WILEY

Systemic and muscular responses to effort-matched short intervals and long intervals in elite cyclists

Nicki W. Almquist [ID] | Håvard Nygaard [ID] | Geir Vegge | Daniel Hammarström [ID] | Stian Ellefsen [ID] | Bent R. Rønnestad [ID]

Section for Health and Exercise Physiology, Inland Norway University of Applied Sciences, Lillehammer, Norway

Correspondence
Nicki W. Almquist, Section for Health and Exercise Physiology, Inland Norway University of Applied Sciences, Lillehammer, Norway.
Email: Nicki.almquist@inn.no

The purpose of this study was to compare the acute effects of time- and effort-matched high-intensity intervals on physiological, endocrine, and skeletal muscle molecular variables in elite cyclists. Eight elite cyclists performed short intervals (SI: 30-seconds) and long intervals (LI: 5-minutes) with work:recovery ratio 2:1, using a randomized crossover design. SI was associated with $14\% \pm 3\%$ higher mean power output (SI; 421 ± 27 vs LI; 371 ± 22 W), and longer working time above 90% of maximal oxygen uptake (VO_{2max}, $54\% \pm 76\%$) and 90% peak heart rate (HR_{peak}, $153\% \pm 148\%$) than LI (all $P < .05$), despite similar degrees of perceived exertion, blood lactate levels and muscle activation measured using EMG root mean square (EMG rms). In blood, SI was associated with more pronounced increases in testosterone and testosterone-to-sex hormone-binding globulin (SHBG) ratios, as well as prolonged cortisol responses ($P < .05$). In skeletal muscle (m. Vastus lateralis), SI and LI led to similar changes in mRNA abundance for a range of transcripts, with the exception of NHE1 mRNA, which decreased after SI ($P < .05$). Overall, SI was associated with more pronounced physiological and endocrine responses than LI in elite cyclists, suggesting that such training might lead to superior adaptations in elite cyclists.

KEYWORDS
EMG, endocrine responses, high-intensity intervals, mRNA expression

1 | INTRODUCTION

Cycling endurance performance is mainly determined by three factors: maximal oxygen uptake (VO_{2max}), fractional utilization of VO_{2max} and efficiency.[1] Accordingly, the scientific community continuously search for training regimes that best target and improve these factors, particularly in well-trained athletes.[2-4] It is a long-standing recognition that such training should include high-intensity interval training (HIT).[5,6] However, the exact composition and nature of HIT remains disputed and evolving. It seems important to maximize the time spent above 90% of VO_{2max}, especially when

the aim is to increase VO_{2max}.[7,8] This is most effectively achieved by HIT comprising of shorter work intervals, as evident from a study by Rønnestad and colleagues, wherein time spent above 90% of VO_{2max} was greater in 30-seconds short intervals (SI) than in 2.8-4.5 minutes long intervals (LI), with the two protocols having similar work-to-recovery ratios (2:1).[4] Indeed, 10 weeks of SI led to superior improvements in VO_{2max} compared to LI in well-trained cyclists and also led to superior changes in power output at 4 mmol·L^{-1} blood lactate [BLa$^-$] and 40-minutes time-trial performance.[9] The underlying mechanisms behind the beneficial effect of SI remain unresolved, though they likely involve both systemic

variables, including cardiovascular adaptations and hormonal responses, and muscular adaptations.

Acutely, SI may trigger greater release of anabolic hormones into the blood than LI, perhaps related to its higher work intensity. While this remains unstudied, high-intensity exercise (HIE) in general induces transient increases in circulating levels of growth hormone (GH) and testosterone,[10] which may act to increase plasma volume and erythropoiesis,[11] through which beneficial adaptations in VO_{2max}[12] as well as in skeletal muscle may occur. As such, circulating levels of GH, testosterone, and insulin-like growth factor 1 (IGF1) correlate positively with VO_{2max}.[13,14] Furthermore, cycling at higher intensities also induces catabolic responses, measured as increased circulating levels of cortisol.[10] This may exert negative effects on cellular plasticity, impairing adaptations.[15] However, elevated levels of cortisol may also act as important signaling cues for recycling of proteins and cellular structures that are damaged during exercise, resulting in a pool of free amino acids, which in turn may facilitate protein synthesis in the anabolic phase.[16] Hormonal responses to HIE intervals in elite cyclists remains largely elusive, and needs further investigation.

In skeletal muscle, several training-induced changes in cellular functions stand out as plausible explanations behind the superior effects of SI on performance. These include improved metabolic functions possibly due to mitochondrial biogenesis and vascularization,[17] improved ion handling,[18] and increased cross-sectional area of the quadriceps muscles.[19] These adaptations are likely to improve the fractional utilization of VO_{2max} and to improve anaerobic energy metabolism and delay fatigue.[20] Mitochondrial function and biogenesis are primarily under control of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), whose regulation is intensity- and muscle activation-dependent, both at the level of transcription and activity.[17,21,22] Although PGC-1α responses to training have been a long-standing focus in exercise physiological research, there are aspects of PGC-1α biology that remain a conundrum. For example, cycling at intensities around 100% of maximal aerobic power output (W_{max}) induces larger increases in PGC-1α mRNA expression than cycling at 73% and 133% of W_{max}.[17] It is thus far from certain that increased training intensities, such as those seen during SI will lead to more pronounced induction of PGC-1α expression than LI. However, SI intervals are performed at intensities close to W_{max},[9] leaving increased PGC-1α expression and activity an intriguing explanation behind its beneficial effects. Alongside this, SI may exert greater effects on vascularization. This angiogenesis likely involves activation of signaling proteins such as vascular endothelial growth factor (VEGFA), which indeed is differentially affected by cycling exercises at different intensities.[23] Also, SI may acutely lead to increased mRNA expression of ion-handling proteins,[18] which may in

turn be important for delaying fatigue during HIE around VO_{2max}.[20,24] While this perspective has gained less attention in previous studies of HIT, content of ion-transporting proteins seem to increase after interventions of repeated sprint training in well-trained subjects.[25] Finally, HIE may acutely increase protein synthesis in muscle fibers, potentially orchestrated through regulation of signaling factors such as PGC-1α4, IGF1, and myostatin.[26] Overall, few studies have investigated the effects of HIT on muscular adaptations in elite cyclists, likely due to skepticism connected to muscle biopsy sampling. The introduction of the less invasive microbiopsy procedure into exercise sciences[27] may thus facilitate invasive measurements in elite cyclists.

The aims of the study were to compare the acute effects of time- and effort-matched SI (30-seconds) and LI (5-minutes) on hormone concentrations in blood and mRNA abundances in m. vastus lateralis in elite cyclists, using a randomized crossover design. We also aimed to verify that the applied SI protocol is associated with higher mean power output and longer time above 90% of VO_{2max} compared to LI, as previously shown by our group,[4] and to investigate if this is accompanied by a higher degree of muscle activation. We hypothesized that SI would lead to more pronounced changes in anabolic hormone concentrations in blood and more pronounced changes in muscle mRNA abundances for genes involved in mitochondrial function, angiogenesis, ion-transport, and protein synthesis.

2 | METHODS

2.1 | Subjects and design

Eight male competitive, elite cyclists volunteered, and all completed the study. Subjects' characteristics are shown in Table 1. Based on VO_{2max}, and W_{max}, the cyclists were regarded as elite cyclists on performance level 4-5.[28] The study was approved by the local ethical committee at Inland Norway University of Applied Sciences and was performed in accordance with the Declaration of Helsinki, except for pre-registration in a public database. Before participation, all cyclists signed an informed consent form. Participants visited

TABLE 1 Physiological parameters from screening

Age (years)	25.3 ± 6.1
Height (cm)	181.1 ± 4.5
Body mass (kg)	74.8 ± 3.8
VO_{2max} (mL·kg^{-1}·min^{-1})	73.9 ± 7.0
W_{max} (W·kg^{-1})	6.2 ± 0.5

Note: Values are mean ± SD.

Abbreviations: SD, standard deviation; VO_{2max}, maximal oxygen consumption; W_{max}, maximal aerobic power produced the last minute during incremental test.

the laboratory on three occasions. On their first visit, they performed a test battery consisting of a standardized blood lactate profile test, an incremental test to exhaustion to determine VO_{2max}, as well as two sets of SI, as described below. On their second and third visit, they performed the two experimental protocols, either SI or LI, allocated to each of the two days in a randomized manner, with 4-7 days between experimental days.

2.2 | Testing procedures

All test procedures and exercise sessions were performed on the same electromagnetically braked cycle ergometer (Lode Excalibur Sport, Lode BV), which was adjusted according to each cyclist's preference and used for both tests. For each participant, all tests and exercises were performed at the same time of day (± 1 hour). The cyclists were instructed to refrain from all types of intense exercise, caffeine, and alcohol 24 hours prior to testing. Further, they were instructed to consume the same type of meal no later than one hour before each test. All tests were conducted in similar environmental conditions (16-18°C and 20%-35% relative humidity) with a fan ensuring circulating air around the cyclist.

2.3 | Blood lactate profile test, VO_{2max} and W_{max}

On the first test day, participants performed a blood lactate profile and a VO_{2max} test, as previously described.[29] Briefly, the blood lactate profile test started with 5 minutes cycling at 125 W. Thereafter, power output was increased by 50 W every 5 minutes. Blood was sampled from a fingertip at the end of each 5-minutes bout and was analyzed for whole-blood lactate [BLa⁻] using a lactate analyzer (Lactate Pro LT-1710, Arcray Inc). The test was terminated when [BLa⁻] was ≥ 4 mmol·L⁻¹. HR was recorded using a Polar S610i HR monitor (Polar). VO_2, respiratory exchange ratio (RER), and HR were measured during the last 3 minutes of each bout. VO_2 was measured (30-seconds sampling time) using a computerized metabolic system with mixing chamber (Oxycon Pro, Erich Jaeger). Gas analyzers were calibrated prior to every test according to manufacturer's description, using certified calibration gases. Calibration of the flow turbine (Triple V, Erich Jaeger) was performed prior to all tests, using a 3-L, 5530 series, calibration syringe (Hans Rudolph) before every test. After termination of blood lactate profile tests, cyclists were given 15 minutes of recovery cycling before completing an incremental cycling test for determination of VO_{2max}. The test was initiated with 1 minute of cycling at a power output corresponding to 3 W·kg⁻¹ (rounded down to nearest 50 W). Thereafter, power output was increased by 25 W every minute

until exhaustion. VO_{2max} was calculated as the average of the two highest consecutive 30-seconds VO_2 measurements. To evaluate if VO_{2max} was achieved, the following criteria were set: HR $\geq 95\%$ of the subjects reported maximal HR, RER ≥ 1.05, and [BLa⁻] ≥ 8.0 mmol·L⁻¹. Wmax was calculated as the mean power output during the last minute of the incremental VO_{2max} test.

2.4 | Experimental protocols and tissue sampling

On experimental days, participants rested for 30 minutes in a supine position before a blood sample was collected from the antecubital vein. Thereafter, a muscle sample was collected from m. Vastus Lateralis (right leg, Pre) using the micro biopsy technique (Bard Magnum, Bard Nordic, Helsingør, Denmark), as previously described[30] using 14-gauge needles (Medax medical devices) under local anesthesia (~2 mL Lidokain, Mylan). 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. Experimental protocols were then initiated, starting with a warm-up procedure consisting of 20-minutes at 175 W, followed by 50 W increases in power output every 3 minutes until >4 mmol [BLa⁻], and concluded by two to three submaximal sprints lasting 20-30 seconds. Then, experimental protocols were performed, either as SI, consisting of 3 sets of 13 × 30-seconds work intervals separated by 15-seconds active recovery periods, or as LI, consisting of 4 × 5-minutes work intervals separated by 2.5-minutes active recovery periods. Power output during active recovery periods between sets was equivalent to 50% of power output during work intervals in both sessions. [BLa⁻] and rate of perceived exertion (RPE) were recorded at the end of every interval. Total time of work intervals amounted to 19.5 and 20 minutes in SI and LI, respectively. Participants were instructed to perform intervals at the highest possible average power output. A supervisor gave strong verbal encouragement during both sessions. Time spent above 90% of VO_{2max} and HR_{Peak} during exercises were defined as accumulation of VO_2 and HR values at or above 90% of VO_{2max} and HR_{Peak}. After finalization of experimental protocols, sampling of blood and muscle biopsies was repeated, with blood being sampled immediately and 30 minutes after termination of the exercise, and muscle biopsies being sampled 3 hours after finalization of the exercise (3 hours post). Blood samples were collected in serum-separating tubes and kept at room temperature for 30 minutes before centrifugation (1500 g for 10 minutes), after which serum was aliquoted into 1.5 mL vials and stored at −80°C until further analyses. Muscle biopsies were after

collection immediately frozen in −80°C cold isopentane and stored for further analysis. Biopsies were only sampled prior to and 3 hours post-exercise, since earlier studies of target proteins, as described later, have shown significant increases in mRNA in this time frame to high-intense exercise.[22,23]

2.5 | EMG procedures

To evaluate muscle activity during experimental protocols, EMG measurements were performed using a wireless EMG-module Telemyo DTS wireless system (Noraxon Inc). Surface electrodes (dual surface electrodes, product 272, inter-electrode distance 20 mm from center to center) were attached as recommended by Konrad 2006,[31] providing data on left leg m. Vastus Lateralis and m. Vastus Medialis activities. EMG recordings were sampled at 1500 Hz and were high-pass filtered using a cutoff frequency of 10 Hz and low-pass filtered using a cutoff frequency 500 Hz, before they were smoothed over 100 milliseconds (root mean square algorithm). EMG rms was calculated as the average of the smoothed EMG data over approximately 20 seconds (each measuring period constituting of cycles starting and ending at the same crank cycle position). EMG rms were then expressed relative to the average signal on a 275 W power output, obtained during standardized warm-up where participants were instructed to maintain the same pedaling frequency. EMG measurements are only presented from 6 participants due to bad connection at the end of HIE intervals in two participants.

2.6 | Blood and muscle analyses

2.6.1 | Hormonal analyses in blood

Serum concentrations of total testosterone, cortisol, GH, sex hormone-binding globulin (SHBG), and IGF1 were measured using an Immulite 1000 analyzer, using kits from the Immulite Immunoassay System Menu (Siemens Medical Solutions Diagnostics). Based on these measurements, we also calculated individual testosterone-to-SHBG ratios, which was used as an indicator of free testosterone in subsequent analyses.

2.6.2 | Gene expression in muscle

To determine expression of target genes in response to the two exercise protocols, real-time RT-PCR was be performed as described elsewhere.[32] Analyses included genes involved in mitochondrial biogenesis (PGC-1α splice 1, PDK4, and TFAM), angiogenesis (VEGFA and Thrombospondin 1),

ion handling (ClC1, MCT1, MCT4, Na^+-K^+ α1, Na^+-K^+ α2 and Na^+-K^+ β1, NHE1), and protein synthesis (IGF1, PGC-1α4, and myostatin), along with 12 potential reference genes (β-actin/ACTB, β2-microglobulin/B2m, charged multivesicular body protein 2A/CHMP2A, follistatin/FST, glyceraldehyde-3-phosphate dehydrogenase/GAPDH, RNA polymerase II subunit A/PolR2A, peptidylprolyl isomerase A/PPIA, Ras-related protein Rab-7a/RAB7A, receptor accessory protein 5/REEP5, ribosomal protein L32/RPL32, TATA-box-binding protein/TBP and Ubiquitin C/UBC). Gene-specific primers were designed using Primer-BLAST and Primer3Plus Sequences as described previously[30] (Table 2).

Total RNA was extracted 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), using 0.5 mm RNase-free Zirconium Oxide beads and a bead homogenizer (Bullet Blender, Next Advanced), according to manufacturer's instructions, as previously described.[33] 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 incubation and subsequent phase separation by centrifugation (12 000 *g*, 10 minutes, 4°C). Four-hundred μL 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). 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). 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).

Samples were reverse transcribed in duplicates (500 ng total RNA) using SuperScript® IV Reverse Transcriptase (Invitrogen, Life technologies AS), using anchored Oligo-dT and random hexamer primers (Thermo Scientific, Life technologies AS), 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) 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.

TABLE 2 Real-time RT-PCR primers utilized for analyses of mRNA expression, including average primer efficiencies and Cq values

| Gene | Primers for qRT-PCR | | Efficiency | Cq |
	Forward primer	Reverse primer	Mean $\pm$ SD	Mean $\pm$ SD
ATP1A1	ATCCTTGAGTACACCTGGCTTG	TTTCCTTGCCATGCGTTTGG	1.62 ± 0.01	32.6 ± 0.8
ATP1A2	TTCCTCGGGGCTTCAAATTC	ATGAGCCCCACAAAGCAAAG	2.16 ± 0.01	23.1 ± 0.6
ATP1B1	ATTTTGGACTGGGCAACTCC	ATTTGGGCTGCAGGAGTTTG	2.09 ± 0.01	23.6 ± 0.4
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
PDK4	CCAGACCAACCAATTCACATCG	TTCAACTGTTGCCCGCATTG	2.06 ± 0.01	25.2 ± 2.0
PGC1As1	TATGGAGTGACATCGAGTGTGC	ACCCAGAAAGCTGTCTGTATCC	1.94 ± 0.01	26.2 ± 0.6
PGC1As4	TGTGCCATATCTTCCAGTGACC	TGCAGTTCCAGAGAGTTCCAC	2.06 ± 0.01	27.5 ± 1.3
RPL32	AAGTTCCTGGTCCACAACG	CGGCACAGTAAGATTTGTTGC	1.97 ± 0.01	22.3 ± 0.1
SLC9A1	TCCATGCAAGTGCTGTTTGG	TTCTTCTGTACAGGCAGCAGAG	1.87 ± 0.01	31.0 ± 0.6
SLC16A1	ACCATTGTGGAATGCTGTCC	TGATGCCCATGCCAATGAAG	1.63 ± 0.01	31.5 ± 1.0
SLC16A3	TTCGGCTGTTTCGTCATCAC	TCCCAAACTCCTGTATGAGCTC	1.96 ± 0.01	27.1 ± 1.2
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	$1.93 \pm 0.0.01$	29.5 ± 2.0
VEGFA	CCTGCAAAAACACAGACTCG	CTCGGCTTGTCACATCTGC	1.99 ± 0.01	26.2 ± 0.9

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-1As1, peroxisome proliferator-activated receptor gamma coactivator-1α splice 1; PGC-1As4, peroxisome proliferator-activated receptor gamma coactivator-1α splice 4; RPL32, ribosomal protein L32; SD, standard deviation; SLC16A1, monocarboxylate transporter 1; SLC16A3, monocarboxylate transporter 4; SLC9A1, 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 microgrobulin.

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.[34] Gene expression data 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. B2m, TBP, and RPL32 were evaluated as suitable reference genes, as their expression 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 elsewhere.[35]

change from pre- to 3 hours post-exercise) and blood hormone levels, wherein effects of time (pre, post, 3 hours post) and exercise condition (LI, SI) and their interaction (time × condition) 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 covariate 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. Descriptive data are presented as mean $\pm$ standard deviation. Gene expression data are presented as Log2 fold change from pre-exercise with a 95% confidence interval.

2.7 | Statistics

Student's paired t test was used to perform pairwise comparisons of effects of SI and LI on physiological measurements (power output, time spent above 90% of VO_{2max} and HR_{peak}, [BLa$^-$] and RPE. A marginal model was used for normalized mRNA levels (measured as Log2 fold

3 | RESULTS

3.1 | Physiological measurements

SI resulted in higher mean power output than LI (14% $\pm$ 3%, $P < .05$, Table 3) and was associated with more time spent above 90% of VO_{2max} (54% $\pm$ 76%, Figure 1A) and more

TABLE 3 Mean power output, blood lactate concentration ([BLa⁻],) and rate of perceived exertion (RPE) for each set and condition

		1st set	2nd set	3rd set	4th set	Mean ± SD
SI	Power output (W)	417 ± 27[a]	424 ± 25[a]	423 ± 30[a]	—	421 ± 27[a]
	[BLa⁻] (mmol·L⁻¹)	7.9 ± 1.7	9.5 ± 2.0	10.7 ± 1.7	—	9.4 ± 1.5
	RPE (6-20)	17.4 ± 0.8	18.7 ± 0.5	19.7 ± 0.5	—	18.6 ± 0.5
LI	Power output (W)	356 ± 25	370 ± 21	374 ± 23	382 ± 29	371 ± 22
	[BLa⁻] (mmol·L⁻¹)	7.6 ± 2.4	9.4 ± 1.9	10.6 ± 1.5	12.3 ± 1.6	10.0 ± 1.5
	RPE (6-20)	17.0 ± 1.0	17.9 ± 0.9	18.6 ± 1.0	19.6 ± 0.5	18.3 ± 0.7

Note: SI, 3 sets of 13 × 30-s work intervals separated by 15-s active recovery; LI,4 × 5-min work intervals separated by 2.5-min active recovery periods; SD, standard deviation.

[a]Indicates significant different ($P < .05$) to LI set, n = 8. Values are mean ± SD.

FIGURE 1 Time spent above 90% of maximal oxygen uptake (VO_{2max}; A, n = 7) and time spent above 90% of peak heart rate (HR_{Peak}; B, n = 6) during short intervals (SI) and long intervals (LI). Columns represent mean values and circles represent individual values, § indicates significant different from LI ($P < .05$)
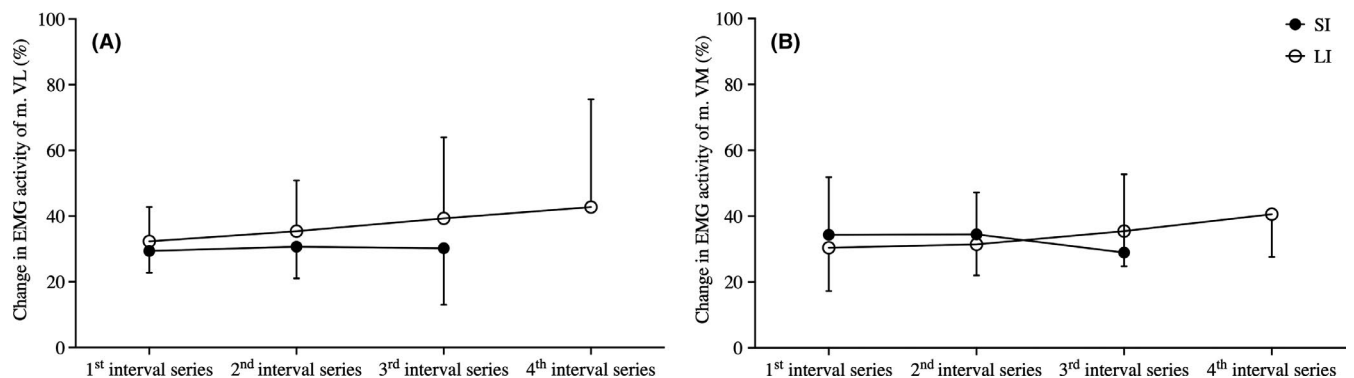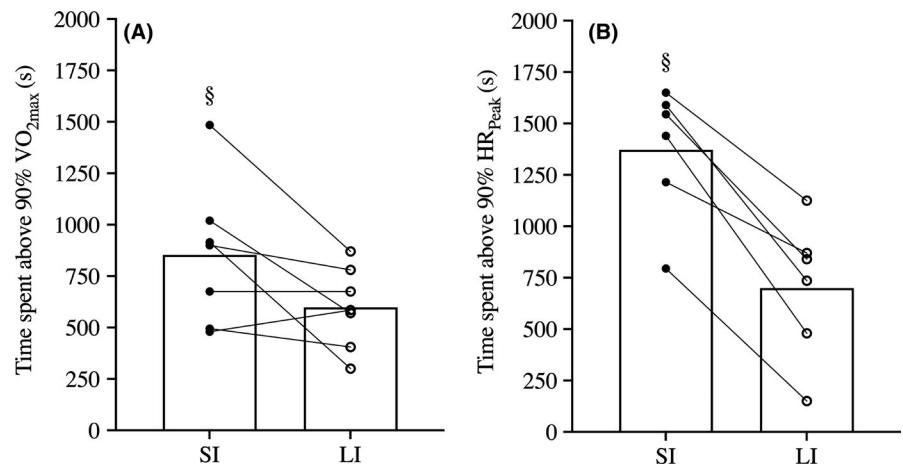

FIGURE 2 Electromyographic (EMG) activity during SI and LI expressed relative to activity during warm-up at 275 W. Panel A; m. Vastus lateralis (m.VL), panel B; m. Vastus medialis (m.VM), n = 6. LI, long intervals; SI, short intervals

time spent above 90% of HR_{peak} (153% ± 148%, $P < .05$, Figure 1B). In SI and LI, mean power output amounted to 90% ± 2% and 80% ± 2% of W_{max}, respectively ($P < .05$). SI and LI resulted in similar changes in [BLa⁻] (Table 3), RPE (Table 3), and muscle activity (Figure 2). Muscle activity was not different between conditions and did not change over the course of the two experimental protocols (Figure 2), but pedaling frequency was higher in SI (109 ± 2 rounds per minute) compared to LI (102 ± 3 rounds per minute, $P < .05$).

3.2 | Blood hormone responses

Short intervals and LI led to increased blood concentrations of cortisol, testosterone, and GH (all $P < .05$, Figure 3A-C), measured immediately after the exercise (post), while only SI led to increased testosterone-to-SHBG ratios ($P < .05$, Figure 3D), and only LI led to increased concentrations of IGF1 and SHBG (both $P < .05$, Figure 3E,F). Of these variables, SI led to more pronounced increases in testosterone concentrations and testosterone-to-SHBG ratios ($P < .05$,
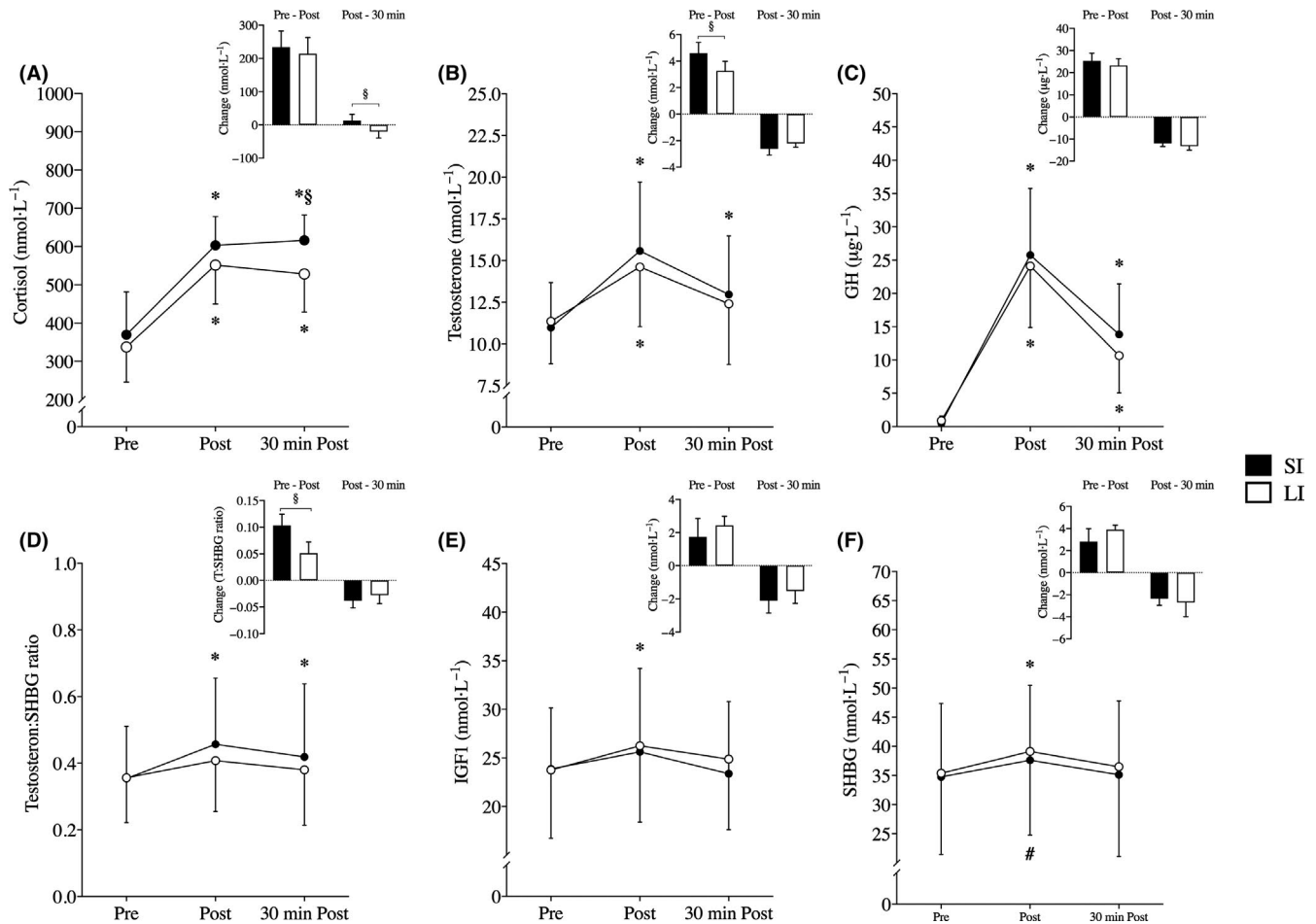
FIGURE 3 Blood hormone levels before, after and 30 min after exercise. The large panels show absolute levels of blood hormones pre, post, and 30 min post-exercise. The small panels indicate absolute changes from Pre to post and from post to 30 min post-exercise, *indicates significantly ($P < .05$) different from pre-exercise, [§]indicates significantly ($P < .05$) different from LI, n = 8. LI, long intervals

Figure 3B,D). At 30 minutes post-exercise, cortisol and GH concentrations remained elevated compared to pre in both intervention groups (both $P < .05$, Figure 3A,C), while testosterone and testosterone-to-SHBG ratios were elevated in SI only (both $P < .05$, Figure 3B,D). At this time point, neither of the training protocols affected IGF1 and SHBG concentrations (compared to pre, Figure 3E,F). In LI, but not in SI, cortisol concentrations decreased from immediately after the exercise to 30-minutes post-exercise, resulting in a differential cortisol response between training protocols in this period ($P < .05$, Figure 3A).

3.3 | Gene expression in m. Vastus lateralis

Short intervals and LI led to increased mRNA levels of selected markers of mitochondrial biogenesis and function (PDK4, PGC-1α splice 1, and TFAM) and angiogenesis (thrombospondin and VEGFA), measured 3 hours after finalization of exercise ($P < .05$, Figure 4A,B). Overall, no difference was observed between experimental conditions

for these transcripts, though a near-significant lower increase in PDK4 mRNA was evident after SI compared to after LI ($P = .054$). SI and LI also led to altered mRNA levels of markers of ion handling and muscle growth. Whereas Na^+-K^+ β1 and PGC-1α splice 4 increased and myostatin decreased in both conditions ($P < .05$, Figure 4C,D), NHE1 decreased after SI only ($P < .05$, Figure 4D). For NHE1 only, the response was different between SI and LI ($P < .05$). Neither SI nor LI led to changes in mRNA levels of ClC1, $Na^+-K^+α1/$ α2 or IGF1 (Figure 4C,D).

4 | DISCUSSION

The present study compared systemic and muscular responses to time- and effort-matched SI and LI in elite cyclists. SI was associated with higher power output and longer working time above 90% of VO_{2max} and 90% of HR_{peak} than LI, without increasing perceived exertion or [BLa^-], adding to and supporting knowledge from previous research.[4,9,36] In line with this, SI and LI were associated with differential
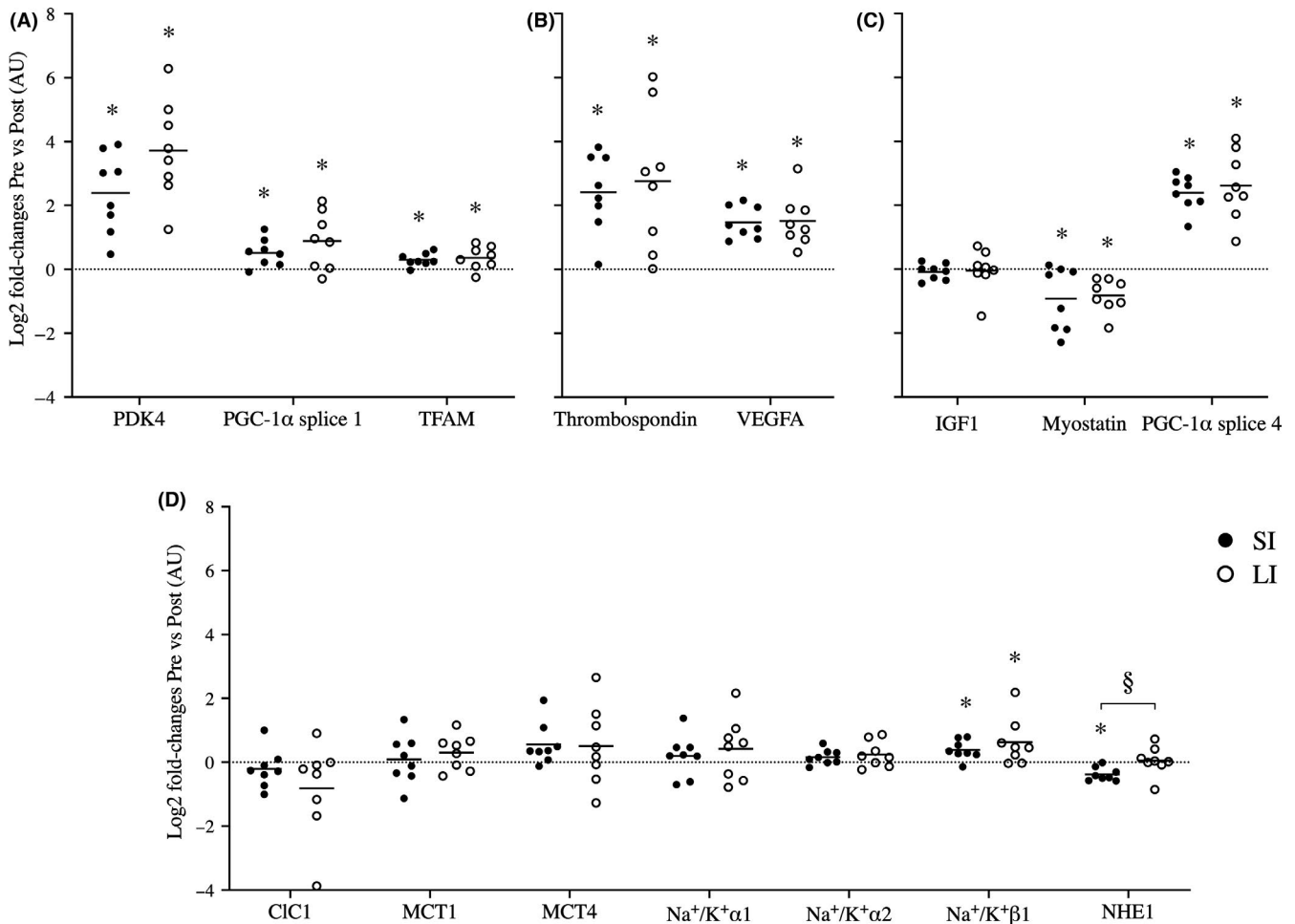
FIGURE 4 Log2 fold changes in mRNA levels of target genes in m. vastus lateralis in response to short intervals (SI; ●) and long intervals (LI; ○), measured from pre to 3 h post-exercise. (A) Genes involved in mitochondrial biogenesis and function, (B) genes involved in angiogenesis, (C) genes involved in regulation of protein synthesis, (D) genes involved in ion handling. Data are mean with individual values. *indicates significantly ($P < .05$) different from pre-exercise, #indicates tendency to different from LI ($P < .1$), §indicates significant different from LI ($P < .05$), n = 8. PDK4; pyruvate dehydrogenase kinase 4, PGC-1α(s4); peroxisome proliferator-activated receptor gamma coactivator-1α (splice 4), TFAM; mitochondrial transcriptional factor A, VEGFA; vascular endothelial growth factor A, ClC1; chloride voltage-gated channel 1, Na^+-$K^+α1/α2/β1$; sodium/potassium-transporting ATPase subunit α1/α2/β1, NHE1; sodium-hydrogen exchanger 1, IGF1; insulin-like growth factor 1

responses of selected endocrine variables, with SI leading to more pronounced increases in testosterone and testosterone-to-SHBG ratios in blood immediately after the exercise and leading to prolonged cortisol responses. Surprisingly, the two intervals led to similar degrees of muscle activation, measured as EMG rms, and were associated with similar mRNA responses in m. Vastus lateralis, with the exceptions of NHE1 mRNA levels. Overall, SI led to augmented physiological and endocrine responses in elite cyclists compared to LI, suggesting that it may convey beneficial effects even in highly trained individuals.

The superior physiological responses to SI, including higher power output and longer time spent above 90% of VO_{2max}, has previously been associated with superior improvements in performance and performance indices in well-trained[9] and elite cyclists.[36] This essentially means that SI elicits more pronounced effects on determinants of

performance such as VO_{2max} and fractional utilization of VO_{2max}. While the effects of SI on VO_{2max} largely resides on systemic cardiovascular adaptations,[12] its effects on fractional utilization of VO_{2max} likely involves muscle adaptations.[37] As for the latter, muscle plasticity can be induced and facilitated by both systemic signaling events and by muscle-specific signaling events caused by the mechanical and metabolic stress of the exercise.[38]

In the present study, we assessed endocrine variables as targets of systemic signaling responses. While both training modalities led to substantial changes in hormone concentrations in blood, SI led to more pronounced responses. This was evident as larger increases in testosterone and testosterone-to-SHBG ratios, suggesting the presence of higher levels of free testosterone immediately after the exercise, and as cortisol responses with prolonged duration. Overall, this supports the notion that these variables respond to

training in an intensity-dependent manner,[10] which may act to facilitate training adaptations in muscle,[16,39] as well as in systemic functions.[11,13] As for cortisol, the prolonged response to SI can be interpreted as exerting a negative effect on muscle cellular plasticity.[15] However, as long-term SI induces superior effects on performance,[9,36] the prolonged cortisol response may facilitate recycling of proteins and cellular structures that are damaged during exercise, resulting in a pool of free amino acids and other micronutrients, that in turn facilitate protein synthesis and cellular plasticity in the anabolic phase.[16] To date, the importance of such acute hormonal changes for long-term adaptations remains inconclusive,[10,15,16] and further research is warranted, including long-term interventions of similar designs to the present study, with more frequent collection of blood samples.

Evidently, SI was associated with higher mechanical and metabolic stress in muscle tissue than LI, as deduced from its higher power output (14% higher than LI) and its prolonged time above 90% of VO_{2max} (54%). It was thus surprising that SI was not associated with increased EMG activity, as expected from previous studies.[17,40] This discrepancy may be due to numerous factors, including the higher pedaling frequency in SI compared to LI, the short breaks (15 seconds) between short work interval (30 seconds), the relative small differences in power output between SI and LI (90% vs 80% of W_{max}), and the small sample size in EMG measurements (n = 6). In SI, the increased time above 90% of VO_{2max} was likely due to the combination of increased intensity during work intervals and the sustained elevation of VO_2 during 15-seconds active pauses.[41]

In general, SI and LI resulted in similar changes in mRNA levels of genes involved in muscle plasticity and functions in m. Vastus lateralis in elite cyclists. For example, SI and LI resulted in similar changes in expression of genes involved in mitochondrial biogenesis (PGC1α splice 1 and TFAM), angiogenesis (thrombospondin and VEGFA), regulation of protein synthesis (myostatin and PGC1α splice 4), and ion handling (Na^+-K^+β1). These similarities may seem counterintuitive, as many of these genes have previously been ascribed intensity-dependent roles in response to acute exercise.[17,18,22,23] However, a few characteristics of the present data may shed light on our finding. First, although SI was performed at higher power outputs, the difference between SI and LI was rather small compared to other studies. For example, in the studies by Edgett et al[17] and Hoier et al,[23] which disclosed intensity-dependent changes in VEGFA and PGC-1α mRNA expression, the difference in power output between conditions was 27 percentage points and 60 percentage points, respectively, compared to the mere 10 percentage point difference in our study. Second, the high fitness level of the present participants may have affected our measured

responses. Indeed, exercise-induced expression of genes involved in mitochondrial biogenesis and angiogenesis seems to be reduced with increasing level of training status,[23,42] though this negative relationship is not evident in all studies.[43] Third, in the present study, SI and LI were associated with similar degrees of muscle activation, measured as EMG activity. While this similarity may be an artifact of the low statistical power of the study, it cannot be ruled out that it had a biological origin that could explain the lack of differences in gene expression patterns between SI and LI.

Of the 15 genes investigated in the present study, only two genes showed or nearly showed differential expression responses to SI and LI: NHE1 and PDK4. For NHE1, SI resulted in decreased expression compared to LI. In a previous review, HIT has been reported to increase NHE1 protein abundances,[20] and hence, one could expect HIE acutely to increase NHE1 mRNA. In muscle fibers, NHE1 facilitates transport of H^+ ions from the cytosol to the extracellular fluid and may thus be important for regulating pH, acting as a potential determinant of HIE performance.[20] The timing of muscle sampling in the present study (3 hours after exercise) might have affected the disclosed changes in NHE1, as HIE-induced changes in mRNA tend to peak 24-48 hours after exercise whereas protein content transiently seem to decrease.[44] The decreased expression of NHE1 in the current study and the unchanged protein abundancy in response to HIT in a previous study,[25] however, leaves the importance of NHE1 unequivocal. As for PDK4, LI led to a near-significant increase in expression compared to SI, with the lack of significance potentially being related to the low statistical resolution of the study. PDK4 activity acts to decrease oxidation of carbohydrates by inactivating the pyruvate dehydrogenase complex and thus shunts metabolism toward beta-oxidation of fatty acids, playing an important role for metabolic functions during both exercise and recovery.[45] As both NHE1 and PDK4 were measured on the level of mRNA (and not protein), one should be cautious to interpret the meaning of these findings. Still, the observed differences in PDK4 and NHE1 responses to SI and LI in muscle tissue of elite cyclists hint toward differential adaptations to the two exercise modalities, though the similarities in mRNA responses were clearly greater than the differences in the data set as a whole. There is need for more research to elucidate the acute and accumulative effects of different HIE protocols in highly trained athletes. Such studies are facilitated by the availability of the microbiospy procedure, which enables biopsy sampling without any appreciable adverse effects and allows sampling of more frequent biopsies, paving the way for data with higher biological resolution.

Taken together, SI elicits higher power output, longer time spent above 90% of VO_{2max} and HR_{peak} compared to LI and a greater endocrine responses despite similar degrees of

muscle activation and perceived exertion, hinting at a beneficial effect of SI over LI in elite cyclists.

4.1 | Perspective

Short intervals allowed elite cyclists to ride at higher power outputs and sustain longer periods at intensities above 90% of VO_{2max} and HR_{peak} compared to LI. This was accompanied by a greater hormonal response, which might lead to superior training adaptations, as previously reported.[9,36] However, SI does not seem to induce greater muscle activation and does not lead to clear differences in mRNA responses. On a practical note, the participants experienced SI and LI as equally exhausting in the current study and SI might therefore be an efficient exercise strategy to augment training stimulus without increasing the subjective experience of exertion. Finally, few studies have investigated muscular adaptations in elite cyclists, possibly due to skepticism connected to muscle biopsy sampling. The less invasive microbiopsy procedure used in the present study should open the avenue for more projects in the future, allowing elucidation of both acute and accumulative effects of different HIE protocols in a greater cohort of elite athletes.

CONFLICT OF INTEREST
None.

ORCID
Nicki W. Almquist https://orcid.org/0000-0002-5994-5186
Håvard Nygaard https://orcid.org/0000-0002-7401-5323
Daniel Hammarström https://orcid.org/0000-0001-8360-2100
Stian Ellefsen https://orcid.org/0000-0003-0467-2018
Bent R. Rønnestad https://orcid.org/0000-0002-6907-1347

REFERENCES
1. Joyner MJ, Coyle EF. Endurance exercise performance: the physiology of champions. *J Physiol*. 2008;586(1):35-44.
2. Sylta O, Tonnessen E, Hammarstrom D, et al. The effect of different high-intensity periodization models on endurance adaptations. *Med Sci Sports Exercise*. 2016;48(11):2165-2174.
3. Westgarth-Taylor C, Hawley JA, Rickard S, Myburgh KH, Noakes TD, Dennis SC. Metabolic and performance adaptations to interval training in endurance-trained cyclists. *Eur J Appl Physiol Occup Physiol*. 1997;75(4):298-304.
4. Ronnestad BR, Hansen J. Optimizing interval training at power output associated with peak oxygen uptake in well-trained cyclists. *J Strength Cond Res*. 2016;30(4):999-1006.
5. Storen O, Bratland-Sanda S, Haave M, Helgerud J. Improved VO_{2max} and time trial performance with more high aerobic intensity interval training and reduced training volume: a case study on an elite national cyclist. *J Strength Cond Res*. 2012;26(10):2705-2711.
6. Hawley JA, Myburgh KH, Noakes TD, Dennis SC. Training techniques to improve fatigue resistance and enhance endurance performance. *J Sports Sci*. 1997;15(3):325-333.
7. Ben Abderrahman A, Zouhal H, Chamari K, et al. Effects of recovery mode (active vs. passive) on performance during a short high-intensity interval training program: a longitudinal study. *Eur J Appl Physiol*. 2013;113(6):1373-1383.
8. Wenger HA, Bell GJ. The interactions of intensity, frequency and duration of exercise training in altering cardiorespiratory fitness. *Sports Med*. 1986;3(5):346-356.
9. Ronnestad BR, Hansen J, Vegge G, Tonnessen E, Slettalokken G. Short intervals induce superior training adaptations compared with long intervals in cyclists - an effort-matched approach. *Scand J Med Sci Sports*. 2015;25(2):143-151.
10. Wahl P, Mathes S, Kohler K, Achtzehn S, Bloch W, Mester J. Acute metabolic, hormonal, and psychological responses to different endurance training protocols. *Horm Metab Res*. 2013;45(11):827-833.
11. Shahani S, Braga-Basaria M, Maggio M, Basaria S. Androgens and erythropoiesis: past and present. *J Endocrinol Invest*. 2009;32(8):704-716.
12. Lundby C, Montero D, Joyner M. Biology of VO_{2max}: looking under the physiology lamp. *Acta Physiol (Oxf)*. 2017;220(2):218-228.
13. Remes K, Kuoppasalmi K, Adlercreutz H. Effect of long-term physical training on plasma testosterone, androstenedione, luteinizing hormone and sex-hormone-binding globulin capacity. *Scand J Clin Lab Invest*. 1979;39(8):743-749.
14. Eliakim A, Brasel JA, Mohan S, Barstow TJ, Berman N, Cooper DM. Physical fitness, endurance training, and the growth hormone-insulin-like growth factor I system in adolescent females. *J Clin Endocrinol Metab*. 1996;81(11):3986-3992.
15. Adlercreutz H, Harkonen M, Kuoppasalmi K, et al. Effect of training on plasma anabolic and catabolic steroid hormones and their response during physical exercise. *Int J Sports Med*. 1986;7(Suppl 1):27-28.
16. Viru A, Viru M. Cortisol–essential adaptation hormone in exercise. *Int J Sports Med*. 2004;25(6):461-464.
17. Edgett BA, Foster WS, Hankinson PB, et al. Dissociation of increases in PGC-1alpha and its regulators from exercise intensity and muscle activation following acute exercise. *PLoS ONE*. 2013;8(8):e71623.
18. Nordsborg NB, Kusuhara K, Hellsten Y, et al. Contraction-induced changes in skeletal muscle Na(+), K(+) pump mRNA expression - importance of exercise intensity and Ca(2+)-mediated signalling. *Acta Physiol (Oxf)*. 2010;198(4):487-498.
19. Vikmoen O, Ellefsen S, Troen O, et al. Strength training improves cycling performance, fractional utilization of VO_{2max} and cycling economy in female cyclists. *Scand J Med Sci Sports*. 2016;26(4):384-396.
20. Hostrup M, Bangsbo J. Limitations in intense exercise performance of athletes - effect of speed endurance training on ion handling and fatigue development. *J Physiol*. 2017;595(9):2897-2913.

21. Granata C, Oliveira RS, Little JP, Renner K, Bishop DJ. Training intensity modulates changes in PGC-1alpha and p53 protein content and mitochondrial respiration, but not markers of mitochondrial content in human skeletal muscle. *FASEB J*. 2016;30(2):959-970.

22. Egan B, Carson BP, Garcia-Roves PM, et al. Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor coactivator-1 mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. *J Physiol*. 2010;588(Pt 10):1779-1790.

23. Hoier B, Passos M, Bangsbo J, Hellsten Y. Intense intermittent exercise provides weak stimulus for vascular endothelial growth factor secretion and capillary growth in skeletal muscle. *Exp Physiol*. 2013;98(2):585-597.

24. Iaia FM, Perez-Gomez J, Thomassen M, Nordsborg NB, Hellsten Y, Bangsbo J. Relationship between performance at different exercise intensities and skeletal muscle characteristics. *J Appl Physiol (1985)*. 2011;110(6):1555-1563.

25. Skovgaard C, Almquist NW, Bangsbo J. The effect of repeated periods of speed endurance training on performance, running economy, and muscle adaptations. *Scand J Med Sci Sports*. 2018;28(2):381-390.

26. Ruas JL, White JP, Rao RR, et al. A PGC-1alpha isoform induced by resistance training regulates skeletal muscle hypertrophy. *Cell*. 2012;151(6):1319-1331.

27. Hayot M, Michaud A, Koechlin C, et al. Skeletal muscle microbiopsy: a validation study of a minimally invasive technique. *Eur Respir J*. 2005;25(3):431-440.

28. De Pauw K, Roelands B, Cheung SS, de Geus B, Rietjens G, Meeusen R. Guidelines to classify subject groups in sport-science research. *Int J Sports Physiol Perform*. 2013;8(2):111-122.

29. Ronnestad BR, Hansen EA, Raastad T. Effect of heavy strength training on thigh muscle cross-sectional area, performance determinants, and performance in well-trained cyclists. *Eur J Appl Physiol*. 2010;108(5):965-975.

30. Hammarström D, Øfsteng S, Koll L, et al. Benefits of higher resistance-training volume depends on ribosome biogenesis. PREPRINT. 2019.

31. Konrad P. *The ABC of EMG*. Vol. 1.4. Scottsdale, AZ: Noraxon INC; 2006.

32. Ellefsen S, Hammarstrom D, Strand TA, et al. Blood flow-restricted strength training displays high functional and biological efficacy in women: a within-subject comparison with high-load strength training. *Am J Physiol Regul Integr Comp Physiol*. 2015;309(7):R767-R779.

33. Hammarstrom D, Ofsteng S, Koll L, et al. Benefits of higher resistance-training volume are related to ribosome biogenesis. *J Physiol*. 2020;598(3):543-565.

34. Ritz C, Spiess AN. qpcR: an R package for sigmoidal model selection in quantitative real-time polymerase chain reaction analysis. *Bioinformatics*. 2008;24(13):1549-1551.

35. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*. 2002;3(7):RESEARCH0034. https://doi.org/10.1186/gb-2002-3-7-research0034

36. Ronnestad BR, Hansen J, Nygaard H, Lundby C. Superior performance improvements in elite cyclists following short intervals vs. effort-matched long intervals training. *Scand J Med Sci Sports*. 2020;30:849-857.

37. Bassett DR Jr, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc*. 2000;32(1):70-84.

38. Fiorenza M, Gunnarsson TP, Hostrup M, et al. Metabolic stress-dependent regulation of the mitochondrial biogenic molecular response to high-intensity exercise in human skeletal muscle. *J Physiol*. 2018;596(14):2823-2840.

39. Hackney AC. Endurance training and testosterone levels. *Sports Med*. 1989;8(2):117-127.

40. Latasa I, Cordova A, Quintana-Ortí G, Lavilla-Oiz A, Navallas J, Rodriguez-Falces J. Evaluation of the Electromyography Test for the Analysis of the Aerobic-Anaerobic Transition in Elite Cyclists during Incremental Exercise. *Appl Sci*. 2019;9:589.

41. Billat V, Petot H, Karp JR, Sarre G, Morton RH, Mille-Hamard L. The sustainability of VO_{2max}: effect of decreasing the workload. *Eur J Appl Physiol*. 2013;113(2):385-394.

42. Perry CG, Lally J, Holloway GP, Heigenhauser GJ, Bonen A, Spriet LL. Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *J Physiol*. 2010;588(Pt 23):4795-4810.

43. Hoier B, Nordsborg N, Andersen S, et al. Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training. *J Physiol*. 2012;590(3):595-606.

44. McGinley C, Bishop DJ. Distinct protein and mRNA kinetics of skeletal muscle proton transporters following exercise can influence interpretation of adaptations to training. *Exp Physiol*. 2016;101(12):1565-1580.

45. Herbst EAF, MacPherson REK, LeBlanc PJ, et al. Pyruvate dehydrogenase kinase-4 contributes to the recirculation of gluconeogenic precursors during postexercise glycogen recovery. *Am J Physiol Regul Integr Comp Physiol*. 2014;306(2):R102-R107.