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Environmental heat stress offsets adaptation associated with carbohydrate periodization in trained male triathletes

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Abstract

Purpose: Carbohydrate (CHO) intake periodization via the sleep low train low (SL-TL) diet–exercise model increases fat oxidation during exercise and may enhance endurance-training adaptation and performance. Conversely, training under environmental heat stress increases CHO oxidation, but the potential of combined SL-TL and heat stress to enhance metabolic and performance outcomes is unknown.

Methods: Twenty-three endurance-trained males were randomly assigned to either control ($n=7$, CON), SL-TL ($n=8$, SL_{Temp}) or SL-TL + heat stress ($n=8$, SL_{Heat}) groups and prescribed identical 2-week cycling training interventions. CON and SL_{Temp} completed all sessions at 20°C, but SL_{Heat} at 35°C. All groups consumed matched CHO intake ($6\,g \cdot kg^{-1} \cdot day^{-1}$) but timed differently to promote low CHO availability overnight and during morning exercise in both SL groups. Submaximal substrate utilization was assessed (at 20°C), and 30-min performance tests (at 20 and 35°C) were performed Pre-, Post-, and 1-week post-intervention (Post+1).

Results: SL_{Temp} improved fat oxidation rates at 60% MAP (~66% VO_{2peak}) at Post+1 compared with CON ($p < 0.01$). Compared with SL_{Temp}, fat oxidation rates were significantly lower in SL_{Heat} at Post ($p = 0.02$) and Post+1 ($p < 0.05$). Compared with CON, performance was improved at Post in SL_{Temp} in temperate conditions. Performance was not different between any groups or time points in hot conditions.

Conclusion: SL-TL enhanced metabolic adaptation and performance compared with CON and combined SL-TL and heat stress. Additional environmental heat stress may impair positive adaptations associated with SL-TL.

KEYWORDS
cycling performance, diet–exercise strategy, endurance training, heat acclimation, metabolism, nutrition

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1 | INTRODUCTION

The primary aim of endurance training is to induce positive physiological adaptation and improve key determinants of endurance performance, including maximal oxygen uptake ($\dot{V}O_{2max}$), physiological thresholds, and exercise economy.[1] Additionally, the stochastic nature of Olympic distance triathlon[2] requires a high degree of metabolic flexibility to efficiently utilize carbohydrates and fat as energy substrates during periods of sub- and supra-maximal exercise, respectively.[3]

Periodically training with low carbohydrate (CHO) availability is a popular diet–exercise strategy among endurance athletes,[4] with sessions commenced with purposefully reduced CHO availability, termed "train low".[5] Despite reduced exercise intensity, train low enhances skeletal muscle cell signaling and transcriptional activity,[6–8] increases fat oxidation rates[9,10] and improves 10 km running time[11] and functional threshold power in trained individuals[12] compared to training with consistently high CHO availability. Conversely, training low may be detrimental to short-duration, supra-maximal exercise performance, with improvements in 1-min peak power absent following 3 weeks of training with low CHO availability compared to participants consuming high CHO intake.[12] Mechanistically, low CHO availability may decrease peak CHO oxidation rates due to increased pyruvate dehydrogenase kinase 4 (PDK4) mRNA expression[6,7]; reducing pyruvate dehydrogenase (PDH) activity, which, combined with reduced glycogenolysis, limits glycolytic end-products available for flux into the mitochondria.[13] Additionally, when exercise is completed with extremely low CHO availability ($<50\,g\cdot day^{-1}$), fat oxidation rates are increased albeit at the expense of maximal CHO oxidation rates and performance compared to high and periodized CHO ($\sim500\,g\cdot day^{-1}$) intake diets.[14] Thus, strategies that promote enhanced molecular adaptation while maintaining metabolic flexibility and improving performance may be desirable to endurance athletes to maximize endurance training adaptations and performance.

A proposed countermeasure for maintaining metabolic flexibility following exercise with low CHO availability is completing exercise under conditions of environmental heat stress. Firstly, it is well established that exercise under heat stress increases CHO metabolism[15] with environmental temperature and exercise intensity critical factors in regulating substrate utilization during exercise in the heat.[16] Moreover, exercise under heat stress may provide a secondary benefit, with positive whole-body metabolic and skeletal muscle mitochondrial adaptation as reported following 2 weeks of endurance training in hot conditions.[17] Therefore, training low under heat stress conditions may alleviate any train low-induced reductions in CHO oxidation, albeit this is yet to be investigated.

An important caveat to this strategy is that in combining strategies, one may detract from the other potentially impairing adaptation.[3] For instance, traditional nutritional guidelines for exercise in hot conditions recommend consistently high CHO intake to account for increased oxidation rates and facilitate muscle glycogen resynthesis between exercise bouts[18] and withholding CHO between sessions may have negative implications for recovery.

The present study aimed to determine the performance and metabolic impact of a 2-week CHO periodization (according to the SL-TL model) and exercise intervention in hot (SL_{Heat}, all sessions completed in 35°C, 50% RH) conditions compared to SL-TL (SL_{Temp}) and control (consistent CHO intake) in temperate conditions (20°C, 50% RH). It was hypothesized that CHO periodization and heat stress would improve performance in hot and temperate conditions with no marked alteration in submaximal substrate utilization. While in line with current literature, SL_{Temp} would increase submaximal fat oxidation rate and improve performance in temperate conditions compared to the control group.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

Ethical approval was granted by the local ethics committee (CPP, Nantes, France; No. 2018-A02544-51), Participants provided written informed consent, and all procedures conformed to the standards of the Declaration of Helsinki 2013.

2.2 | Participants

Twenty-four trained male triathletes, free of musculoskeletal and neurological disease and not receiving pharmacological treatment, were recruited for the study (participant characteristics presented in Table 1). One person withdrew, citing personal reasons reducing the control group to $n = 7$. A priori sample size estimation indicated a minimum sample size of 21 participants ($n = 7$ per group) was required to detect a small to moderate effect for mean power during performance tests with 80% statistical power using the G*Power (v 3.1) software. The effect size used for this calculation was based on small to moderate effect sizes in performance outcomes following 3 weeks of SL-TL intervention in previous studies.[11,12]

2.3 | Study design

The present study implemented a randomized control trial design conducted during the non-summer months

TABLE 1 Participant characteristics for the control (CON), sleep low (SL), and sleep low and heat acclimation (SL$_{Heat}$) groups.

	CON	SL$_{Temp}$	SL$_{Heat}$
Number	7	8	8
Age (years)	34 ± 7	28 ± 5	28 ± 7
Height (cm)	182 ± 7	180 ± 7.0	181 ± 4
Weight (kg)	71.6 ± 6.7	73.6 ± 8.3	74 ± 5
$\dot{V}O_{2max}$ (L·min^{-1})	4.7 ± 0.5	4.5 ± 0.6	4.5 ± 0.6
$\dot{V}O_{2max}$ (ml·kg.$^{-1}$min^{-1})	65.7 ± 6.7	61 ± 5.5	60.4 ± 7.1
MAP (W)	369 ± 42	352 ± 33	358 ± 48
Experience in triathlon (years)	6 ± 4	5 ± 2	7 ± 4
Habitual training Volume (h·week^{-1})	11 ± 6	10 ± 3	11 ± 5

Note: All data are presented as mean $\pm$ SD.

Abbreviations: MAP, Maximal aerobic power; $\dot{V}O_{2max}$, Maximal oxygen uptake.

(October–April) in Paris, France. During Week 1, participants completed a familiarization of the physiological testing battery, including incremental maximal exercise test, submaximal substrate utilization, and 30-min maximal capacity tests in hot (35°C, 50% relative humidity [rH]) and temperate (20°C, 50% rH) conditions. During the second week, participants maintained regular training and dietary intake recorded via an online training platform (Playsharp, Clermont-Ferrand, France) and a written dietary log. Week 3 constituted the "pre-test" week, and participants repeated the physiological battery. Participants then commenced a 2-week diet–exercise intervention following random allocation to the control group (CON), SL$_{Temp}$, or SL$_{Heat}$ groups. CON and SL$_{Temp}$ completed all training sessions in temperate conditions (20°C, 50% rH) with consistently moderate-to-high CHO intake or periodized CHO intake, respectively. SL$_{Heat}$ followed a periodized CHO strategy; however, all sessions were under environmental heat stress (35°C, 50% rH). Following the intervention, the physiological battery was repeated twice, once immediately following the intervention (Week 6) and again following 1 week of habitual diet and training (Week 8). The study timeline with all procedures is presented in Figure 1.

Before each laboratory visit, participants were provided with comprehensive pre-visit standardization protocols, including no exhaustive exercise or alcohol for 48 h and no caffeine consumption for the 24 h before each testing battery. In addition, participants were asked to replicate pre-visit dietary intake 24 h before each visit. They were given a standardized pre-trial meal (CHO: 2.0 g·kg BM^{-1}, Protein [PRO]: 0.3 g·kg BM^{-1}, Fat: 0.3 g·kg BM^{-1}) to be consumed 2 h before attending the laboratory. Consistency of nutritional intake was confirmed via serum metabolomics,[19] revealing no significant difference in metabolites immediately before exercise.

On arrival, participants provided a rating of fatigue[20] (did not exceed 4 [little fatigued] in any case) then provided a mid-stream urine sample to confirm hydration status via urine-specific gravity (USG) assessment by portable refractometer (URC-NE, 1.000–1.050, Atago, Tokyo, Japan). No participant returned hypohydrated samples (U$_{SG}$ >1.020). Finally, nude body mass was recorded using a digital platform scale with a cable remote display (SECA, USA).

2.4 | Assessment of maximal oxygen uptake ($\dot{V}O_{2max}$)

$\dot{V}O_{2max}$ and maximal aerobic power (MAP) were assessed by incremental cycle test performed on an electronically braked ergometer (Excalibur Sport, Lode, Groningen, Netherlands). Following a 10-min warm-up at 100 W, workload was increased by 30 W every 2 min until volitional exhaustion. Breath-by-breath oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) were obtained using an online gas analysis system (Quark Cosmed, Rome, Italy), and heart rate (HR) (Polar H10, Kempele, Finland) recorded throughout the exercise. MAP (W) was calculated per Hawley & Noakes.[21]

2.5 | Training program

Participants were prescribed identical 2-week training programs in their assigned environmental condition, consisting of daily low-intensity sessions (LIT) (1 h at 60% MAP) for six consecutive days with high-intensity interval training (HIT) (6 × 5 min at 85% MAP) scheduled on alternate evenings. All sessions were completed on Wattbike Pro stationary cycle requiring participants to maintain the prescribed power output. HR was collected
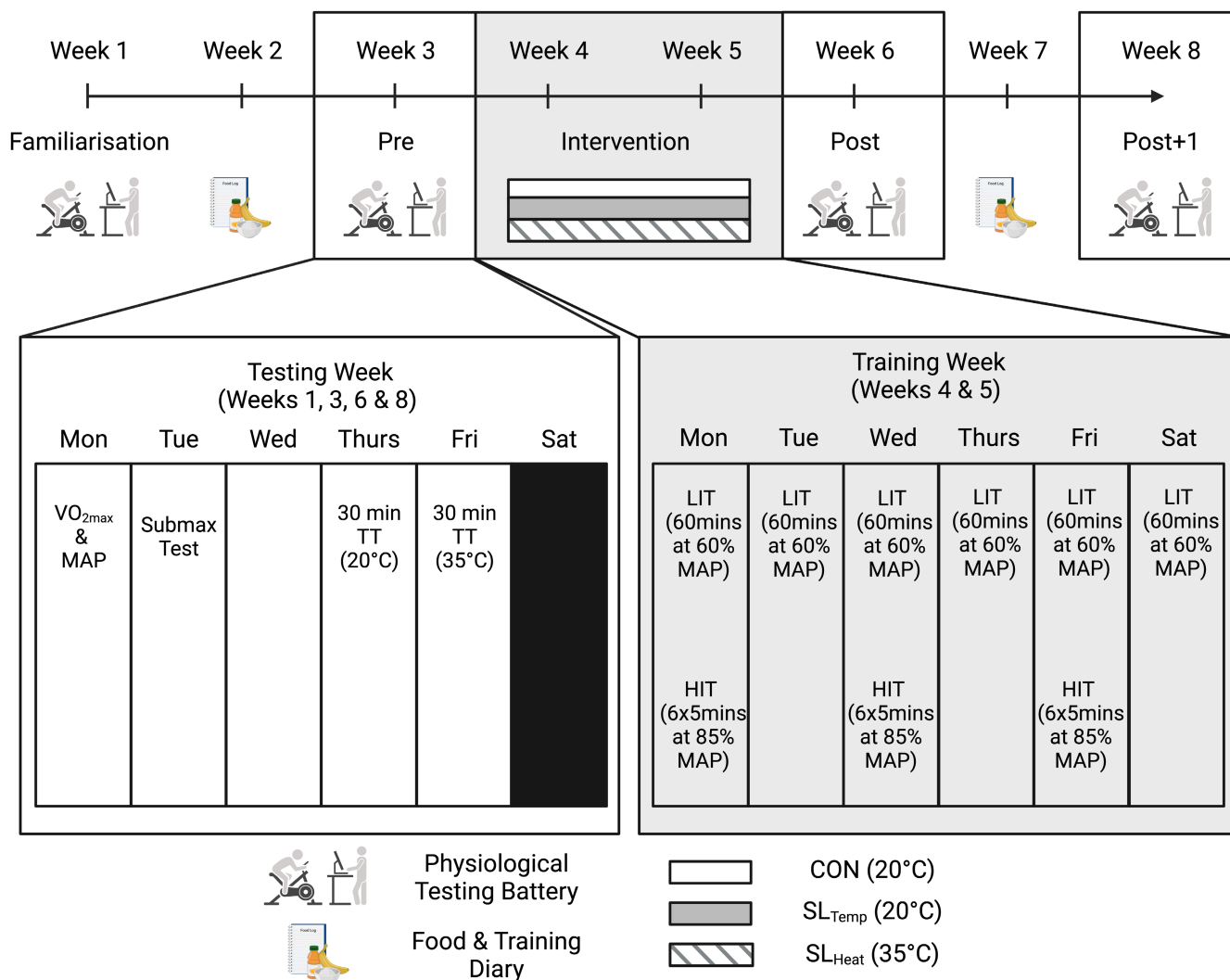
FIGURE 1 Schematic overview of study design spanning 8 weeks of intervention. The physiological testing battery consisted of maximal intensity cycling tests to determine oxygen uptake ($\dot{V}O_{2max}$) and maximal aerobic power (MAP), submaximal substrate utilization tests (Submax Test), and two 30-min-maximal exercise tests (30 min TT) in temperate (20°C) or hot (35°C) conditions.

during exercise via provided HR monitors (Polar H10, Kempele, Finland). HR and power data were recorded via a smartphone application (Wattbike, Nottingham, UK) and uploaded to an online training monitoring platform (Playsharp, Clermont-Ferrand, France) for analysis.

2.6 | Nutritional guidelines

Baseline dietary intake was recorded via written food diary for the week preceding pre-tests (baseline) and throughout the 2-week intervention, excluding days 7 and 14. Participants weighed all uncooked foods and noted quantities and preparation/cooking techniques. A qualified, registered sports dietician analyzed all nutritional data.

Dietary guidelines, including standardized dietary instructions and meal plans, were provided to participants to alter CHO availability for specific sessions during the week. Total daily CHO intake was characterized as moderate to high based upon the hours of prescribed exercise and intensity ($6\,g\cdot kg^{-1}\cdot day^{-1}$).[22] Crucially, daily CHO intake was matched between groups but timed differently to achieve either "normal" or low CHO availability before specific training sessions. SL_{Temp} and SL_{Heat} groups were prohibited from consuming CHO following each HIT session (no CHO at dinner) until after the fasted LIT session the following morning, according to the SL-TL model. Following the LIT session, participants consumed $6\,g\cdot kg^{-1}$ of CHO before the next HIT session on the same day (Table 2). To maintain overnight satiety, all participants consumed a high protein, low CHO gel (CHO: 1.4 g, PRO: 20 g, Fat 1 g; SIS WHEY 20, Science in Sport, Lancashire, UK) before bed. Direct contact with the research team, and dieticians were available to assist with dietary concerns.

TABLE 2 Overview of prescribed exercise (in bold) and CHO intake ($g \cdot kg^{-1}$) during the intervention for control (CON), sleep low (SL_{Temp}) and sleep low + heat (SL_{Heat}) groups.

Group	Day 1 & 8		Day 2 & 9		Day 3 & 10		Day 4 & 11		Day 5 & 12		Day 6 & 13	
	CON	SL_{Temp} & SL_{Heat}	CON	SL_{Temp} & SL_{Heat}	CON	SL_{Temp} & SL_{Heat}	CON	SL_{Temp} & SL_{Heat}	CON	SL_{Temp} & SL_{Heat}	CON	SL_{Temp} & SL_{Heat}
Morning (before 10:00)	Breakfast (2g); **LIT**		Breakfast (2g); **LIT**	**LIT** (Fasted); Breakfast (2g)	Breakfast (2g); **LIT**		Breakfast (2g); **LIT**	**LIT** (Fasted); Breakfast (2g)	Breakfast (2g); **LIT**		Breakfast (2g); **LIT**	**LIT** (Fasted); Breakfast (2g)
Midday	Lunch (1.5g)	Lunch (2g)	Lunch (1.5g)		Lunch (1.5g)	Lunch (2g)	Lunch (1.5g)	Lunch (2g)	Lunch (1.5g)	Lunch (2g)	Normal Diet	
Afternoon (before 17:00)	Snack (0.5g)	Snack (2g)	Snack (0.5g)		Snack (0.5g)	Snack (2g)	Snack (0.5g)	Snack (2g)	Snack (0.5g)	Snack (2g)		
Evening (after 17:00 and before 21:00)	**HIT**; Dinner (2g)	Dinner (0g)	Dinner (2g)		**HIT**; Dinner (2g)	Dinner (0g)	Dinner (2g)	Dinner (2g)	**HIT**; Dinner (2g)	Dinner (0g)		

2.7 | Submaximal cycling test

A two-stage submaximal cycling test whereby a 10-min warm-up at 100 W preceded two successive 6-min stages at 60% (66% $\dot{V}O_{2max}$) and 70% (77% $\dot{V}O_{2max}$) of MAP was completed by all participants. Breath-by-breath (Quark Cosmed, Rome, Italy) respired gases ($\dot{V}O_2$ and $\dot{V}CO_2$) and HR (Polar H10, Kempele, Finland) were recorded throughout the exercise. Whole-body CHO and fat oxidation ($g \cdot min^{-1}$) rates were calculated from final 1-min averaged $\dot{V}O_2$ and $\dot{V}CO_2$ and nonprotein respiratory exchange ratio (RER) values of the intensity of interest.[23] Energy expenditure (EE) ($kcal \cdot min^{-1}$) was obtained from the rate of $\dot{V}O_2$ and based on the thermal equivalent of O_2 for nonprotein RER as per Brouwer.[24]

2.8 | Thirty-minute time trial (TT)

Participants completed the TT in thermoneutral (20°C, 50% rH) and hot (35°C, 50% rH) conditions.

2.8.1 | Exercise protocol

Following a 15-min self-paced warm-up (standardized within participants between trials), six phases of 4 min 50 s interspersed with 10-s maximal sprints was completed. Participants were instructed to ride as hard as possible throughout the test to obtain their best mean power output (MPO, including sprints). The test was conducted on participants' bicycles mounted to a validated stationary cycle home trainer (Hammer H2, Cycleops, USA).[25]

2.8.2 | Core temperature

Core temperature was measured via a validated suppository telemetric pill (E-Celcius, BodyCap, Hérouville Saint-Clair, France).[26] Participants self-inserted the pill beyond the anal sphincter approximately 1 h before exercise as recommended by the national athletic trainers association.[27] Data were recorded at 1-min intervals via a logger (e-Viewer, BodyCap, Hérouville Saint-Clair, France).

2.8.3 | Perceptual measures

Participants reported perceived exertion,[28] thermal comfort and thermal sensation before each sprint, using a color-coded, audio-visual scale.[29]

2.9 | Statistical analysis

All data are reported as mean $\pm$ SD. Where data was collected at pre, post, and post+1 (TT's and submaximal cycling test), a two-way analysis of covariance (ANCOVA) was conducted to identify significant differences between experimental groups (CON vs SL vs SL_{Heat}) on performance and substrate utilization controlling for inter-individual variability using baseline test data (pre) as a covariate. Additionally, mixed-effects ANOVA was used to assess the effect of group and time on training responses. Post-hoc Bonferroni, multiple comparison tests were performed when significant effects were present. Statistical significance was $p < 0.05$ for all tests. Where significant main effects were present, effect sizes were calculated using partial eta squared (η^2_p) with values of 0.01, 0.06, and over 0.14 considered small, medium, and large, respectively.[30] ANCOVA was performed using Stata Statistical Software: Release 18 (StataCorp LLC, TX, USA), and ANOVA conducted using GraphPad Prism v.9 (GraphPad Software, CA, USA).

3 | RESULTS

3.1 | Training response

All participants completed all prescribed training sessions. RPE-based training load differed between groups; SL_{Heat} had a significantly ($p < 0.001$, $\eta^2_p = 0.02$) higher training load (813 ± 29 a.u.) compared with CON (724 ± 29 a.u.) and SL_{Temp} (731 ± 59 a.u.). When considering session-specific differences, during LIT exercise, SL_{Heat} had a significantly ($p < 0.001$, $\eta^2_p = 0.03$) higher training load (363 ± 49 a.u.) compared with CON (262 ± 33 a.u.) and SL_{Temp} (278 ± 40 a.u.). There were no differences in RPE-based training load during the HIT exercise between groups.

Relative power output (%MAP) during LIT sessions was reduced in SL_{Heat} and SL_{Temp} groups compared wiht CON ($p < 0.05$, $\eta^2_p = 0.1$). Power output was reduced in SL_{Heat} on days 2, 3, 4, 6, 9, and 13 ($p < 0.05$) and days 2 and 4 in SL_{Temp} (Figure 2A). Heart rate (%HRmax) was not significantly different between groups during the training intervention, albeit a significant effect of time ($p < 0.001$, $\eta^2_p = 0.12$) was present (Figure 2B). In line with the RPE-based training load, RPE was significantly elevated ($p < 0.0001$, $\eta^2_p = 0.27$) in the SL_{Heat} group compared with SL_{Temp} and CON groups (Figure 2C). The subjective thermal comfort rating was significantly higher in the SLHeat group than in the SL_{Temp} and CON groups ($p < 0.0001$, $\eta^2_p = 0.17$). During the second week of intervention, the difference in thermal comfort was reduced between

groups, with significant differences between SL_{Heat} and CON on Days 10 and 13 (Figure 2D).

During the HIT exercise, mean power output was not significantly different between groups during any session. However, power output was reduced during recovery periods (R-4, 5, and 6) compared with SL_{Temp} (Figure 3A). During HIT session 4, only during R-5 and R-6 were there differences in power output between SL_{Heat}, CON and SL_{Temp} (Figure 3B). By the final HIT session (6), there were no differences in mean power during any interval (Figure 3C).

3.2 | Submaximal cycling test

When controlling for pre-test HR, there was a significant mean effect for group ($p = 0.03$, $\eta^2_p = 0.16$) at 60% MAP. No difference was identified when controlling for pre-test HR at 70% MAP.

At 60% MAP, there was a significant main effect of group on respiratory exchange ratio (RER) when controlling for pre-exercise RER ($p = 0.002$, $\eta^2_p = 0.26$). RER in SL_{Temp} (0.85 ± 0.03) was significantly different than CON (0.92 ± 0.04) at Post+1 ($p < 0.01$). At Post+1, RER showed a trend toward significance ($p = 0.06$) between SL_{Temp} and SL_{Heat}. No main effects of group or time were present when controlling for Pre-intervention RER at 70% MAP.

When controlling for baseline, there was a significant main effect of group ($p < 0.01$, $\eta^2_p = 0.22$) at 60% MAP. SL_{Temp} fat oxidation rates (0.74 ± 0.16 g·min^{-1}) were significantly higher than CON (0.39 ± 0.25 g·min^{-1}) at 60% MAP ($p = 0.02$). At 70% MAP, there was a significant main effect of group ($p < 0.01$, $\eta^2_p = 0.028$) when controlling for baseline fat oxidation rate. Fat oxidation rate was significantly lower in SL_{Heat} at Post+1 (0.45 ± 0.24 g·min^{-1}) compared to SL_{Temp} (Post+1 = 0.72 ± 0.71 g·min^{-1}) ($p < 0.01$). Physiological responses during submaximal cycling tests are presented in Figure 4 and Data S1.

3.3 | Thirty-minute time trial in temperate conditions (20°C, 50% RH)

When controlling for mean baseline power, there was a significant main effect for group ($p < 0.001$ $\eta^2_p = 0.33$). There was a significant difference between CON and SL_{Temp} immediately following the intervention (Post, $p = 0.042$). Mean power output was increased from Pre to Post by $7.9\% \pm 3.2\%$ in SL_{Temp} compared to $2.4\% \pm 4.4\%$ in CON. Significant differences were also identified at Post+1 ($p = 0.012$) between SL_{Temp} and SL_{Heat}. Meam power output increased from Pre to Post+1 by $8.13\% \pm 4.6\%$ in SL_{Temp} compared to $1.1\% \pm 6\%$ in SL_{Heat} (Figure 5A). Mean HR
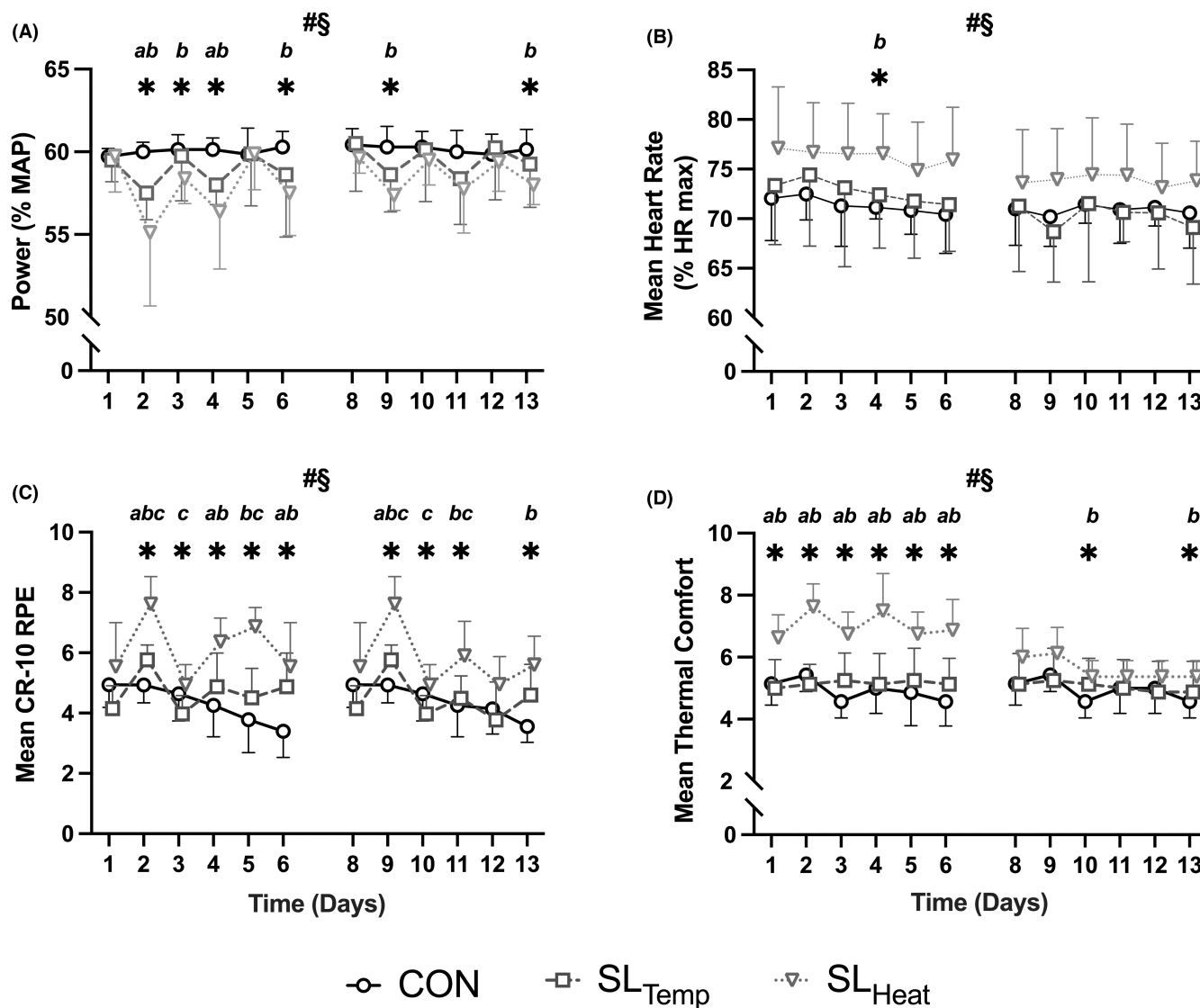
FIGURE 2 Training Data for daily low-intensity training (LIT) (A) Mean power output relative to maximal aerobic power (% MAP); participants were asked to maintain 60% for the hour. (B) Mean heart rate (beats·min^{-1}). (C) Mean rating of perceived exertion. (D) Mean thermal comfort. The main group statistical effect is represented by "#" and the time effect by "§." Post-hoc analysis is represented as significantly different by a *. Pairwise comparisons "a" denotes a significant difference between CON and SL$_{Temp}$, "b" denotes a significant difference between CON and SL$_{Heat}$, and "c" denotes a significant difference between SL and SL$_{Heat}$.

decreased in all groups from Pre to Post; however, when controlling for pre-intervention HR, there were no significant differences between groups (Figure 5C). In addition, there was no difference in mean core body temperature between or within groups when controlling for Pre-intervention mean core body temperature (Figure 5E).

3.4 | Thirty-minute time trial in hot conditions (35°C, 50% RH)

Mean power output (Figure 5B) and mean HR (Figure 5D) were not different between groups when

controlling for baseline measures of each variable. There was a significant main interaction effect (group × time) for mean core body temperature when controlling for Pre-intervention mean core body temperature ($p = 0.01$, $\eta^2_p = 0.21$). SL$_{Temp}$ (37.6 ± 0.3°C) mean core body temperature was significantly lower than CON (37.9 ± 0.3°C) at Post+1 ($p = 0.03$). Additionally, there was a significant difference in mean core body temperature between SL$_{Temp}$ (37.8 ± 0.3°C) and SL$_{Heat}$ (37.6 ± 0.2°C) at Post ($p = 0.02$) with no significance at Post+1 (37.9 ± 0.2°C, $p = 0.052$). Mean core body temperature significantly differed in SL$_{Heat}$ between Post and Post+1 ($p < 0.01$, Figure 5F).
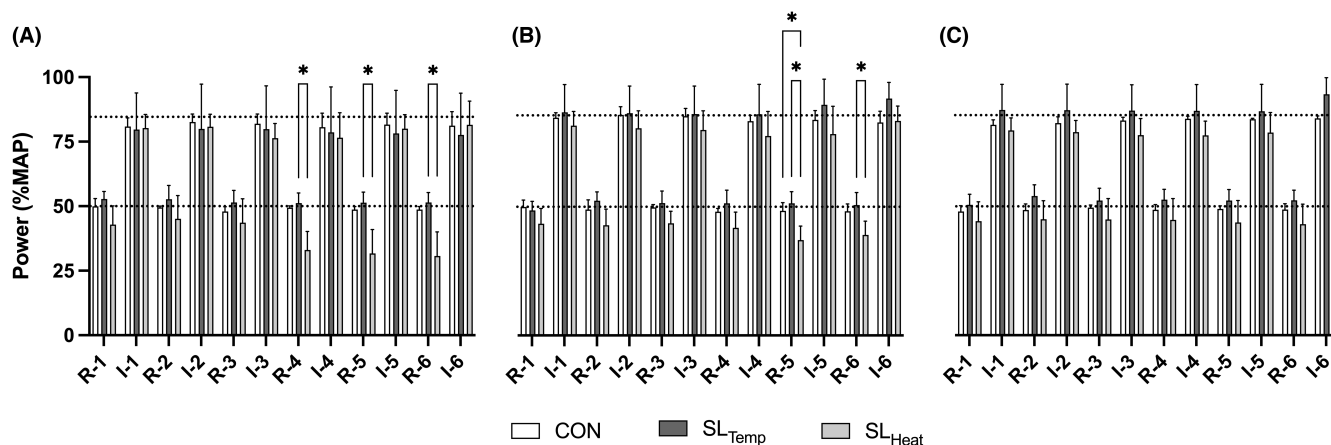
FIGURE 3 Relative mean power output (% MAP) during first (A), fourth (B) and final (C) HIT sessions. R and I refer to recovery and interval, respectively. Horizontal dotted lines at 85% and 50% denote target exercise intensity for recovery and exercise intervals. Data reported as mean ± SD. *denotes significant difference ($p < 0.05$) with groupwise difference shown via brackets.
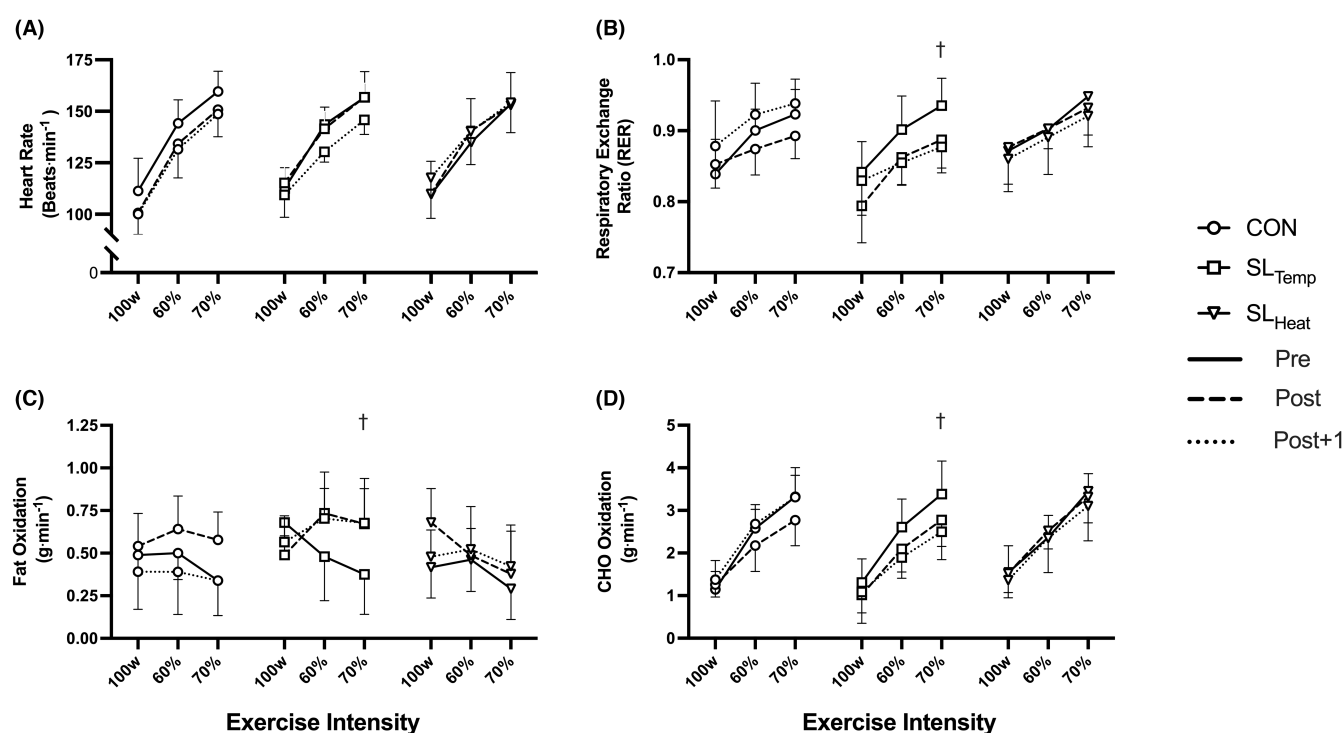

FIGURE 4 Metabolic data recorded during the submaximal cycling test. (A) Mean heart rate (beats·min^{-1}). (B) Respiratory exchange ratio. (C) Fat oxidation (g min^{-1}). (D) CHO oxidation (g min^{-1}). Data reported as mean ± SD. Significant Bonferroni post-hoc differences represented by † (significantly different from CON at Post+1).

3.5 | Dietary intake

Energy intake was increased during the prescribed training intervention compared with baseline for all groups ($p < 0.01$, $\eta^2_p = 0.008$), with significant increases in CHO from baseline across all groups ($p < 0.01$, $\eta^2_p = 0.005$). In addition, protein intake was significantly increased during training in the SL$_{Temp}$ and SL$_{Heat}$ groups ($p < 0.05$,

$\eta^2_p = 0.03$) with no difference in CON. Mean daily energy and macronutrient intake are reported in Table 3.

4 | DISCUSSION

The primary aim of this study was to assess whether adding environmental heat stress to a diet–exercise

FIGURE 5 Mean power output (A and B), heart rate (C and D), and core body temperature (E and F) recorded during 30-min TT in temperate and hot conditions across Pre, Post, and Post+1 timepoints. All data are presented in grouped mean bar charts spanning all time points (Pre, Post, Post+1) with individual data represented by points and individual changes between time points represented by a connecting line. Significant Bonferroni post-hoc differences represented by * (significantly different from CON at Post) † (significantly different from CON at Post +1), + (significantly different from SL_{Temp} at Post) ‡ (significantly different from SL_{Temp} at Post+1).
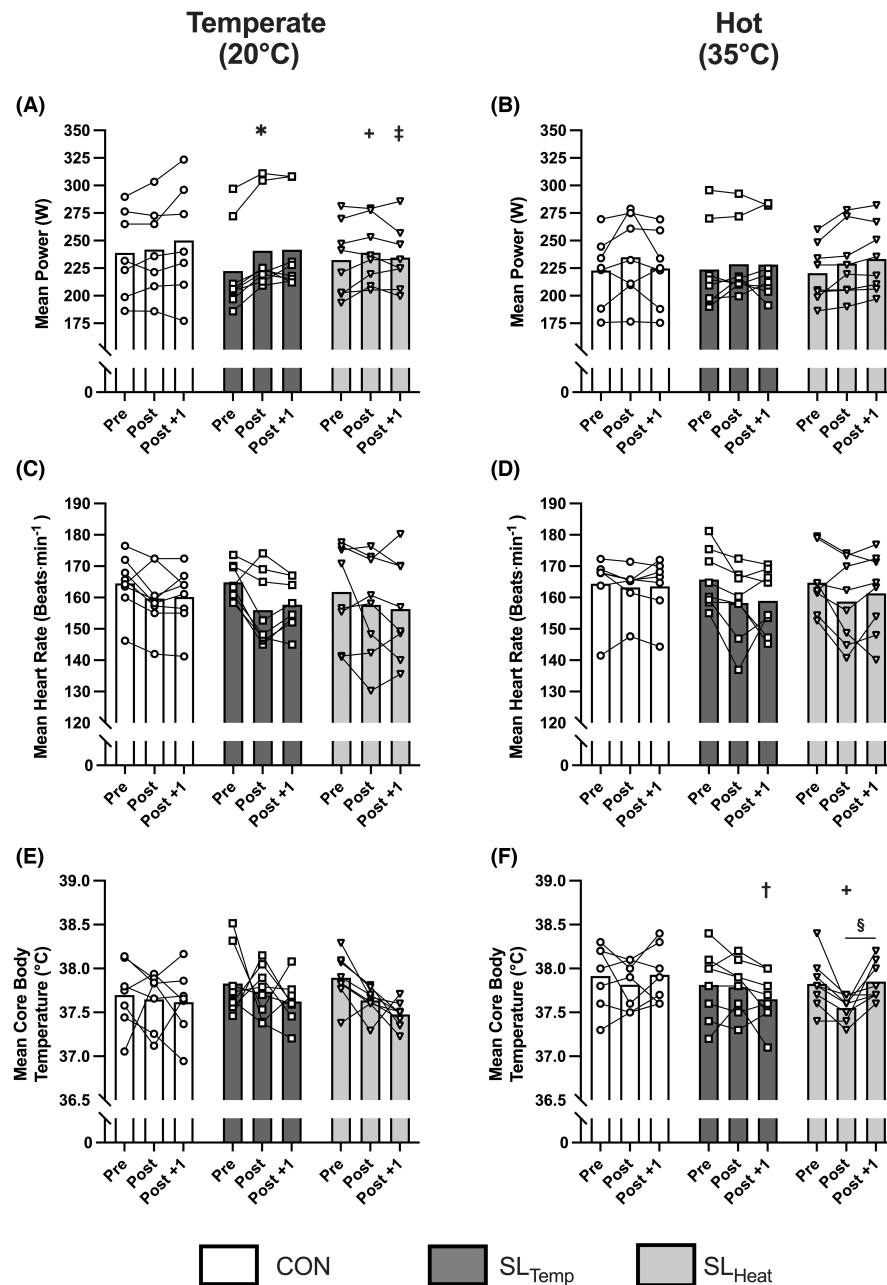
intervention where CHO availability is voluntarily reduced around training sessions (i.e., according to the SL-TL model) would confer greater performance improvement in hot and temperate conditions without alterations in whole-body substrate metabolism. The main findings of this study were: (1) under environmental heat stress (35°C, 50% RH), endurance training completed alongside the SL-TL strategy does not improve performance in hot or temperate conditions; (2) it fails to induce an increase in fat oxidation rate; and (3) SL-TL in temperate conditions (20°C, 50% RH) improved performance and increased submaximal whole-body fat oxidation rates. This study adds to the existing literature that training with periodized CHO intake improves performance and increases whole-body fat oxidation rates. Furthermore,

the data presented here also reveals that heat stress offsets the increase in fat oxidation observed following low CHO training. Nevertheless, the lack of resulting performance improvement compared with SL-TL in temperate conditions questions the utility of training with low CHO under environmental heat stress.

Multiple strategies, including heat stress, hypoxia, and substrate manipulation, are implemented alongside endurance training to augment post-exercise adaptive responses and improve training outcomes.[31] These strategies are undertaken in the belief that greater "metabolic stress" and extreme homeostatic disturbances will maximize skeletal muscle adaptation, enhance endurance-training outcomes, and optimize critical determinants of endurance performance.[31] Against this backdrop, we hypothesized

TABLE 3 Daily energy and macronutrient intake for control (CON), sleep low (SL$_{Temp}$), and sleep low and heat (SL$_{Heat}$) groups before the training program (baseline) and during the diet–exercise–environmental intervention.

		Energy (kcal·day^{-1})	CHO (g·kg^{-1}·day^{-1})	Fat (g·kg^{-1}·day^{-1})	Protein (g·kg^{-1}·day^{-1})
CON	Baseline	2280 ± 231	3.5 ± 0.4	1.2 ± 0.1	1.8 ± 0.2
	Training	2890 ± 265[a]	5.8 ± 0.1[a]	1.0 ± 0	2.0 ± 0
SL$_{Temp}$	Baseline	2339 ± 436	3.4 ± 0.6	1.3 ± 0.2	1.5 ± 0.1
	Training	2919 ± 306[a]	5.6 ± 0.5[a]	1.1 ± 0.1	1.9 ± 0.2[a]
SL$_{Heat}$	Baseline	2208 ± 265	3.2 ± 0.5	1.2 ± 0.0	1.5 ± 0.1
	Training	2922 ± 295[a]	5.6 ± 0.4[a]	1.0 ± 0.1	1.9 ± 0.2[a]

Note: All data are presented as mean ± SD.

[a]Denotes a significant difference from baseline.

that completing SL-TL under heat stress would provide a greater performance benefit than SL-TL alone. It has recently been suggested that completing endurance training under heat stress suppresses peak fat oxidation rates during exercise in temperate conditions,[17] and evidence here supports that hypothesis. Combining SL-TL and heat stress did not induce metabolic adaptation and increased fat oxidation rates in temperate conditions. The blunted metabolic response observed here may be partly due to the reduced contribution of lipid metabolism during training in hot conditions.[32] Conversely, changes in relative exercise intensity during the submaximal exercise test may account for short-term changes in substrate utilization. While maximal oxygen uptake ($\dot{V}O_{2max}$) and maximal aerobic power (MAP) were unaltered in any group following the intervention, when expressed relative to $\dot{V}O_{2max}$ (L·min^{-1}), SL$_{Heat}$ increased fractional utilization of $\dot{V}O_{2max}$ at 60% MAP of their pre-test MAP during the submaximal cycling test from pre- (63% ± 6%) to post-intervention (70% ± 9%), a trend not observed in either control or SL$_{Temp}$ groups. This increase in relative exercise intensity is likely a contributing factor to changes in substrate utilization given the intensity-dependent regulation of substrate utilization.[33]

Unfortunately, the current study did not allow intramuscular analysis and interrogation of the mechanisms underpinning changes in substrate utilization during exercise, nor were blood lactate samples collected during this test which may have allowed for insight into the energy systems utilized during each stage of the test.

Alongside no change in whole-body substrate utilization rates, performance did not improve in the SL$_{Heat}$ group in either environmental condition. It is widely accepted that exercise under heat stress induces a heat-acclimated phenotype and improves performance in hot conditions.[34] Despite not implementing a traditional heat acclimation protocol *per se*,[35] repeated heat exposures did induce a positive acclimation response evidenced by reduced heart rate and increased power across the training program (Figure 2A,B), improved thermal comfort (Figure 2D), and reduced core body temperate during both time trials

(Figure 5E,F). Despite hallmarks of acclimation, performance in th heat was not improved significantly following the training intervention (Figure 5B). While mechanical workload is undoubtedly reduced, HR is consistently elevated in the SL$_{Heat}$ group, indicating higher physiological strain during exercise in hot environmental conditions. Superimposing additional stressors during exercise likely constituted a period of intensified training load in our participants, potentially inducing a state of overreaching.

Defined as an accumulation of training and non-training stress resulting in a short-term decrement in performance capacity,[36] recovery from overreaching can occur within 2 weeks.[37,38] Lack of immediate performance improvement or changes in whole-body substrate utilization, coupled with hallmarks of overreaching,[37,39] potentially account for initial short-term impairments in exercise performance. To account for this, participants repeated performance tests following 1 week of recovery, albeit no significant improvements in performance were observed following 1 week. Nevertheless, accounting for the training status of the participants and relatively short intervention period, SL$_{heat}$ increased mean power output by 4% and 6% at post and post+1, respectively, compared with ~1%–2% improvements in the CON and SL$_{Temp}$ groups. These differences could be considered practically relevant improvements and are supported by the moderate effect size ($\eta^2_p = 0.06$) for mean power output during the 30 min TT at 35°C. It must be noted that the present study did not measure markers of overreaching such as heart rate variability (HRV); however, a trend toward increased HRV has been observed following endurance training in hot conditions[17] with intense heat acclimation protocols resulting in potential maladaptation.[40]

The present study also adds to the literature on metabolic adaptation induced by chronic low CHO availability during training. Commencing endurance exercise with reduced CHO availability increases acute fat oxidation rates[7,41] with the chronic application of the "twice-daily" CHO periodization strategy (whereby athletes train twice daily to commence the second session with reduced CHO

availability), increasing whole-body fat oxidation rates and relative contribution of intramuscular fatty acids to total energy expenditure during exercise in well-trained cyclists.[9,10] Despite positive metabolic adaptation in response to the "twice daily" approach, limited evidence shows long-term metabolic alterations following repeated SL-TL bouts.[11,42] In line with our initial hypothesis, in agreement with previous work by Marquet et al.,[11] we have evidenced that strategically training with low CHO availability decreased HR, RER and CHO utilization at fixed submaximal exercise intensity—conversely, Riis et al.[41] reported that 4 weeks of SL-TL did not alter the maximal fat oxidation rate. However, the precise nutritional status of the participants before the exercise tests are unclear, and any differences in intake and timing between trials may significantly impact substrate utilization.

Unfortunately, the omission of a heat acclimation-only group makes conclusive statements regarding the role of each stressor on performance difficult. The inclusion of a group that completed all sessions with "normal" CHO availability (in line with CON) in hot conditions (35°C, 50% RH) would permit the validation of the present heat acclimation protocol and identify the potential differences between groups. Nevertheless, based on the current literature, a lack of improvement during exercise is unprecedented and undoubtedly raises concerns when considering nutritional intervention during exercise and training intervention in the heat. Furthermore, a constant work rate regimen (daily exposures at 60% MAP) may not have allowed optimal adaptation compared with progressive overload approaches such as controlled hyperthermia (targeted core temperature of 38.5°C).[43] Utilizing the latter strategy would allow for continuous adaptation due to the forcing function (i.e., metabolic heat production) continually increasing through manipulating endogenous thermal load (as work capacity increases over time). Similarly, a controlled HR approach to prescribing exercise intensity would provide a suitable alternative. An initial decrease in power output would be diminished as heat acclimation induction occurs (16), maintaining comparable physiological loads between hot and hot temperate conditions.[44] Furthermore, the constant work rate approach (fixed power output) used in this study likely induced a state of physiological habituation as tolerance to heat stimulus increases over time,[43] the influence of which progressively reduces as adaptations develop.[45]

4.1 | Perspectives

Based on the data presented, it is inadvisable to exercise with intentionally low CHO availability in hot conditions. This practice could potentially hinder heat acclimation and impede performance in hot conditions. It is widely accepted that CHO oxidation is increased during exercise under heat stress,[15] hence nutritional guidelines recommending consistently high CHO intake.[18] Similarly, repeated exposures to heat stress reduce the physiological impact of heat stress and thus reduce CHO utilization,[32] albeit the time course of this metabolic adaptation remains unknown. The question remains, should high CHO intake be maintained indefinitely in the heat, or is there a critical level of acclimation; that, once achieved, would allow for the manipulation of substrate availability per the "fuel for the work required" paradigm?[5]

4.2 | CONCLUSION

The present study provides evidence to support metabolic adaptation induced by SL-TL, which translated to improved exercise performance despite reductions in training intensity in thermoneutral conditions. We have also provided novel data reporting impaired metabolic adaptation when ST-TL is completed in a hot environment. Despite the hallmarks of heat acclimation, there was no benefit to performance immediately after. While SL-TL appears to be a beneficial strategy for metabolic and performance adaptation, the efficacy of such an approach in a hot environment is still being determined. Future research should investigate whether the performance and metabolic responses observed here are consistent with athletes who have completed prior heat acclimation and appropriately adjusted nutritional guidelines for exercise in the heat.

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

PATIENT CONSENT STATEMENT
All participants were provided with written informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.