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

Samuel Bennett1,2 | Eve Tiollier2 | Elodie Guibert2 | Antonio Morales-Artacho2 | Paul Lalire3 | Daniel J. Owens1 | James P. Morton1 | Franck Brocherie2 | Julien Louis1,2

1Research Institute for Sport and Exercise Science (RISES), Liverpool John Moores University, Liverpool, UK
2Laboratory Sport, Expertise and Performance (EA 7370), French Institute of Sport, Paris, France
3French Triathlon Federation (FFTri), Saint-Denis, France

Correspondence
Julien Louis, Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Tom Reilly Building, Byrom St Campus, Liverpool L3 3AF, UK.
Email: j.b.louis@ljmu.ac.uk

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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·kg\(^{-1}\)·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\(_{2\text{peak}}\)) 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
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 (VO\textsubscript{2max}), physiological thresholds, and exercise economy.\textsuperscript{1} Additionally, the stochastic nature of Olympic distance triathlon\textsuperscript{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.\textsuperscript{3}

Periodically training with low carbohydrate (CHO) availability is a popular diet–exercise strategy among endurance athletes,\textsuperscript{4} with sessions commenced with purposely reduced CHO availability, termed “train low.”\textsuperscript{5} Despite reduced exercise intensity, train low enhances skeletal muscle cell signaling and transcriptional activity,\textsuperscript{6–8} increases fat oxidation rates\textsuperscript{9,10} and improves 10 km running time\textsuperscript{11} and functional threshold power in trained individuals\textsuperscript{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.\textsuperscript{12}

Mechanistically, low CHO availability may decrease peak CHO oxidation rates due to increased pyruvate dehydrogenase kinase 4 (PDK4) mRNA expression\textsuperscript{6,7; reducing pyruvate dehydrogenase (PDH) activity}, which, combined with reduced glycogenolysis, limits glycolytic end-products available for flux into the mitochondria.\textsuperscript{13} Additionally, when exercise is completed with extremely low CHO availability (<50 g·day\textsuperscript{-1}), fat oxidation rates are increased albeit at the expense of maximal CHO oxidation rates and performance compared to high and periodized CHO (500 g·day\textsuperscript{-1}) intake diets.\textsuperscript{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\textsuperscript{15} with environmental temperature and exercise intensity critical factors in regulating substrate utilization during exercise in the heat.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{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\textsubscript{Heat}, all sessions completed in 35°C, 50% RH) conditions compared to SL-TL (SL\textsubscript{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\textsubscript{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.\textsuperscript{11,12}

2.3 | Study design

The present study implemented a randomized control trial design conducted during the non-summer months...
(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 48h and no caffeine consumption for the 24h before each testing battery. In addition, participants were asked to replicate pre-visit dietary intake 24h 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 2h 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 fatigue20 (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 (USG > 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 (VO2max)

VO2max 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 100W, workload was increased by 30W every 2min until volitional exhaustion. Breath-by-breath oxygen uptake (VO2) and carbon dioxide output (VCO2) 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) (1h at 60% MAP) for six consecutive days with high-intensity interval training (HIT) (6 × 5min 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

<table>
<thead>
<tr>
<th>TABLE 1 Participant characteristics for the control (CON), sleep low (SL), and sleep low and heat acclimation (SL Heat) groups.</th>
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</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>VO2max (L·min⁻¹)</td>
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<tr>
<td>VO2max (ml·kg⁻¹·min⁻¹)</td>
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<tr>
<td>MAP (W)</td>
</tr>
<tr>
<td>Experience in triathlon (years)</td>
</tr>
<tr>
<td>Habitual training Volume (h·week⁻¹)</td>
</tr>
</tbody>
</table>

*Note: All data are presented as mean ± SD.*

*Abbreviations: MAP, Maximal aerobic power; VO2max, Maximal oxygen uptake.*
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·kg⁻¹·day⁻¹). 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·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.

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 (VO₂max) 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.
2.7 | Submaximal cycling test

A two-stage submaximal cycling test whereby a 10-min warm-up at 100W preceded two successive 6-min stages at 60% (66% \( \dot{V}O_{2\text{max}} \)) and 70% (77% \( \dot{V}O_{2\text{max}} \)) 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·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·min\(^{-1}\)) was obtained from the rate of \( \dot{V}O_2 \) and based on the thermal equivalent of O\(_2\) for non-protein 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 50s 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-Celsius, 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 ± 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 (η²_p) with values of 0.01, 0.06, and over 0.14 considered small, medium, and large, respectively. 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, η²_p = 0.02) higher training load (813 ± 29 u.a.) compared with CON (724 ± 29 u.a.) and SL Temp (731 ± 59 u.a.). When considering session-specific differences, during HIT exercise, SL Heat had a significantly (p < 0.001, η²_p = 0.03) higher training load (363 ± 49 u.a.) compared with CON (262 ± 33 u.a.) and SL Temp (278 ± 40 u.a.). 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 with CON (p < 0.05, η²_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, η²_p = 0.12) was present (Figure 2B). In line with the RPE-based training load, RPE was significantly elevated (p < 0.0001, η²_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 SL Heat group than in the SL Temp and CON groups (p < 0.0001, η²_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, η²_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, η²_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, η²_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, η²_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 η²_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% ± 3.2% in SL Temp compared to 2.4% ± 4.4% in CON. Significant differences were also identified at Post+1 (p = 0.012) between SL Temp and SL Heat. Mean power output increased from Pre to Post+1 by 8.13% ± 4.6% in SL Temp compared to 1.1% ± 6% in SL Heat (Figure 5A). Mean HR
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 ($p = 0.01$, $\eta^2_p = 0.21$). SLTemp ($37.6 \pm 0.3^\circ$C) mean core body temperature was significantly lower than CON ($37.9 \pm 0.3^\circ$C) at Post+1 ($p = 0.03$). Additionally, there was a significant difference in mean core body temperature between SLTemp ($37.8 \pm 0.3^\circ$C) and SLHeat ($37.6 \pm 0.2^\circ$C) at Post ($p = 0.02$) with no significance at Post+1 ($37.9 \pm 0.2^\circ$C, $p = 0.052$). Mean core body temperature significantly differed in SLHeat between Post and Post+1 ($p < 0.01$, Figure 5F).
Energy intake was increased during the prescribed training intervention compared with baseline for all groups ($p < 0.01$, $\eta_p^2 = 0.008$), with significant increases in CHO from baseline across all groups ($p < 0.01$, $\eta_p^2 = 0.005$). In addition, protein intake was significantly increased during training in the SL_{Temp} and SL_{Heat} groups ($p < 0.05$, $\eta_p^2 = 0.03$) 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...
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. 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. Against this backdrop, we hypothesized
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, and evidence under heat stress suppresses peak fat oxidation rates recently been suggested that completing endurance training, when expressed relative to $\dot{V}O_2_{\text{max}}$ (L·min$^{-1}$), $SL_{\text{Heat}}$ (MAP) were unaltered in any group following the intervention period.

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Energy (kcal·day$^{-1}$)</th>
<th>CHO (g·kg$^{-1}$·day$^{-1}$)</th>
<th>Fat (g·kg$^{-1}$·day$^{-1}$)</th>
<th>Protein (g·kg$^{-1}$·day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CON</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2280 ± 231</td>
<td>3.5 ± 0.4</td>
<td>1.2 ± 0.1</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Training</td>
<td>2890 ± 265$^a$</td>
<td>5.8 ± 0.1$^a$</td>
<td>1.0 ± 0.0</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td><strong>SL$_{\text{Temp}}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2339 ± 436</td>
<td>3.4 ± 0.6</td>
<td>1.3 ± 0.2</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Training</td>
<td>2919 ± 306$^a$</td>
<td>5.6 ± 0.5$^a$</td>
<td>1.1 ± 0.1</td>
<td>1.9 ± 0.2$^a$</td>
</tr>
<tr>
<td><strong>SL$_{\text{Heat}}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2208 ± 265</td>
<td>3.2 ± 0.5</td>
<td>1.2 ± 0.0</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Training</td>
<td>2922 ± 295$^a$</td>
<td>5.6 ± 0.4$^a$</td>
<td>1.0 ± 0.1</td>
<td>1.9 ± 0.2$^a$</td>
</tr>
</tbody>
</table>

*Denotes a significant difference from baseline.

Note: All data are presented as mean ± SD.

Despite hallmarks of acclimation, performance in the heat was not improved significantly following the training intervention (Figure 5B). While mechanical workload is undoubtedly reduced, HR is consistently elevated in the SL$_{\text{Heat}}$ 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, recovery from overreaching can occur within 2 weeks. Lack of immediate performance improvement or changes in whole-body substrate utilization, coupled with hallmarks of overreaching, 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$_{\text{Heat}}$ increased mean power output by 4% and 6% at post and post+1, respectively, compared with ~1%–2% improvements in the CON and SL$_{\text{Temp}}$ groups. These differences could be considered practically relevant improvements and are supported by the moderate effect size ($\eta_p^2 = 0.06$) 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 with intense heat acclimation protocols resulting in potential maladaptation.

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 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.\textsuperscript{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.\textsuperscript{11,42} In line with our initial hypothesis, in agreement with previous work by Marquet et al.,\textsuperscript{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.\textsuperscript{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).\textsuperscript{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.\textsuperscript{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,\textsuperscript{45} the influence of which progressively reduces as adaptations develop.\textsuperscript{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,\textsuperscript{15} hence nutritional guidelines recommending consistently high CHO intake.\textsuperscript{18} Similarly, repeated exposures to heat stress reduce the physiological impact of heat stress and thus reduce CHO utilization,\textsuperscript{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?\textsuperscript{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.

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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.
REFERENCES


**SUPPORTING INFORMATION**

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

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