, Hoechberg, Germany) which
213 was calibrated every hour with standard calibration procedures. Blood was sampled from the
214 fingertip at the end of each 5-min bout and analysed for whole blood $[BLa^-]$ using a lactate analyser
215 (Biosen C_line, EKF Diagnostic, Germany). Based on these measures, the power output at 4

mmol·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·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. 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(37): Power input = VO_2 L·s^{-1} · (4840 J·L^{-1} · RER + 16,890 J·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(26). 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 N·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 in the 5-min test used at Pre was

replicated on Rec to ensure similar pacing conditions. Gels (Enervit Sport Gel, Sweden) and energy-drink (Squeezy, Norway) without caffeine were provided ad libitum after the incremental test to exhaustion and throughout the remainder of the test protocol. Nutritional intake was recorded at Pre and repeated at Rec. Mean power output during 30-s sprints was 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(42), 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(43). 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(44):

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

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

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

$$BV\ (mL) = RBCV \times 100\ /Hct$$

$$PV\ (mL) = BV - RBCV$$

Recovery-Stress state

The short version of the Recovery-Stress Questionnaire for Athletes (RESTQ-36-R-Sport) was used(45) to map the recovery-stress 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 the previous day's session using rate of perceived exertion (session RPE)(46) evaluated on a modified version (1-10) of the original Borg-scale(47). Likewise, on their motivation to exercise, we used a modified version of our previously published 9-point scale on "perceived wellbeing in legs"(48) to evaluate "how motivated are you to exercise today?". This 9-point scale spanned from 1: very, very unmotivated, through 5: normal, to 9: very, very motivated.

Muscle analyses

Western blotting

Preparation and analyses of muscle tissue was conducted using the same protocol as previously described(31). 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 TBST, 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; anti-phosphofructokinase-1, 1:500 (sc166722), and Thermo Fischer Scientific; Na^+-K^+ β1, 1:1000 (MA3-930).

Enzyme activity

CS and 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(49). 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. Recovery-stress 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. Hopkins' effect sizes (ES) using pooled SD were calculated to compare the practical significance of differences in changes between conditions(50). 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 (SPR vs CON: 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 (SPR vs CON: 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 SPR showed a moderate non-significant increase (p=0.07, ES: 0.9), whereas it remained unchanged in CON (p=0.35, Figure 3B). [BLa^-] measured 1 min after the 5-min test changed differently between groups from Pre to Rec (p<0.01). However, neither SPR (Pre: 12.2 ± 2.7 vs Rec: 14.2 ± 2.8, p=0.05) nor CON changed [BLa^-] (Pre: 12.6 ± 2.7 vs Rec: 10.7 ± 2.7, p=0.06). RPE did not change differently (p=0.16), and remained unchanged in both SPR (Pre: 19.2 ± 1.0 vs Rec: 19.6 ± 0.5, p=0.51) and CON (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 decreased in CON from Pre to Rec only in the semi-fatigued state (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 and CON from Pre to REC. RBCV did not change differently between groups (p=0.38, ES: 0.1), but only CON showed a significant increase from Pre to Rec (SPR: 2.6 ± 4.7%, p=0.07; CON:, 3.9 ± 4.5%, p=0.02).

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 both SPR (2 ± 18%, p=0.96) and CON (-9 ± 8%, p=0.06, figure 4A) from Pre to Post. 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 (CON vs SPR: -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 4^{th} and the 5^{th} 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 a 4% larger improvement in 30-s sprint power from Pre to Rec compared to CON, and 5-

min mean power was also ~4% higher in SPR compared to CON at Rec. 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·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 increases in 30-s sprint and 5-min mean power in SPR compared to CON, were accompanied by larger increases in [BLa^-] and a moderate, non-significant increase in $\%VO_{2max}$ 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(28, 30). 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(26). 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(26). 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(28). 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(25). The more pronounced benefits of adding sprints to LIT-sessions for endurance performance in the current study might be explained by cessation of HIT or sprint training in the

control group, the longer duration of LIT-sessions (>4 h), and the more anaerobic nature of the 5-min test compared to the much longer ~40-45-min tests in previous studies(25, 28).

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(51), 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(52, 53), 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 differently affected 5-min mean power in SPR compared to CON. It thus seems necessary to maintain a certain level of high-intense training (i.e., HIT or sprinting) during periods dominated by LIT to sustain high levels of gross efficiency as previously suggested(51, 54), 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(27, 28). 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(34). 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

478 remained unchanged. These discrepancies between our data and other studies might be due to a
479 combination of difference in fitness-level and duration of the intervention. A study on trained
480 subjects including sprints during ~1 h LIT-sessions for 8 wks has been shown to lead to greater
481 increases in CS protein content and PFK-activity compared to LIT only(28). Interestingly, in the
482 present study, CS content showed a numerical decrease in CON-only during the training camp, with
483 the effect size being small to moderate compared to SPR (ES: 0.6), reiterating on the potential
484 importance of maintaining high-intense exercise stimuli such as sprinting during periods of
485 predominantly LIT in elite cyclists. Furthermore, whereas Na^+-K^+ β1 protein content was maintained in
486 SPR, it decreased in CON, despite the overall increase in LIT-based training volume, with the response
487 being different between groups. As such, previous studies have highlighted that there is an
488 association between training intensity and changes in Na^+-K^+-ATPase expression in muscle in well-
489 trained individuals, with increased training intensity being necessary to increase expression(55),
490 contrasting the more readily occurring changes seen in untrained individuals(56). Ion-transportation
491 capacities have been suggested to play a role during all-out performances(29). A decrease in ion-
492 transporting proteins might therefore, hypothetically, accelerate the off-set of skeletal muscle
493 homeostasis during high-intensity exercises such as the 5-min test in the present study. This could
494 contribute to explaining the difference in 5-min mean power between SPR and CON. Notably,
495 muscle characteristics are scarcely investigated in elite cyclists and the present study gives one of
496 the first insights into the muscular adaptation to a habitual alteration in training load in the
497 preparatory period of elite cyclists.

498 Total stress and recovery measures did not change during the intervention in either SPR or
499 CON and did not differ between groups, suggesting that implementing sprints during LIT during a
500 period of augmented training volume was well-tolerated by the elite participants. Notably, the total
501 stress was low, with corresponding high levels of total recovery, corroborating well with levels

previously reported for elite athletes during a training camp(57). 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(17). Interestingly, the relative monotony of LIT in CON reduced 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}(18), which might relate to a negative energy balance(22). In the current study, the participants maintained their body composition throughout the training camp and the subsequent recovery period, indicating maintenance of energy balance despite the substantial increase in training load. Measures of mental state (RESTQ) and performance were also unaltered or improved during the intervention, thus giving no indications of a state of overreaching. Therefore, elite cyclists seem to be able to match energy intake and expenditure during training camps, and to tolerate the habitual increases in training load, in a manner that is not necessarily seen in lesser trained cyclists(18). Interestingly, inclusion of sprints during habitual LIT-sessions were initially experienced as more strenuous, which probably reflected a higher training load on SPR-sessions compared to CON-session, although not significantly different (SPR: 88 ± 26 vs CON: 63 ± 27 AU, p=0.057). However, 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 as previously reported from our lab using sessions of a similar design(37). The gradual psychological (and physiological) habituation to performing 30-s sprints during LIT-sessions compared to LIT

526 only indicates that this regimen might be beneficial to implement in the preparatory period without
527 affecting the time to recover.
528 In general, the 14-d training camp was associated with 48% increases in training load,
529 followed by a subsequent 10-d recovery period of -56% reduced training loads compared to
530 habitual training. In both SPR and CON, this led to increased RBCV and VO_2 at 4 mmol·L^{-1} [BLa$^-$],
531 though without affecting BV, %VO_{2max} and power output at 4 mmol·L^{-1} [BLa$^-$] or VO_{2max}. Only
532 CON showed a non-significant increase in Hb-mass (~2%), but was not different from SPR. This
533 borderline increase was small compared to reports in elite cyclists (~5%) after a 5-wk exercise-heat
534 acclimation intervention and was of similar magnitude to the typical error of measurement reported
535 in our lab(58). If this minor change in Hb-mass was of relevance for endurance performance, it
536 should arguably have affected 5-min mean power, which was not observed. The increases in RBCV
537 in both SPR and CON support previous evidence of increased levels of reticulocytes(59) and
538 RBCV(60) after periods of training overload and recovery in well-trained athletes. Unsurprisingly,
539 the observed increases in RBCV in our highly trained elite cyclists were slightly lower than those
540 seen after 2-4 wks of increased endurance training in untrained or moderately trained subjects(61).
541 The high fitness level of our participants may have limited the adaptive capacity to habitual changes
542 in training load and could also explain why we did not observe changes in VO_{2max}. In this context,
543 the general lack of effects of the intervention on BV, %VO_{2max} and power output at 4 mmol·L^{-1}
544 [BLa$^-$], VO_{2max} and W_{max} may also be attributed to the lack of more demanding HIT in both groups
545 during the intervention period. Although the repeated sprint exercises were performed at maximal
546 effort, time spent above 90% of VO_{2max} is minimal(62) and was likely insufficient to lead to
547 increases in VO_{2max} in elite cyclists. Regretfully, the current study did not include a negative control
548 group i.e. elite cyclists that did not change their total training load. However, the present results still

549 indicate that habitual training camps focusing on LIT-only do not improve competition-relevant
550 cycling performance in elite cyclists.
551
552 **Methodological considerations**
553 The present study quantified the training load based on iTRIMP and did, unfortunately, not include
554 power-meter data. Anecdotally, cyclists perform several power-bursts during their long low-
555 intensity sessions as a function of the terrain, to maintain a wheel or accelerate after a corner. These
556 sprints are less likely to be picked up by the iTRIMP load quantification as this is based on HR-
557 measures and not on power data. Hence, it cannot be excluded that the maintenance of sprint
558 performance in CON could be a result of this habitual riding behaviour. However, if this is the case,
559 this further underlines the positive effects of adding an additional small number of 30-s sprints in
560 improving sprint performance in elite cyclists.
561
562 **Conclusion**
563 In conclusion, including 30-s sprints within five LIT-sessions during a training camp in the
564 preparatory period improved repeated sprint-ability more than LIT-only and 5-min performance
565 changed differently, without affecting total stress/recovery in elite cyclists. In addition, SPR was
566 associated with maintained Na^+-K^+ β1 protein content in muscle compared to CON. Collectively,
567 this suggests that inclusion of sprints during LIT improves competition-relevant performances and
568 performance-related indices in elite cyclists. Finally, the 14-day training camp with an overall
569 increase in training load in both SPR and CON, followed by a subsequent recovery period led to
570 increased RBCV and VO_2 at 4 $mmol \cdot L^{-1}$ [BLa^-] in both groups but had no effect on BV, $\%VO_{2max}$
571 and power output at 4 $mmol \cdot L^{-1}$ [BLa^-], VO_{2max} or W_{max}. This suggests that training camps focusing
572 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.

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). Mean daily exercise time ($h \cdot d^{-1}$) is indicated above each group for each period. 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 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 and individual values are presented. * indicates main effect of time ($p<0.05$). § 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 ($p<0.05$).