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The higher oxygen consumption during multiple short intervals is sex-independent and not influenced by skeletal muscle characteristics in well-trained cyclists

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Abstract

It has been suggested that time spent at a high fraction of maximal oxygen consumption ($\%\dot{V}O_{2max}$) plays a decisive role for adaptations to interval training. However, previous studies examining how interval sessions should be designed to achieve a high $\%\dot{V}O_{2max}$ have exclusively been performed in males. The present study compared the $\%\dot{V}O_{2max}$ attained during three different 6 × 8 min interval protocols, in female ($n = 11$; $\dot{V}O_{2max}$, 62.5 (6.4) mL · min^{-1}·kg^{-1}) and male ($n = 8$; $\dot{V}O_{2max}$, 81.0 (5.2) mL · min^{-1}·kg^{-1}) cyclists. Mean power output during work intervals were identical across the three interval protocols, corresponding to the cyclist's 40 min maximal effort (PO_{40min}): (1) 30 s intervals at 118% of PO_{40min} interspersed with 15 s active recovery at 60% (30/15), (2) constant pace at 100% of PO_{40min} (CON), and (3) altering between 60 s intervals at 110% and 60 s at 90% of PO_{40min} (60/60). Additionally, the study explored whether the *m. vastus lateralis* characteristics of the cyclists (fiber type proportion, capillarization, and citrate synthase activity) were associated with the $\%\dot{V}O_{2max}$ attained during the interval sessions. Overall, mean $\%\dot{V}O_{2max}$ and time ≥90% of $\dot{V}O_{2max}$ were higher during 30/15 compared to CON (86.7 (10.1)% and 1123 (787) s versus 85.0 (10.4)% and 879 (779) s, respectively; both $p \leq 0.01$) and 60/60 (85.6 (10.0)% and 917 (745) s, respectively; both $p \leq 0.05$), while no difference was observed between 60/60 and CON (both $p \geq 0.36$). During interval sessions, $\%\dot{V}O_{2max}$ and time ≥90% of $\dot{V}O_{2max}$ did not differ between sexes. Skeletal muscle characteristics were not related to $\%\dot{V}O_{2max}$ during interval sessions. In conclusion, well-trained cyclists demonstrate highest $\%\dot{V}O_{2max}$ during 30/15, irrespective of sex and skeletal muscle characteristics.

All tests and exercise training sessions were conducted at the physiological test laboratory at Inland Norway University of Applied Sciences, campus Lillehammer.

KEYWORDS
endurance training, interval training, oxygen consumption, sex differences, skeletal muscle characteristics

Highlights

- Among the workload-matched sessions investigated (30/15, 60/60, and CON), 30/15 intervals resulted in the highest oxygen consumption and longest periods with oxygen consumption above 90% of the participant's maximal oxygen consumption.
- For each of the three interval sessions investigated, no significant differences between female and male participants were displayed for average fraction of maximal oxygen consumption achieved during the intervals.
- The *m. vastus lateralis* fiber type proportion, capillarization and citrate synthase activity of the participants were not related to the average fraction of maximal oxygen consumption achieved during the different interval sessions.

1 | INTRODUCTION

Exercise training at high intensities stresses the oxygen delivery and utilization system, and is considered to be an effective physiological stimulus for increasing maximal oxygen consumption ($\dot{V}O_{2max}$) and enhancing endurance performance (Buchheit & Laursen, 2013; Midgley et al., 2006; Wenger & Bell, 1986). Interval training is usually performed in the severe intensity domain (endurance time up to �30–45 min (Burnley & Jones, 2007)) and is an essential training component for endurance athletes (Coates et al., 2023). To maximally enhance $\dot{V}O_{2max}$, it is recommended to perform interval sessions that accumulate as much time as possible at intensities ≥90% of $\dot{V}O_{2max}$ (Buchheit & Laursen, 2013; Midgley & McNaughton, 2006). Following this rationale, several studies with male endurance athletes as participants have focused on optimizing interval sessions through maximizing the time spent ≥90% of $\dot{V}O_{2max}$ (e.g., Almquist et al., 2020; Bossi et al., 2020; Held et al., 2023; Rønnestad et al., 2021; Thevenet et al., 2007). In these studies, the combinations of different workloads and -durations, different numbers of work intervals as well as different work-rest patterns have been explored. However, it is currently unknown whether these approaches to maximize time ≥90% of $\dot{V}O_{2max}$ also apply to female endurance athletes. Female and male endurance athletes are reported to possess dissimilar fractional utilization of $\dot{V}O_{2max}$ at lactate threshold (LT; Maldonado-Martin et al., 2004; Støa et al., 2020). The latter can theoretically affect the ability to attain a high fraction of $\dot{V}O_{2max}$ (%$\dot{V}O_{2max}$) during exercise, which may indicate that the oxygen consumption ($\dot{V}O_2$) responses to distinct interval sessions are sex-dependent.

Previous studies on male endurance athletes have identified interval sessions with alternating higher and lower workloads within each work interval (Bossi et al., 2020; Rønnestad et al., 2021), and series with multiple short work intervals with active recovery in between (Almquist et al., 2020; Rønnestad & Hansen, 2016) to be well-suited for extending the time ≥90% of $\dot{V}O_{2max}$. The physiological mechanisms underlying the longer time sustained ≥90% of $\dot{V}O_{2max}$ during series of multiple short work intervals and variable work intervals compared to traditional constant pace work intervals (CON) are not fully understood. Nevertheless, it has been postulated that factors such as higher levels of intramuscular adenosine diphosphate (Wilson, 2015), the larger use of respiratory muscles (Bossi et al., 2020), and the higher activation of type II muscle fibers (Vanhatalo et al., 2010) contribute to the greater energy demand observed during variable workloads compared to constant workload efforts. Particularly, interval sessions with series of multiple short intervals (e.g., 30 s) with active recovery in between (e.g., 15 s) have been observed to be favorable for achieving and sustaining a long time ≥90% of $\dot{V}O_{2max}$ (Almquist et al., 2020; Rønnestad & Hansen, 2016). As an example, in Almquist et al. (2020), elite male cyclists completed 3 sets of 13 × 30 s work intervals separated by 15 s active recovery periods (the 30/15 protocol) where they achieved higher average power output (PO) and, consequently, a higher $\dot{V}O_2$ and longer time ≥90% of $\dot{V}O_{2max}$ compared to 4 × 5 min constant pace work intervals, without perceiving greater exertion. To our knowledge, responses to the 30/15 protocol have never been compared to a CON protocol with a similar mean PO during work intervals. However, a comparison between protocols using similar mean PO has been made between 6 × 5 min work intervals with varied workloads within each work interval (i.e., alternating 30 s work periods at the lowest theoretical PO that elicits $\dot{V}O_{2max}$, separated by 60–90 s work periods at a PO just above the LT) and 6 × 5 min constant pace (Bossi et al., 2020). The study demonstrated that the protocol with variable workload intervals led to higher $\dot{V}O_2$ than a CON protocol in males, despite having a similar mean PO. A potential mechanism for this phenomenon is that the periods with higher PO will activate and exhaust energy-inefficient type II muscle fibers to a greater extent than during CON intervals, potentially resulting in a more pronounced $\dot{V}O_2$ slow component, as indicated by Vanhatalo et al. (2010). However, given that females in general typically possess lower proportions of type II muscle fibers (Hicks et al., 2001; Simoneau & Bouchard, 1989), and tend to exhibit a smaller performance fatigue development compared to males (Ansdell et al., 2019; Fulco et al., 1999; Hunter, 2014), it is reasonable to hypothesize that the mean $\%\dot{V}O_{2max}$ and the accumulated time ≥90% of $\dot{V}O_{2max}$ in work-matched interval sessions with series of multiple short intervals, variable work intervals, and CON will display smaller differences in females than in males. The effect of sex and skeletal muscle characteristics on the $\%\dot{V}O_{2max}$ achieved during different interval session protocols remains unexplored.

Hence, this study aimed to investigate the isolated effect of PO distribution on $\%\dot{V}O_{2max}$ and the accumulated time ≥90% of $\dot{V}O_{2max}$ during three different interval sessions. By controlling for variables such as total exercise duration and mean PO, we aimed to provide a clearer understanding of the specific influence of PO distribution during work intervals. All interval sessions consisted of six 8 min work intervals performed as (1) series of multiple short intervals continuously alternating between 30 s work separated by 15 s active recovery periods (30/15), (2) constant pace work intervals (CON), or (3) continuously alternating 60 s work intervals (60/60). We hypothesized that both the 30/15 and the 60/60 sessions would result in a higher mean $\%\dot{V}O_{2max}$ and accumulate more time ≥90% of $\dot{V}O_{2max}$ compared to the CON session, despite having identical total exercise duration and mean PO. Moreover, we aimed to assess whether the $\dot{V}O_2$ responses to the different workload-matched interval sessions would be dissimilar between female and male cyclists. Additionally, we sought to explore potential relationships between $\%\dot{V}O_{2max}$ elicited during the different interval sessions and *m. vastus lateralis* characteristics (fiber type I proportion, capillarization, and citrate synthase (CS) activity).

2 | MATERIALS & METHODS

2.1 | Study ethics and participants

The study was conducted in accordance with the Declaration of Helsinki and received approval from both the local ethical committee at Inland Norway University of Applied Sciences (reference no: Case 4-2020, 20/03749) and the Norwegian Social Science Data Services (NSD, reference no: 552564). Prior to inclusion, participants were informed of the content of the study and they all provided written informed consent to participate.

The study had nineteen participants, of whom 11 females (♀) and 8 males (♂), completed the study protocol (Table 1). The participants' main sport discipline was road cycling (n = 12; ♀, n = 6; ♂, n = 6), mountain biking (n = 6; ♀, n = 4; ♂, n = 2), and triathlon (n = 1♀). Based on initial $\dot{V}O_{2max}$ (mL · min^{-1}·kg^{-1}) and maximal 1-min PO during an incremental test (W_{max}; W · kg^{-1}), the female participants were categorized as performance level 3 (i.e., "trained"; n = 2), 4 (i.e., "well-trained"; n = 1), and 5 (i.e., "professional"; n = 8) according to the criteria of Decroix et al. (2016), whereas all the eight male participants were classified as performance level 5 (i.e., "professional") based on the criteria of De Pauw et al. (2013).

2.2 | Experimental design

Participants visited the laboratory on five separate occasions, including two screening visits and three experimental trials. During the initial screening visit, participants underwent a test battery comprising a standardized blood lactate profile test and an incremental exercise test performed to exhaustion to determine $\dot{V}O_{2max}$ and W_{max}. The following day, participants returned for

TABLE 1 Participant characteristics.

	Overall (n = 19)	Females (n = 11)		Males (n = 8)
General				
Age (years)	22.6 (4.1)	23.8 (5.0)		21.0 (1.4)
Body height (cm)	174.6 (7.8)	171.5 (8.1)	⚥	179.0 (4.8)
Body mass (kg)	66.9 (7.4)	66.6 (9.5)		67.3 (3.6)
Endurance measures				
$\dot{V}O_{2max}$ (mL · min^{-1}·kg^{-1})	70.3 (11.0)	62.5 (6.4)	⚥	81.0 (5.2)
W_{max} (W · kg^{-1})	5.9 (1.0)	5.2 (0.7)	⚥	6.8 (0.4)
$\%\dot{V}O_{2max}$ at LT (%)	81.6 (3.2)	83.3 (2.7)	⚥	79.4 (2.5)
M. vastus lateralis phenotypic measures				
Fiber type I proportion (%)	69.4 (11.4)	69.3 (12.8)		69.5 (10.2)
Capillary density (capillaries/mm^2)	652 (105)	641 (123)		668 (80)
Capillary-to-fiber ratio	3.3 (0.7)	3.2 (0.7)		3.5 (0.6)
CS activity (mIU · mg^{-1} protein)	312 (203)	280 (145)		358 (273)

Note: Data are means (SD). ⚥, sex difference ($p < 0.05$).

Abbreviations: $\%\dot{V}O_{2max}$, fraction of $\dot{V}O_{2max}$; CS, citrate synthase; LT, lactate threshold, defined as the power output at a blood lactate concentration of 4 mmol · L^{-1}; $\dot{V}O_{2max}$, maximal oxygen consumption; W_{max}, maximal 1-min power output during the incremental test.

their second visit, during which they completed a 40 min maximal cycling trial, and a muscle micro-biopsy from the *m. vastus lateralis* of a randomized leg was collected 40 min before the cycling trial. The experimental trials were conducted over three consecutive days. During this period, three different interval sessions were carried out. Each of these sessions was matched in terms of duration and mean PO and consisted of six 8 min work intervals. During each of these sessions, $\dot{V}O_2$ and carbon dioxide production, heart rate (HR), blood lactate concentration ([La$^-$]), and ratings of perceived exertion (RPE) were measured to quantify physiological, metabolic, and perceptual responses. To counterbalance the interval session order between participants, they were randomized to perform the three sessions in one of three different orders. To minimize the influence of circadian rhythm, tests, and sessions were carried out at the same time of day (±2 h) for each participant.

2.3 | Interval training sessions

All three interval sessions included 6 × 8 min work intervals separated by 3 min of active recovery. The mean PO during all 8 min work intervals was equal across the three interval sessions and corresponded to each participant's maximal average PO during the 40 min cycling trial (PO_{40min}). Between the three interval sessions, the PO distribution within the work intervals varied (Figure 1): (1) multiple short intervals continuously alternating between 30 s work at 118% of PO_{40min} separated by 15 s active recovery periods at 60% of PO_{40min} (i.e., 30/15), (2) constant work at a PO corresponding to 100% of PO_{40min} (i.e., CON), and (3) continuously altering 60 s work intervals at 110% and 90% of PO_{40min}, respectively (i.e., 60/60). The PO during the 3 min recovery periods between the 8 min work intervals corresponded to 35% of PO_{40min}. The cadence during the sessions was freely chosen. All three interval sessions were

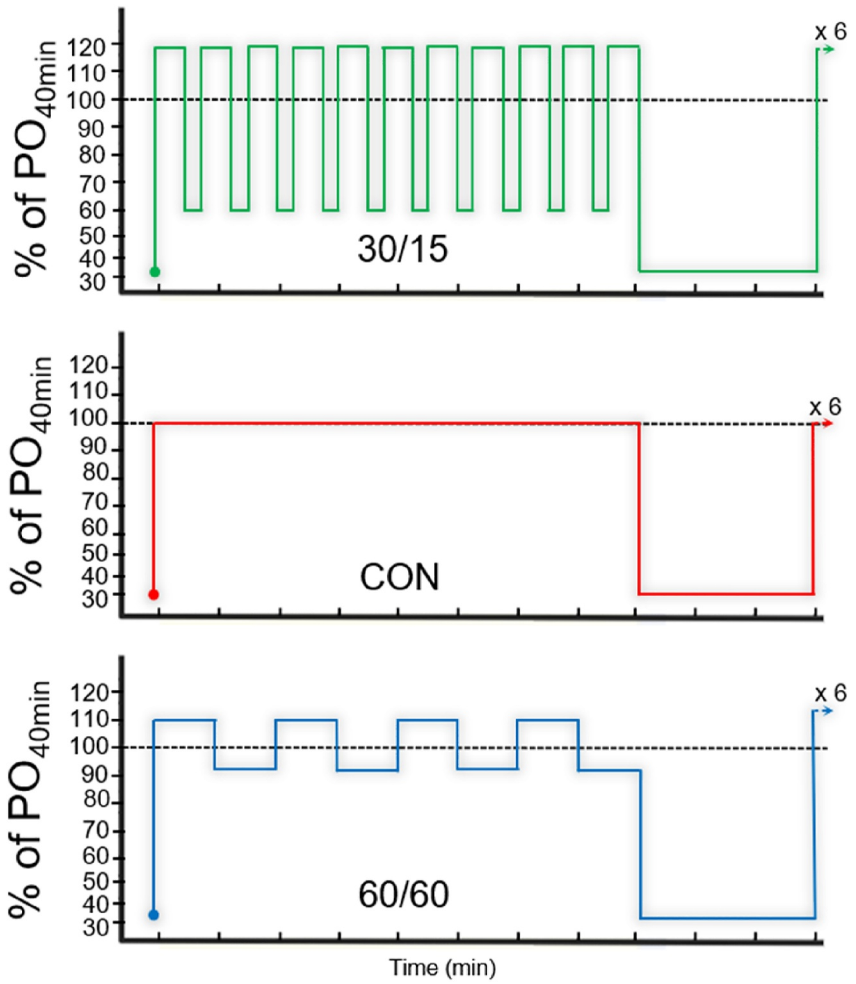

FIGURE 1 Overview of the three different interval sessions used in the present study. All sessions consisted of 6 × 8 min work intervals separated by 3 min recovery periods. The intensity during the work intervals was prescribed and corresponded to each participant's maximum average power output during a 40 min maximal cycling trial (PO_{40min}). 30/15, 30 s work intervals separated by 15 s active recovery periods performed continuously in each 8 min work interval; CON, constant workload during the 8 min work intervals; 60/60, two different 60 s work intervals alternating continuously in each 8 min work interval.

performed on the participant's personal bike connected to an individual stationary trainer device (Tacx Neo 2T Smart T2875 or T2850). The PO accuracy of the Tacx Neo 2T are reported to be within 1% by the manufacturer (Garmin, 2023). All interval sessions were supervised by the same test leader, who controlled the PO during the interval sessions using an app (Tacx Training™, version 4.26.2, Garmin Ltd.) connected to the trainer device. Prior to all interval sessions, a 20 min standardized warm-up protocol was performed. The warm-up protocol was based on the Borg 6–20 RPE scale (Borg, 1982) and consisted of 5 min at an intensity corresponding to a RPE of 11, succeeded by 5 min at a RPE of 13, 3 min at a RPE of 15, 2 min at a RPE of 9–10, 30 s at a RPE of 16, 30 s at a PO corresponding to 120% of PO_{40min}, and at last, 4 min at a RPE of 9–10.

Throughout all interval sessions, HR (Garmin Edge 530, Garmin Ltd.), $\dot{V}O_2$, minute ventilation (V_E), and breathing rate (B_R) were measured continuously with a 10 s sampling time during all 8 min work intervals. $\dot{V}O_2$, V_E, and B_R were measured using a metabolic cart with mixing chamber (Oxycon Pro, Erich Jaeger), which was calibrated in line with the manufacturer's description prior to each interval session. This calibration process included the use of certified calibration gases with known concentrations to calibrate the gas analyzers and a 3L calibration syringe (Hans Rudolph) to calibrate the air flow turbine (Triple V, Erich Jaeger). Across all work intervals during each interval session, mean $\dot{V}O_2$, mean HR, mean V_E, and mean B_R were calculated and expressed as a fraction of $\dot{V}O_{2max}$ (%$\dot{V}O_{2max}$), maximal HR (%HR_{max}), maximal V_E (%V_{Emax}), and maximal B_R (%B_{Rmax}), respectively. Immediately after completing each work interval, RPE was recorded, and a fingertip blood sample was obtained for determination of blood lactate concentration ($[La^-]$; Biosen C-line Lactate Analyzer, EKF Diagnostic GmbH), which also was calculated and expressed as a fraction of maximal $[La^-]$ (%$[La^-]_{max}$). Amounts of water, sports drink, and solid foods consumed during the first interval session were noted and repeated at the two following interval sessions. Ten minutes after finishing each interval session, the cyclists reported their session RPE using a 10-point scale (Foster et al., 2001).

2.4 | Physiological screening

On the first screening visit, the test procedure was performed on an electromagnetically braked bicycle ergometer (Lode Excalibur Sport) which was adjusted according to individual preferences. The screening visit began with an incremental lactate profile test. Female participants started at 80 watts (W), while male participants began at 125 W (175 W if self-estimated 4 $mmol \cdot L^{-1}$ $[La^-] > 275$ W). The workload increased by 40 W for females and 50 W for males every fifth min. $\dot{V}O_2$ was measured continuously during the last 3 min of each bout (30 s sampling time), and a fingertip blood sample was collected to determine $[La^-]$ with 30 s left of each bout. When a $[La^-]$ of 2 $mmol \cdot L^{-1}$ was measured, PO increases were adjusted to 20 W for females and 25 W for males until reaching the LT, defined as the PO when a $[La^-]$ of 4 $mmol \cdot L^{-1}$ was achieved. $\dot{V}O_2$ corresponding to the LT was calculated by linear interpolation between the two data points of $[La^-]$ and $\dot{V}O_2$ measured from the last two steps performed and expressed as %$\dot{V}O_{2max}$ at LT. Following 10 min of active recovery at 100 W, an incremental test was initiated to determine $\dot{V}O_{2max}$ and W_{max}. Female participants started at 160 W, while male participants began at 200 W (250 W if 4 $mmol \cdot L^{-1}$ $[La^-] > 320$ W). The workload increased by 20 W for females and 25 W for males every minute until exhaustion, defined as cadence dropping <60 revolutions per minute. Maximal HR (HR_{max}) was measured during the test and W_{max} was calculated as the mean PO during the last minute of the incremental test. $\dot{V}O_{2max}$ was calculated as the mean of the two highest consecutive 30 s $\dot{V}O_2$ measurements. For each participant, the same HR monitor, lactate analyzer, and metabolic cart as during the interval sessions were used. The metabolic cart was calibrated before each test, following the same protocol as used prior to the interval sessions.

During the second screening visit, participants performed a 40 min cycling trial using their own bikes connected to the same stationary trainer device used during the interval sessions. The participants were instructed to aim for their highest possible 40 min mean PO and adjusted the PO themselves using the Tacx Training™ app connected to the trainer device. Prior to the 40 min cycling trial, they performed the same 20 min warm-up protocol as before the interval sessions.

2.5 | Muscle biopsy sampling and histochemical analyses

Muscle microbiopsies were sampled from *m. vastus lateralis* under local anesthesia (Lidocaine, 10 mg/mL, AstraZenaca AS) using a 12-gauge needle (Universal Plus, Medax) operated with a spring-loaded biopsy instrument (Bard Magnum, Bard), as previously described (Hammarström et al., 2020). Biopsies were sampled at 1/3 of the distance from the patella to the anterior superior iliac spine. The collected tissue was quickly dissected free of blood and visible connective tissue in ice-cold sterile saline solution (0.9% NaCl). Samples for immunohistochemistry were transferred to a 4% formalin solution for fixation for 24–72 h before further preparation.

2.5.1 | Immunohistochemistry

Formalin-fixed muscle biopsies were processed, sectioned, and stained for dystrophin, myosin heavy chain 1, and capillaries (CD34) as previously described (Almquist et al., 2022). Images of stained cross-sections were captured using a high-resolution camera (Axiocam, Zeiss) mounted on a light microscope (Axioskop-2, Zeiss), with a fluorescent light source (X-Cite 120, EXFO Photonic Solutions Inc.). Multiple images were taken using 10× objectives to capture the entirety of each cross section. All analyses were performed using an automated procedure in the CellProfiler 4.2.1 software (Carpenter et al., 2006) ensuring an unbiased quantification. On average, 578 (198) fibers were analyzed per muscle sample.

2.5.2 | Citrate synthase activity

Muscle samples were homogenized as detailed elsewhere (Meinild Lundby et al., 2018) and subsequently determined for total protein concentration (Thermo Fisher Scientific). CS activity was assayed in muscle lysates using a commercially available kit (C3260; Sigma-Aldrich) according to the manufacturer's instructions. CS activity was normalized to protein concentration and expressed in international units (IU; $mIU \cdot mg^{-1}$ protein).

2.6 | Data analysis and statistics

All descriptive data are presented as means with standard deviations (SD) unless otherwise stated. To compare various characteristics between female and male participants, including age, body height, body mass, $\dot{V}O_{2max}$, W_{max}, $\%\dot{V}O_{2max}$ at LT, fiber type proportion, muscle capillarization, and oxidative enzyme activity independent sample *t*-tests were used. To compare physiological and perceptual responses between the three interval sessions, pairwise comparisons from mixed linear models with Tukey-adjusted *p*-values were used (lme4 version 1.1 and emmeans version 1.8.2 packages for R, version 4.2.1). To investigate sex-related differences between the three interval sessions, the interaction between sex and type of interval session were included in the mixed models. In explorative analyses, to investigate potential characteristics that may be accountable for the $\%\dot{V}O_{2max}$ achieved during the different interval sessions, mixed linear models were fitted with $\%\dot{V}O_{2max}$ at interval sessions as the dependent variable, while type of interval session and participant characteristics ($\%\dot{V}O_{2max}$ at LT and different skeletal muscle characteristics [i.e., fiber type I proportion, capillary density, number of capillaries per muscle fiber, and CS activity]) were treated as fixed effects. In all statistical models performed, random intercepts for each participant were specified. The residual plots were used to verify model assumptions. Inferential statistics are presented using a 95% confidence interval (95% CI: [lower bound, upper bound]), *p*-values, and point-estimates of the effect. *p*-values <0.05 were considered statistically significant.

3 | RESULTS

The average PO_{40min} for all participants was 269 (51) W. In the first interval session, one male participant had to reduce the PO after the first work interval, which was then replicated at his subsequent interval sessions. Consequently, the mean PO during all interval sessions corresponded to 100 (0.0)% of PO_{40min} (69.1 (4.1)% of W_{max}) for the female participants and 99.1 (2.4)% of PO_{40min} (68.5 (2.0)% of W_{max}) for the male participants, where absolute mean PO was higher for male than for female participants (Table 2). The upper and lower workloads during 30/15 were 316 (57) W and 161 (29) W, respectively. During 60/60, the respective values were 295 (53) W and 241 (43) W.

3.1 | Physiological and perceptual responses during interval sessions

Overall, $\%\dot{V}O_{2max}$ were higher during 30/15 compared to both CON (86.7 (10.1)% versus 85.0 (10.4)%, respectively; $p = 0.001$) and 60/60 (85.6 (10.0)%; $p = 0.032$), while no difference was observed between 60/60 and CON ($p = 0.361$; Figure 2A,B; Table 2). There were no differences in mean $\%\dot{V}O_{2max}$ between female and male participants during any of the interval sessions (⚥30/15, $p = 0.922$; ⚥CON, $p = 0.991$; ⚥60/60, $p = 0.998$; Table 2).

Overall, 30/15 elicited longer time ≥90% of $\dot{V}O_{2max}$ compared to both CON ($p = 0.001$) and 60/60 ($p = 0.042$), while there was no difference between 60/60 and CON ($p = 0.892$; Table 2). For this variable as well, there were no statistically significant sex differences observed between any of the sessions (⚥30/15, $p = 0.962$; ⚥CON, $p = 1.000$; ⚥60/60, $p = 1.000$; Table 2). Both the absolute and relative values of V_E, B_R, and $[La^-]$ were greater in the 30/15 session compared to both CON and 60/60 sessions (Table 2; Figure 3A). Of these, the only measures which were significantly different between females and males were the absolute mean V_E within all sessions (⚥30/15, $p < 0.001$; ⚥CON, $p < 0.001$; ⚥60/60, $p = 0.001$), and the absolute mean $[La^-]$ within the 60/60 session (⚥30/15, $p = 0.064$; ⚥CON, $p = 0.086$; ⚥60/60, $p = 0.047$; Table 2). $\%HR_{max}$ did not differ between interval sessions (all $p > 0.306$; Table 2; Figure 4B), which was also applicable for time ≥90% of HR_{max} (all $p > 0.332$; Table 2), and there were no statistically significant sex differences observed between any of the sessions for these variables (all $p > 0.426$; Table 2). Regarding participants' perceptual responses, both mean RPE across all work intervals and session RPE were higher in the 30/15 session compared to CON ($p = 0.005$ and $p = 0.005$, respectively), while no significant differences were observed between the 30/15 session and 60/60 ($p = 0.228$ and $p = 0.139$, respectively) or the 60/60 session and CON ($p = 0.216$ and $p = 0.139$, respectively; Table 2). Both mean RPE across all work intervals and session RPE were comparable between sexes (Table 2).

3.2 | Explorative analyses of potential factors accountable for the fraction of $\dot{V}O_{2max}$ during interval sessions

There were no significant overall relationships between $\%\dot{V}O_{2max}$ during the interval sessions and $\%\dot{V}O_{2max}$ at LT, or between $\%\dot{V}O_{2max}$

TABLE 2 Physiological and perceptual responses elicited during the work intervals of INT sessions.

	30/15				CON			60/60		
	♀		♂		♀		♂	♀		♂
Mean power output (W)	235 (27)	⚥	313 (32)		235 (27)	⚥	313 (32)	235 (27)	⚥	313 (32)
Physiological responses										
$\%\dot{V}O_{2max}$ (%)	85.9 (10.2)		87.8 (10.0)	*#	84.5 (10.8)		85.6 (9.7)	85.2 (10.3)		86.1 (9.5)
Time ≥90% of $\dot{V}O_{2max}$ (s)	995 (814)		1300 (713)	*#	863 (833)		902 (699)	925 (806)		906 (651)
$\%HR_{max}$ (%)	91.6 (4.4)		89.8 (5.4)		90.0 (5.5)		90 (5.4)	89.6 (5.3)		90.4 (5.2)
Time ≥90% of HR_{max} (s)	2199 (318)		1511 (885)		1740 (900)		1785 (595)	1473 (816)		1872 (779)
Mean V_E ($L \cdot min^{-1}$)	104 (18)	⚥	152 (32)	*#	95 (17)	⚥	139 (27)	98 (17)	⚥	140 (24)
$\%V_{Emax}$ (%)	70 (10)		75 (8)	*#	64 (10)		69 (6)	65 (8)		70 (5)
Mean B_R ($breath \cdot min^{-1}$)	49 (11)		55 (9)	*#	45 (11)		50 (8)	46 (10)		52 (9)
$\%B_{Rmax}$ (%)	84.4 (8.2)		87.1 (7.2)	*#	77.8 (9.2)		79.1 (7.6)	80.1 (7.0)		81.9 (7.8)
Mean $[La^-]$ ($mmol \cdot L^{-1}$)	6.3 (1.9)		9.1 (3.2)	*#	4.5 (1.4)		7.1 (1.8)	4.6 (2.0)	⚥	7.5 (1.8)
$\%[La^-]_{max}$ (%)	57.2 (20.8)		65.9 (21.0)	*#	39.8 (12.9)		52.1 (12.6)	40.3 (16.1)		54.4 (10.1)
Perceptual responses										
Mean RPE (6–20)	16.4 (1.0)		17.1 (0.8)	*	15.9 (1.5)		15.9 (1.2)	15.9 (1.4)		16.7 (1.1)
Session RPE (1–10)	7.0 (1.3)		7.4 (1.1)	*	6.4 (1.8)		5.9 (1.1)	6.6 (1.9)		6.5 (1.2)

Note: Data are means (SD). ♀, females; ♂, males. #, different from 60/60 ($p < 0.05$). *, different from CON ($p < 0.05$). ⚥, sex difference ($p < 0.05$).
Abbreviations: $\%\dot{V}O_{2max}$, mean fraction of maximal oxygen consumption ($\dot{V}O_{2max}$); $\%[La^-]_{max}$, mean fraction of maximal $[La^-]$; $\%B_{Rmax}$, mean fraction of maximal B_R; $\%HR_{max}$, mean fraction of maximal heart rate (HR_{max}); $\%V_{Emax}$, mean fraction of maximal V_E; $[La^-]$, blood lactate concentration; 30/15, 30 s work intervals separated by 15 s active recovery periods performed continuously in each 8 min work interval; 60/60, two different 60 s work intervals alternating continuously in each 8 min work interval; B_R, breathing rate; CON, constant workload during the 8 min work intervals; RPE, rate of perceived exertion; V_E, minute ventilation.

during interval sessions and the skeletal muscle characteristics (fiber type I proportion, capillary density, number of capillaries per muscle fiber, and CS activity) of the participants (Figure 4). However, a significant negative interaction was found between 30/15 and CON in response to a higher $\%\dot{V}O_{2max}$ at LT (Figure 4A). No other significant interactions were observed between any of the interval sessions.

4 | DISCUSSION

In this study, participants demonstrated a higher $\%\dot{V}O_{2max}$ and longer time ≥90% of $\dot{V}O_{2max}$ during the 30/15 session compared to both the CON and 60/60 sessions, while no such differences were observed between the 60/60 and the CON session. There were no significant differences observed between female and male participants in $\%\dot{V}O_{2max}$ or time ≥90% of $\dot{V}O_{2max}$ across the three different interval sessions. These findings suggest that 30/15 intervals are particularly favorable for facilitating a large physiological stimulus in both well-trained female and male cyclists. Furthermore, the study demonstrates that $\%\dot{V}O_{2max}$ during the different interval sessions were neither associated with any of the assessed skeletal muscle characteristics (fiber type I proportion, capillary density, number of capillaries per muscle fiber, and CS activity), nor associated with $\%\dot{V}O_{2max}$ at LT. However, in explorative analyses, a negative interaction was observed between 30/15 and CON in response to a higher $\%\dot{V}O_{2max}$ at LT. Possibly, the latter indicates that for achieving a high $\%\dot{V}O_{2max}$ during interval sessions, 30/15 is particularly favorable for cyclists with a lower $\%\dot{V}O_{2max}$ at LT, whereas the type of interval session is of less importance for cyclists with a higher $\%\dot{V}O_{2max}$ at LT.

It was initially hypothesized that both the 30/15 and 60/60 sessions would result in higher mean $\%\dot{V}O_{2max}$ values and longer accumulated time ≥90% of $\dot{V}O_{2max}$ compared to the CON session. This hypothesis was based on previous studies that supported such an outcome with similar interval protocols (Almquist et al., 2020; Bossi et al., 2020; Rønnestad & Hansen, 2016; Rønnestad et al., 2021). However, it was only the 30/15 session that elicited a higher $\%\dot{V}O_{2max}$ and longer time ≥90% of $\dot{V}O_{2max}$ compared to CON in the present study. These differences could be attributed to variations in protocol design, including the work–rest patterns and the amplitude of PO variations: when comparing to CON in the present study, all work intervals were initiated with an in average 47 (9) W higher PO during the 30/15 session, and 26 (5) W higher PO during the 60/60 session. A higher PO at the onset of a work interval will further increase the rate of oxidative metabolism in the active muscle, leading to faster $\dot{V}O_2$ kinetics, and ultimately increased $\dot{V}O_2$ (Jones et al., 2011; Margaria et al., 1965; Rossiter et al., 2002). As shown in Figure 2A, the 30/15 session displayed a visually higher $\dot{V}O_2$ compared to CON at the onset of the 8 min work intervals,

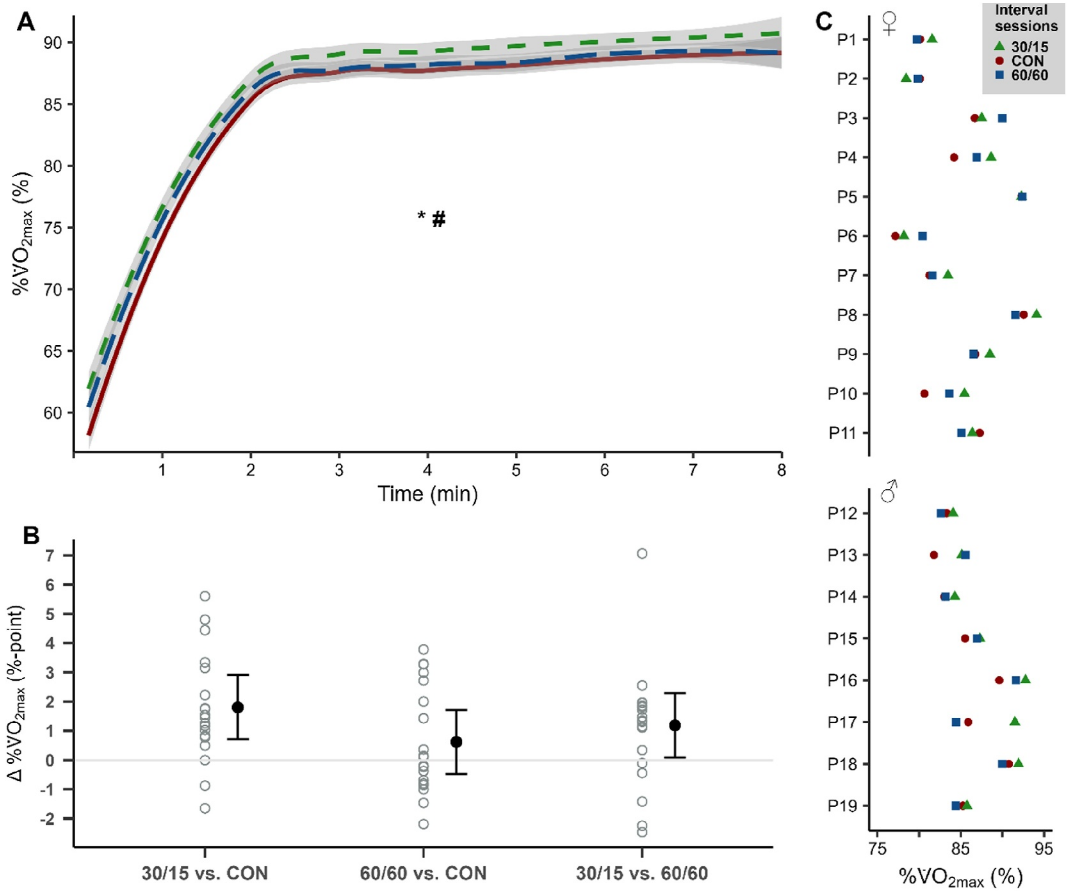

FIGURE 2 (A) Overall mean fraction of maximal oxygen consumption (%$\dot{V}O_{2max}$) elicited during the six 8 min work intervals with (1) continuous alternating 30 s work intervals separated by 15 s active recovery periods (30/15, green dashed line), (2) constant pace work intervals (CON, red solid line), and (3) two different continuously alternating 60 s work intervals (60/60, blue long dashed line). (B) Pairwise comparisons of the %-point differences (Δ) in %$\dot{V}O_{2max}$ between the different interval sessions. (C) The %$\dot{V}O_{2max}$ of each female (♀) and male (♂) participant during the different interval sessions. The light gray areas in (A) and the whiskers in (B) indicate 95% confidence intervals. *, 30/15 was different from CON ($p < 0.05$); #, 30/15 was different from 60/60 ($p < 0.05$).

which may have contributed to the higher mean %$\dot{V}O_{2max}$ and longer time ≥90% of $\dot{V}O_{2max}$. In contrast, the 60/60 session displayed only a marginal, numerically higher $\dot{V}O_2$ compared to CON at the beginning of the 8 min work intervals (Figure 2A). This suggests that for interval protocols with variable work intervals or series of multiple short work intervals, each work interval series should be initiated at a sufficiently high PO, and that the absolute PO during the first work period in the current 60/60 session may have been too low. Consistent with this, Bossi et al. (2020) observed a greater $\dot{V}O_2$ response in an interval protocol with variable work intervals compared to CON when the mean PO during interval sessions was equal, but the variable work intervals were initiated at an in average 63 W higher PO compared to CON.

Visually, the 30/15 session also displayed a higher $\dot{V}O_2$ during the last ~6 min of the work intervals, with partially non-overlapping CIs (not clearly depicted in the figure) compared to both CON and 60/60 (Figure 2A). To explain this, a few potential mechanisms need to be highlighted. First, 30/15 resulted in both higher absolute and relative mean V_E and mean B_R compared to both CON and 60/60, indicating greater pulmonary mechanical work and, thus, conceivably higher oxygen cost (Aaron et al., 1992). Second, the high PO during the 30 s work intervals during the 30/15 session could potentially lead to a larger $\dot{V}O_2$ slow component (Coyle et al., 1992; Krustrup et al., 2008; Vanhatalo et al., 2010), resulting in higher oxygen demand for the same work rate. A larger $\dot{V}O_2$ slow component may have been generated by activating more of the type II muscle fibers, which are generally assumed to be less energy-efficient (Coyle et al., 1992; Krustrup et al., 2008). In addition to progressive muscle fiber recruitment, a larger $\dot{V}O_2$ slow component can be generated by a higher oxygen cost for fatigued muscle fibers (Vanhatalo et al., 2010). The high workload during the 30 s work intervals could have induced greater muscle fatigue, which in turn may have increased the metabolic demand during the subsequent 30 s work intervals, as well as during the 15 s active recovery periods. Therefore, the large PO differences between the surges within the work intervals of variable and multiple short interval sessions, combined with the sufficiently high average absolute workload during these work intervals, likely accounted for the greater $\dot{V}O_2$ responses

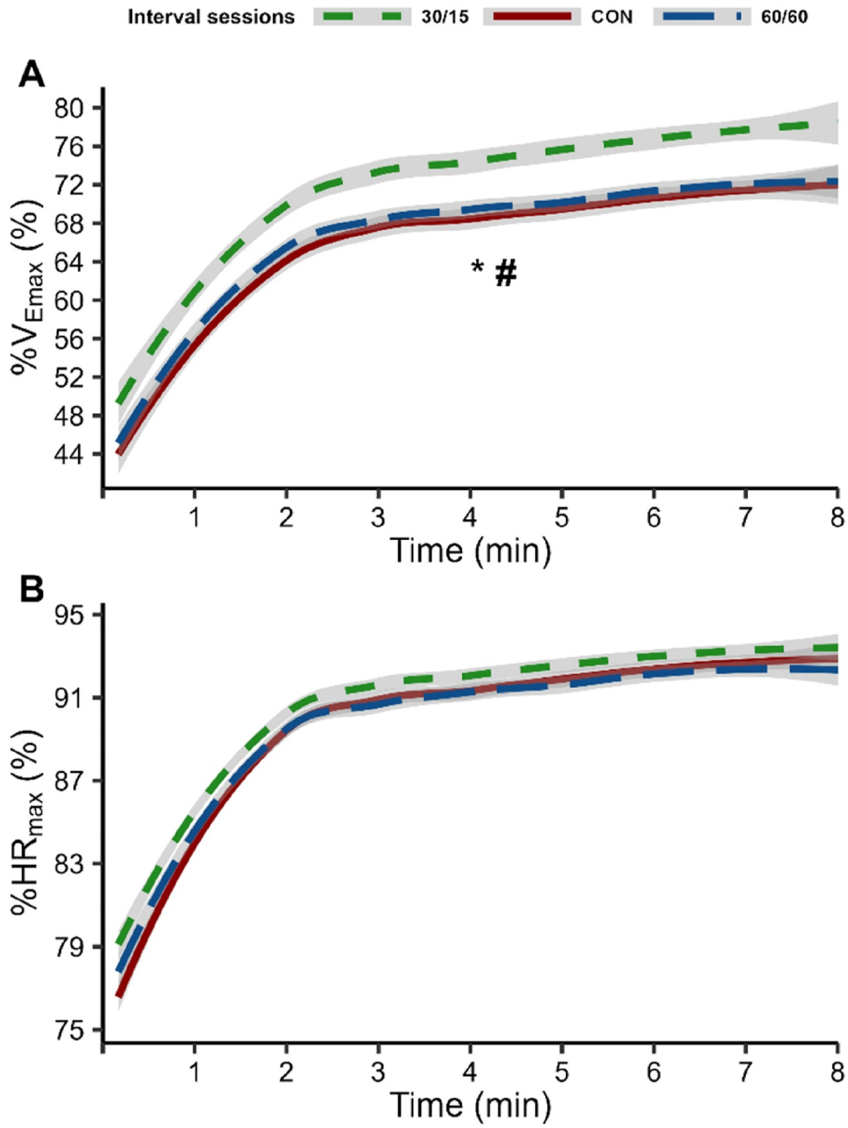

FIGURE 3 (A) Overall mean fraction of maximal minute ventilation (%V_{Emax}) and (B) overall mean fraction of maximal heart rate (%HR_{max}) elicited during the six 8 min work intervals with (1) continuously alternating 30 s work intervals separated by 15 s active recovery periods (30/15, green dashed line), (2) constant pace work intervals (CON, red solid line), and (3) two different continuously alternating 60 s work intervals (60/60, blue long dashed line). The light gray areas indicate 95% confidence intervals. *, 30/15 was different from CON ($p < 0.05$); #, 30/15 was different from 60/60 ($p < 0.05$).

compared to CON protocols. Furthermore, in light of our exploratory data indicating that individuals with a low %$\dot{V}O_{2max}$ at LT gain greater advantages from the 30/15 session for achieving a high %$\dot{V}O_{2max}$ during interval sessions, it is plausible that this is linked to the necessity for cyclists with such a characteristic to initiate their work intervals at a higher PO to induce a rapid increase in $\dot{V}O_2$. Conversely, for cyclists with a high %$\dot{V}O_{2max}$ at LT, the type of interval session seems to be of less importance for achieving a high %$\dot{V}O_{2max}$ during interval sessions. Theoretically, this could be related to a less pronounced $\dot{V}O_2$ slow component, and a faster mean response time, in these participants. However, further research is needed to verify this.

To our surprise, no previous studies investigating the acute effects of protocols with variable work intervals and series of multiple short intervals have included females. This study is therefore the first to demonstrate that interval protocols with variable work intervals and series of multiple short intervals induce comparable $\dot{V}O_2$ responses between female and male cyclists. Considering the more pronounced $\dot{V}O_2$ slow component in subjects with a greater proportion of type II fibers in their vastus lateralis muscle (Vanhatalo et al., 2010) and the fact that females typically possess lower proportions of type II muscle fibers (Hicks et al., 2001; Simoneau & Bouchard, 1989), it could be reasonable to assume that interval protocols with variable work intervals and series of multiple short

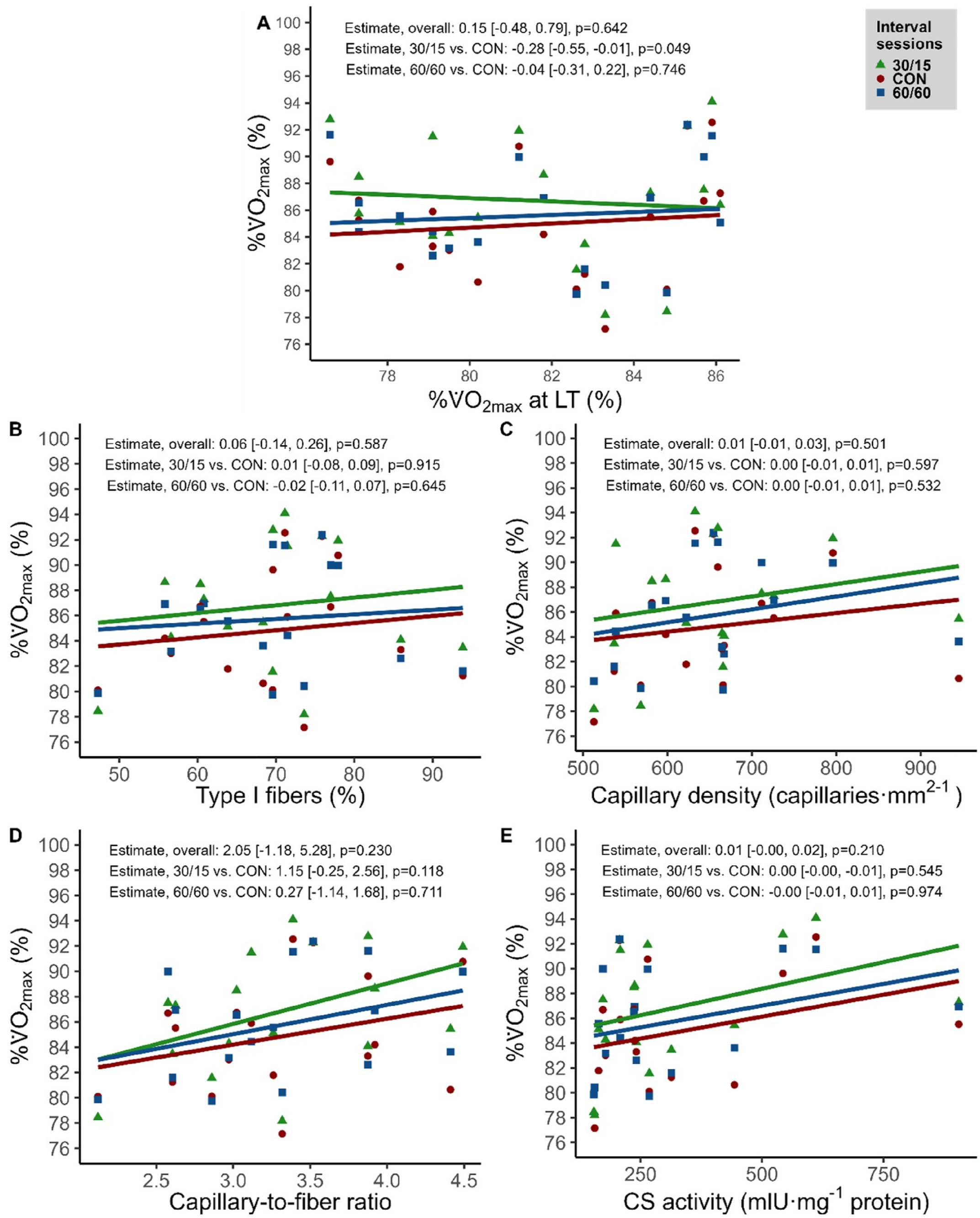

FIGURE 4 Participants' (A) fraction of maximal oxygen consumption (%$\dot{V}O_{2max}$) at the lactate threshold (LT), defined as the power output at which a blood lactate concentration of 4 mmol · L^{-1} is achieved, (B) fiber type I proportion, (C) capillary density, (D) capillary-to-fiber ratio, and (E) citrate synthase (CS) activity were examined as predictors of %$\dot{V}O_{2max}$ elicited during the three different interval sessions in which the six 8 min work intervals were performed as (1) continuous alternating 30 s work intervals separated by 15 s active recovery periods (30/15, green triangle), (2) constant pace work intervals (CON, red circle), and (3) two different continuously alternating 60 s work intervals (60/60, blue square). Estimated slopes for each condition are shown. "Estimate, overall" represents the overall estimated change in %$\dot{V}O_{2max}$ at interval sessions per unit change in the predictive variable; "Estimate, 30/15 versus CON" and "Estimate, 60/60 versus CON" indicate interactions with CON (red line) for 30/15 (green line) and 60/60 (blue line), respectively. Point-estimates are presented with 95% confidence intervals ([lower bound and upper bound]) and *p*-value.

intervals may be less effective in eliciting high $\dot{V}O_2$ responses in female cyclists. However, the present study did not find such disparities in fiber type proportions between female and male cyclists, and %$\dot{V}O_{2max}$ during interval sessions did not show any relationship with muscle fiber type I proportion. %$\dot{V}O_{2max}$ during the three interval sessions was also similar between females and males. Nevertheless, in this study, females displayed a higher %$\dot{V}O_{2max}$ at LT than their male counterparts, which is consistent with previous findings

(Maldonado-Martin et al., 2004; Støa et al., 2020). Since we also observed a negative interaction in the elicited %$\dot{V}O_{2max}$ during interval sessions between 30/15 and CON in response to a higher %$\dot{V}O_{2max}$ at LT (Figure 4A), it could be speculated that interval protocols with series of multiple short work intervals are less effective for eliciting high $\dot{V}O_2$ responses in female cyclists. In this study, however, this did not appear to be the case. Therefore, it is reasonable to recommend the 30/15 session for both females and males if the training session goal is to maximize the $\dot{V}O_2$ during the interval session.

Another argument in favor of protocols with variable work intervals and series of multiple short intervals compared to CON is the previous observations of participants eliciting more time ≥90% of $\dot{V}O_{2max}$ without feeling more exhausted (Almquist et al., 2020; Bossi et al., 2020). Consistent with this, the participants in the present study reported equal RPE and session RPE during 60/60 and CON. However, in contrast to previous findings, the participants reported higher RPE and session RPE during 30/15 compared to CON in the current study. This could potentially be a result of differences in interval session design and pacing strategy (effort-matched in the previous study vs. PO-matched in the present study), longer total work duration, as well as greater statistical power due to more participants in the present study (n = 19 vs. n = 8; Almquist et al., 2020). The higher levels of RPE and session RPE during 30/15 compared to CON in the present study are supported by the concomitantly higher absolute and relative mean V_E, mean B_R, and mean [La^-]. This is in line with the reports of a strong association between the respiratory rate and RPE (Nicolò et al., 2017), as well as between RPE and both [La^-] and HR (Oliveira et al., 2013). Taken together, following a PO-matched approach as in the current study, the 30/15 session induce more time ≥90% of $\dot{V}O_{2max}$ than CON, but at the expense of slightly higher physiological and perceptual demands.

Considering the practical implication of the present study, it is important to highlight that both the %$\dot{V}O_{2max}$ achieved and the time sustained ≥90% of $\dot{V}O_{2max}$ during an interval session as criteria for judging the efficacy of the training session are arguably lacking direct scientific support (Midgley et al., 2006; Thevenet et al., 2007). However, given that performing interval sessions designed to elicit a high %$\dot{V}O_{2max}$ and a long time ≥90% of $\dot{V}O_{2max}$ have been observed to improve $\dot{V}O_{2max}$ (Rønnestad et al., 2015) and measures of endurance performance (Rønnestad et al., 2015, 2022) to a larger degree than CON intervals, well-trained cyclists of both sexes should consider including the described 30/15 session rather than the 60/60 and CON session in their exercise training programs. As previously reported, the reliability of time ≥90% HR_{max} is rather poor (Rønnestad et al., 2022). The present results indicate that the HR were not different between the three interval sessions despite higher $\dot{V}O_2$ during the 30/15 session compared to the other sessions. Along with the finding of greater variability in HR measurements, this supports the assumption that one should be very careful in using HR measurements as a surrogate measure of $\dot{V}O_2$. Further, the present findings indicate that the skeletal muscle characteristics investigated in this study are not important when prescribing interval sessions for endurance athletes. To summarize, based on previous and present findings, there are not any weighty arguments for sex-specific or muscle-phenotypic interval prescriptions.

The present study had its limitations; no specific familiarization was conducted and the sessions were performed over three consecutive days. This raises the possibility of participants experiencing both a learning effect and accumulated fatigue. However, it must be emphasized that the participants included in the study were well-trained cyclists who were well familiar with interval training, as well as the specific interval protocols used. Moreover, the sessions were randomized, which minimize any familiarization effect and mitigate the influence of accumulated fatigue. The phase of menstrual cycle for female cyclists was not controlled for in this study. However, the fact that the sessions were carried out on three consecutive days might limit the potential impact of substantial hormonal fluctuations on the outcomes, even though we acknowledge that endogenous and exogenous hormones can exhibit day-to-day variations (Sims & Heather, 2018). Notably, it is recently demonstrated that variables determining performance do not significantly differ between menstrual cycle phases (Mattu et al., 2020; Taylor et al., 2024).

5 | CONCLUSIONS

When mean PO is similar in interval sessions consisting of six 8 min work intervals, the multiple short interval 30/15 session display higher %$\dot{V}O_{2max}$ and longer accumulated time ≥90% of $\dot{V}O_{2max}$ compared to both the 60/60 and the CON session. This suggests that the 30/15 session provides the largest exercise stimulus among the three interval sessions, but notably, it also displayed slightly larger respiratory, metabolic, and perceptual demands. For each of the interval sessions, no differences between female and male cyclists were observed for %$\dot{V}O_{2max}$ or time ≥90% of $\dot{V}O_{2max}$. Skeletal muscle characteristics (fiber type I proportion, capillarization, and CS activity) were not related to the achieved $\dot{V}O_2$ during the different interval sessions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PATIENT CONSENT STATEMENT

Prior to inclusion, participants were informed of the content of the study, and they all provided written informed consent to participate.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not applicable.

CLINICAL TRIAL REGISTRATION

Not applicable.

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