Overnight fasting compromises exercise intensity and volume during sprint interval training but improves high-intensity aerobic endurance

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Running title: Fasted vs. fed sprint interval training (SIT)

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ABSTRACT

BACKGROUND: The combined effects of sprint interval training (SIT) and exercising in the fasted state are unknown. We compared the effects of SIT with exogenous carbohydrate supplementation (SIT_{CHO}) and SIT following overnight fast (SIT_{Fast}) on aerobic capacity (peak oxygen consumption: $\dot{V}O_{2peak}$) and high-intensity aerobic endurance (time-to-exhaustion at 85% $\dot{V}O_{2peak}$ [$T_{85\%}$]). **METHODS**: Twenty male cyclists were randomized to SIT_{CHO} and SIT_{Fast}. Both groups performed 30-second all-out cycling followed by 4-minute active recovery 3 times per week for 4 weeks, with the number of sprint bouts progressing from 4 to 7. Peak power output (PPO) and total mechanical work were measured for each sprint interval bout. The SIT_{CHO} group performed exercise sessions following breakfast and consumed carbohydrate drink during exercise, whereas the SIT_{Fast} group performed exercise sessions following overnight fast and consumed water during exercise. Before and after training, $\dot{V}O_{2peak}$ and $T_{85\%}$ were assessed. Blood glucose, non-esterified fatty acids, insulin and glucagon concentrations were measured during $T_{85\%}$. **RESULTS**: Overall PPO and mechanical work were lower in SIT_{Fast} than SIT_{CHO} (3664.9 vs. 3871.7 Joules/kg; p=0.021 and 10.6 vs. 9.9 Watts/kg; p=0.010, respectively). Post-training $\dot{V}O_{2peak}$ did not differ between groups. Baseline-adjusted post-training $T_{85\%}$ was longer in SIT_{Fast} compared to SIT_{CHO} (19.7 ± 3.0 vs. 16.6 ± 3.0 minutes, ANCOVA p=0.038) despite no changes in circulating energy substrates or hormones. **CONCLUSIONS**: Our results suggest that SIT_{Fast} compromises exercise intensity and volume but still can have a greater impact on the ability to sustain high-intensity aerobic endurance exercise compared to SIT_{CHO}.

Key words: exercise, aerobic power, submaximal performance, Wingate, time to fatigue, carbohydrate supplementation

Introduction

Aerobic capacity (i.e., peak oxygen consumption; $\dot{V}O_{2peak}$) and high-intensity aerobic endurance (i.e., ability to sustain work at intensities above 85% $\dot{V}O_{2peak}$) constitute fundamental aspects of performance associated with endurance sports.[1] Thus, training strategies to improve these parameters continue to be an important area of research in sport science. Traditionally, continuous aerobic endurance training with aerobic intervals (>4 minutes in length) has been used to increase aerobic capacity and endurance in trained athletes.[2-4] However, anaerobic sprint interval training (SIT) of shorter duration (30 seconds) has recently emerged and adopted as an alternative strategy to induce rapid muscular aerobic adaptations, such as increased muscle glycogen content, oxidative enzyme expression, and mitochondrial biogenesis.[5,6] SIT supplemented into regular training has been shown to increase $\dot{V}O_{2peak}$ in well-trained athletes[7] and high-intensity aerobic endurance in recreationally active individuals.[8,9]

Modification to nutritional availability has also been proposed as a potent strategy to modulate exercise training responses. It has been well established that carbohydrate supplementation can acutely increase aerobic cycling endurance.[10] However, training with reduced carbohydrate availability have advantages in enhancing glycogen content[11-13] and activating signaling pathways[12] that lead to the activation of aerobic enzymes[14] to a greater extent than the same exercise performed with ample carbohydrate supplementation. Aerobic exercise training under restricted exogenous carbohydrate availability has been shown to improve supramaximal cycling capacity[15] and time trial performance[16] to a greater extent than the same training performed with high carbohydrate availability. Thus, while exercise with increased

carbohydrate availability can have positive immediate effects on aerobic performance and/or recovery, high carbohydrate availability during exercise training may hamper some of the important adaptive responses induced by exercise training.

To date, the effects of SIT and fasted-state exercise have been studied independently. While both types of training appear to share overlapping mechanisms for muscular adaptations (e.g., increased muscular glycogen content and aerobic enzymatic activities), it is unknown if the combination of SIT and fasted-state exercise training (SIT_{Fast}) can induce greater improvements in aerobic capacity and high-intensity aerobic endurance compared to SIT performed under ample carbohydrate condition (SIT_{CHO}). The primary purpose of the study was to compare the effects of SIT_{CHO} and SIT_{Fast} on aerobic capacity and high-intensity aerobic endurance. The secondary purpose was to compare the effects of SIT_{CHO} and SIT_{Fast} on circulating substrates and hormone concentrations involved in energy metabolism during high-intensity aerobic endurance cycling. We hypothesized that SIT_{Fast} would induce greater increases in aerobic capacity and high-intensity endurance compared to SIT_{CHO}.

Material and methods

Participants

Male cyclists were recruited through advertisement. The inclusion criteria for the study were: 1) cycle more than 3 hours per week; 2) non-smokers; 3) not currently performing fasted-state training; 4) between 18 and 45 years of age; and 5) able to train in the morning 3 days per week. We recruited participants who cycle more than 3 hours per week to minimize changes in cycling economy over the exercise training period. Potential participants completed a questionnaire to verify their eligibility. Physical Activity Readiness Questionnaire (PAR-Q)[17] was also completed to screen for any contraindications to performing high-intensity exercise. Participants gave

written informed consent prior to participation. All experimental procedures performed were in accordance with Declaration of Helsinki and were approved by University of Alberta Health Research Ethics Board.

Experimental design

A randomized parallel group trial was conducted. After screening for eligibility, baseline aerobic capacity ($\dot{V}O_{2peak}$) was assessed, followed by high-intensity aerobic endurance cycling (time-to-exhaustion at 85% $\dot{V}O_{2peak}$ [$T_{85\%}$]) on a subsequent day at least 48 hours apart. Participants were then randomized to either SIT_{CHO} or SIT_{Fast} by a researcher not involved in the study. Without being blinded to the conditions, both groups performed 3 training sessions per week with 48-72 hours between each session for 4 weeks (e.g., training on Monday, Wednesday, and Friday morning). After successful completion of 4-week SIT, $\dot{V}O_{2peak}$ and $T_{85\%}$ tests were repeated using the same protocols used in baseline testing. The post-training $T_{85\%}$ took place approximately 72 hours following the last day of SIT. The post-training assessment of $\dot{V}O_{2peak}$ occurred on a subsequent day, at least 48 hours apart. Overview of experimental protocol is summarized in **Figure 1**. All exercise testing and sessions were completed on a stationary bike ergometer (Monark, Ergomedic 894E, Varberg, Sweden).

Training protocols

All participants trained 3 days per week for 4 weeks in controlled laboratory conditions. Training sessions were interspersed with at least 48 hours of recovery. The progressive SIT training included 30 seconds of all-out cycling efforts[18] with a resistance equivalent to 0.075 kg x body mass (kg), separated by 4 minutes of unloaded pedaling. Participants performed 4 bouts per

session in week 1, 5 bouts per session in week 2, 6 bouts per session in week 3, and 7 bouts per session in week 4 (Figure 1). Before each high-intensity bout, an instruction was given to increase pedaling speed as high as possible, and the appropriate load was applied promptly. Participants were given verbal encouragement to maintain maximal pedaling speed throughout the 30-second period.

Participants in the SIT_{CHO} group completed 3-day dietary record (breakfast only) before participating in SIT. If breakfast carbohydrate intake was <2.5g/body mass (kg),[19] participants were instructed to supplement carbohydrate intake by adding cereal and/or orange juice to their breakfast. Breakfast was consumed at least 1 hour prior to each training session to minimize gastrointestinal distress. During each training session, 591 mL Gatorade (0 g fat, 35g carbohydrate, and 0 g protein; Pepsico Beverages Canada, 5205 Satellite Drive Mississauga, ON, Canada) was also provided to the SIT_{CHO} group. The SIT_{Fast} group reported to the laboratory after ≥10 hours of overnight fast, and consumed only water during exercise sessions.

To examine if restricted exogenous carbohydrate availability compromised training volume and power output (PO), we measured peak power output (PPO), mean PO, and fatigue index during each sprint interval bout. Reflective makers were placed on the flywheel, and a photo cell interfaced to a computer determined the number of flywheel revolutions during a sprint interval bout using a custom designed software program. PO was calculated as the product of resistance and flywheel revolutions every 0.1 second. PPO was determined from the first 5 seconds,[20,21] and fatigue index was computed from the difference between 5-second PPO and the PO during the last 5 seconds of the test divided by the PPO multiplied by 100.[18] Mechanical work of each sprint interval bout was quantified for each participant by multiplying the mean PO by total sprint time (i.e., work = Joules/seconds x 30 seconds). Weekly training volume was

calculated by summing mechanical work of all sprint intervals performed in the week, which was adjusted for participants' body mass to allow between-group comparison. PPO was also adjusted for different body mass.

Aerobic capacity ($\dot{V}O_{2peak}$) test

Participants performed an incremental test to exhaustion on a cycle ergometer at a self-selected cadence to determine $\dot{V}O_{2peak}$ and VT using a TrueMax® (ParvoMedics, Sandy, UT) metabolic measurement system. First and second VT (VT_1 and VT_2) were determined as previously described[22] by a researcher blinded to conditions. The initial 3 stages of the incremental test were 2 minutes each, and the weight applied to the flywheel were 1.0, 1.5 and 2.0 kg, respectively. Resistance was increased by adding 0.3 kg per minute thereafter until volitional exhaustion. $\dot{V}O_{2peak}$ was determined as the highest value averaged over 20 seconds before reaching volitional exhaustion. The cadence, saddle and handle bar positions used for the baseline test were recorded and reproduced in subsequent tests.

High-intensity aerobic endurance ($T_{85\%}$) test

Endurance performance was defined as the length of time that cycling could be maintained at an intensity requiring 85% of VO_{2peak}. Participants reported to the laboratory after an overnight fast (≥10 hours) and were provided with standardized breakfast: a granola bar (8 g fat, 28 g carbohydrate, and 3 g protein; Nature Valley granola bar, General Mills, Mississauga, ON, Canada) and meal replacement drink (6 g fat, 32 g carbohydrate, and 9 g protein; Ensure regular, Abbott, Saint-Laurent, QC, Canada). Participants were given 30 minutes of rest, during which a catheter was inserted in the antecubital vein. The intravenous line was kept patent with saline

solution flushes. Participants then performed 5-minute warm-up at pedaling resistance corresponding to 25% of resistance prescribed for the $T_{85\%}$ test. The $T_{85\%}$ test was performed at the same cadence selected for the $\dot{V}O_{2peak}$ test while adjusting workload to match the intensity at 85% of individually determined $\dot{V}O_{2peak}$. Participants were blinded to the elapse of time and remained seated for the entire testing duration. No verbal encouragement was provided. The test was terminated when cadence fell below the predetermined speed for more than 5 seconds and followed by 5 minutes cool-down.

Blood samples were collected into a 10-mL EDTA vacutainer tube (Becton, Dickson and Company, ON, Canada) immediately before warm-up, every 10 minutes during the test, and immediately following cool-down. Respiratory gases were analysed using metabolic measurement system between 6^{th} and 9^{th} minutes every 10 minutes to determine the rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Rating of perceived exhaustion (RPE) was recorded immediately before the respiratory gas collection period using the Borg 6-20 RPE scale.[23]. Heart rate was also recorded at this time.

Biochemical analyses

Two mL of collected blood samples were transferred into a tube with 6.7 µL aprotinin (Millipore, MA, USA), and 0.25 mL were transferred into a tube containing 1.0 mL ice-cold 8% perchloric acid. Aprotinin was added to inhibit proteases known to interfere with the determination of glucagon concentration, while perchloric acid was added to deproteinize the sample. Tubes were centrifuged and acquired plasma was cooled and stored in a -80 °C freezer until analysis. NEFA (Wako Diagnostics, CA, USA), plasma glucose, and lactate concentrations were determined enzymatically with spectrophotometric assays. Glucagon and insulin concentrations were

measured using a Multi-Spot® Assay System with a Sector® Imager 2400 (Meso Scale Discovery®, MD, USA). All assays were run in duplicate and mean values were used for subsequent analyses. The intra-assay coefficient of variation for glucagon, insulin, NEFA, plasma glucose, and lactate were 6.5%, 4.2%, 5.6%, 4.4%, and 4.0%, respectively.

Statistical analysis

At baseline, independent t-tests were used to compare participant characteristics between SIT_{CHO} and SIT_{Fast}. While differences did not reach statistical significance, relative $\dot{V}O_{2peak}$ and $T_{85\%}$ in SIT_{Fast} were lower compared to SIT_{CHO} at baseline (53.1 ± 7.8 vs. 57.3 ± 7.8; p=0.248 and 12.6 ± 8.0 vs. 17.4 ± 7.5; p=0.184, respectively). Typically, poor baseline measures lead to greater improvements.[24] We therefore performed analysis of covariance (ANCOVA) on post-training measures with baseline measures included as covariates to determine if training outcomes differed between SIT_{CHO} and SIT_{Fast}.[25] These analyses are presented as adjusted means (also known as least square means) throughout the article. For circulating substrates and hormones, we separately performed ANCOVA on measures obtained before exercise, during exercise, and after exercise.

For the analysis of fatigue index, mechanical work and PPO adjusted for body mass, weekly measures were compared between SIT_{CHO} versus SIT_{Fast} by using repeated measure ANOVA. Data are expressed as mean ± standard deviation (SD). Normality of residual distributions was examined with Shapiro-Wilk test. Insulin concentration was log-transformed to achieve normality. Data were analysed using IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA).

Results

Participants

Twenty-five participants completed the baseline tests. Of these, 5 withdrew from the study. Two participants randomized to SIT_{CHO} withdrew due to schedule incompatibility. Three participants, 1 from SIT_{CHO} and 2 from SIT_{Fast} withdrew from the study due to severe dizziness and nausea following the first SIT session. As a result, 9 participants in the SIT_{CHO} group and 11 participants in the SIT_{Fast} group were included in the analyses. All participants completed 12 exercise training sessions, except for 1 in SIT_{Fast} who missed 1 session in the 4^{th} week. There were no significant differences between SIT_{CHO} and SIT_{Fast} in baseline characteristics, $\dot{V}O_{2peak}$, $T_{85\%}$, VT_1 or VT_2 (**Table 1**).

Training volume, PPO, and fatigue index

Weekly mean mechanical work and PPO adjusted for body mass, and fatigue index for both groups are summarized in **Figure 2**. Repeated measure ANOVA showed that, with an addition of a sprint interval bout per week, total mechanical work increased every week in both groups ($p<0.001$). Overall mechanical work adjusted for body mass was significantly higher in SIT_{CHO} compared to SIT_{Fast} (3871.7 vs. 3664.9 Joules/kg; $p=0.021$, Cohen's $d=0.29$). Similarly, overall PPO adjusted for body mass was significantly higher in SIT_{CHO} compared to SIT_{Fast} (10.6 vs. 9.9 Watts/kg; $p=0.010$, Cohen's $d=0.57$). Fatigue index did not differ between the groups.

Aerobic capacity ($\dot{V}O_{2peak}$) and VT

There was no difference in post-training $\dot{V}O_{2peak}$ between SIT_{CHO} and SIT_{Fast} (**Figure 3**). Pre and adjusted post-training $\dot{V}O_2$ at VT_1 were 33.7 ± 9.2 vs. 33.7 ± 5.1 $mL \cdot O_2$/kg/min for SIT_{CHO} and

30.1 ± 8.2 vs. 33.1 ± 5.9 mL·O_2/kg/min for SIT_{Fast}, respectively. Pre and adjusted post-training $\dot{V}O_2$ at VT_2 were 46.3 ± 9.0 vs. 43.8 ± 7.2 mL·O_2/kg/min for SIT_{CHO} and 42.3 ± 9.0 vs. 43.2 ± 6.7 mL·O_2/kg/min for SIT_{Fast}, respectively. There were no differences in post-training VT_1 or VT_2 between groups.

High-intensity aerobic endurance ($T_{85\%}$)

Oxygen consumption expressed as a percentage of baseline $\dot{V}O_{2peak}$ was 87.8 ± 6.0% and 86.2 ± 4.0% during pre- and post-$T_{85\%}$, respectively, in SIT_{CHO}. In SIT_{Fast}, pre and post %$\dot{V}O_{2peak}$ were 86.4 ± 7.7% and 89.7 ± 8.4%, respectively. Mean PO for SIT_{CHO} and SIT_{Fast} during $T_{85\%}$ were 261.8 ± 23.4 and 261.8 ± 23.4 Watts, respectively. There was no significant difference between groups.

Adjusted mean post-training $T_{85\%}$ was significantly longer in SIT_{Fast} than SIT_{CHO} (19.7 ± 3.0 min vs. 16.6 ± 3.0 min, p=0.038, effect size estimated by partial Eta squared=0.230; **Figure 4**). The rate of perceived exertion, heart rate, or respiratory exchange ratio (RER) did not change or differ between groups (**Table 2**). We only analysed RER during the first 10 minutes because second metabolic cart measures (i.e., between 16-19 minutes) were only available from 6 participants. In addition, because 2 participants reached volitional exhaustion before the first metabolic cart measurement, these participants were not included in the RER analysis.

Blood sample analyses

The results of blood sample analyses are summarized in **Table 2**. At baseline, there were no significant differences in plasma glucose, lactate, NEFA, insulin or glucagon concentrations before, during, and immediately after $T_{85\%}$ tests between SIT_{CHO} and SIT_{Fast}. However, adjusted glucose and glucagon concentrations measured immediately before the post-training $T_{85\%}$ test

were significantly lower in SIT_{Fast} compared to SIT_{CHO} (4.2 ± 0.8 vs. 5.1 ± 0.3 mmol/L, p=0.024, partial Eta squared=0.28 and 29.5 ± 12.7 vs. 53.2 ± 25.7 pmol/L, p=0.022, partial Eta squared=0.30). Immediately following the post-training $T_{85\%}$ test, adjusted glucose concentrations were lower in SIT_{Fast} than SIT_{CHO} (5.1 ± 1.1 vs. 6.0 ± 1.0 mmol/L, p=0.011, partial Eta squared=0.34).

Discussion

To the best of our knowledge, this is the first study to compare the effects of sprint interval training performed under both limited and ample exogenous carbohydrate conditions on aerobic capacity and high-intensity aerobic endurance. By manipulating exogenous carbohydrate availability during training, we showed that SIT performed after an overnight fast leads to better high-intensity aerobic endurance performance compared to SIT performed with additional carbohydrate intake after adjusting for baseline differences. This may have training implications for athletes since the ability to sustain power outputs at high intensity (i.e., >85% $\dot{V}O_{2peak}$) has been suggested as a critical determinant factor of success in endurance events.[19,26] Interestingly, the SIT_{Fast} group increased $T_{85\%}$ more than the SIT_{CHO} group despite lower body mass-adjusted mean mechanical work and PPO during training.

Previous studies have shown as little as 6 sessions of SIT over 2 weeks are a potent stimulus to enhance skeletal muscle buffering capacity, oxidative capacity, and glycogen content to a similar degree as 90 to 120 minutes of endurance exercise training[5] and double time-to-exhaustion at 80% $\dot{V}O_{2peak}$[27] in healthy individuals. Our results indicated that performance of experienced cyclists can be enhanced using very similar protocols with a potential of added performance benefit by training under fasted state. Exercise intensity in our study during the

$T_{85\%}$ test was ~88% $\dot{V}O_{2peak}$, which corresponds to the exercise intensity used in a previous study investigating high-intensity aerobic endurance performance in well-trained competitive cyclists.[1] Despite similar exercise intensity relative to individually-determined $\dot{V}O_{2peak}$, cycling duration of the current study were shorter than the previous study (i.e., ~15 minutes at baseline vs. ~45 minutes), possibly due to lower overall training status or aerobic capacity (55.3 ± 7.5 vs. 67.3 ± 3.9 ml·O_2/kg/min) in our participants. Despite shorter time-to-exhaustion, our results translate into an improved capacity to cover additional 1.1 ± 1.7 and 2.1 ± 1.4 km at given exercise intensity in SIT_{CHO} and SIT_{Fast} groups, respectively. The mean difference of an extra kilometre performed in the SIT_{Fast} strengthens the potential benefit of fasted state training on cycling performance.

Our results showing increased high-intensity aerobic endurance despite no changes in $\dot{V}O_{2peak}$ is similar to previous studies in well-trained runners and cyclists.[28,29] Considering previously reported central adaptation (i.e., increased left ventricular mass and stroke volume) and increased $\dot{V}O_{2peak}$ in response to higher volume SIT (5 sessions per week for 8 weeks),[30] we suspect that our SIT volume was not sufficient to induce changes in $\dot{V}O_{2peak}$. Alternatively, because changes in $\dot{V}O_{2peak}$ have predominantly been observed in untrained participants,[6,31-35] a greater training stimulus may be required to induce physiological adaptation in more fit participants such as ours. Likewise, while a previous study showed increases in VT_1 and VT_2 in response to SIT,[7] there was no group level changes in threshold in our study in either SIT_{CHO} or SIT_{Fast}. This discrepancy may also be attributable to lower training volume in our study (i.e., 66 SIT bouts vs. 96 SIT bouts). We found no correlations between the baseline $\dot{V}O_{2peak}$ and changes in $T_{85\%}$.

At baseline, there were no differences in the concentrations of circulating hormones and energy substrates. However, after training, glucose concentrations measured immediately before and after $T_{85\%}$ were significantly lower in SIT_{Fast} compared to SIT_{CHO}. Additionally, glucagon concentration measured immediately before post-training $T_{85\%}$ was also lower in SIT_{Fast} compared to SIT_{CHO}. We do not have clear explanation on why glucagon concentration was lower in SIT_{Fast} than SIT_{CHO} following training. However, others have demonstrated that antecedent episode of increased cortisol concentration[36] can blunt counter regulatory responses, including glucagon, in response to hypoglycaemia on a subsequent day. Thus, repeated episodes of augmented cortisol concentration induced by fasted-state exercise[37] may have suppressed glucagon concentration following SIT_{Fast}. Alternatively, higher energy demands on the liver during SIT_{Fast} may have increased AMP to ATP ratio in the liver. This could have stimulated hepatic 5' AMP-activated protein kinase (AMPK) activity, thereby increasing glucagon sensitivity in the liver[38] and reducing glucagon concentrations. This lower glucagon concentration is likely to be a contributing factor for lower glucose concentration in SIT_{Fast} before post-training $T_{85\%}$.

As acutely manipulating substrate availability can exert profound effects on muscle energy stores and patterns of fuel metabolism during exercise, when repeated over time such interventions can have the potential to modulate numerous adaptive processes in skeletal muscle.[39] Our results of longer aerobic cycling endurance following SIT_{Fast} than SIT_{CHO} without changes in energy substrates mobilization or oxidation rates suggest that the change was due to unmeasured parameters. While we did not perform muscle biopsy, available evidence has shown that commencing exercise with limited exogenous carbohydrate intake[13,40] or withholding glucose intake during exercise[40,41] increases muscle AMPK activity, which stimulates

downstream regulators, such as peroxisome proliferator-activated receptor-γ 1α (PGC-1α),[42,43] a protein known to facilitate mitochondrial biogenesis.[44-47] Additionally, compared to the same exercise training under a fed condition, the fasted-state exercise facilitates glycogenolysis,[48] and thereby increases glycogen content to a greater extent once replenished.[11,49,50] Altogether, given that both SIT and fasted-state exercise can enhance oxidative enzyme activities and glycogen contents, we speculate that the effects of SIT and fasted-state exercise may be additive and resulted in a greater degree of improvement in $T_{85\%}$.

A limitation of our study includes relatively large heterogeneity in baseline $\dot{V}O_{2peak}$ and $T_{85\%}$. We performed ANCOVA to adjust for these baseline differences instead of analysing percent changes as percent changes are generally more pronounced for participants with poorer baseline measures. Nonetheless, ANCOVA does not eliminate the possibility that differing physiological and/or biochemical responses occurred as a result of different starting points. Additionally, while we instructed participants not to alter their dietary intake, we did not capture macronutrient contents of their dietary intake during the training period. Thus, the potential influence of residual confounding arising from different dietary intake cannot be ignored. Lastly, we were unable to provide mechanisms underpinning performance changes. Further study is warranted to determine the specific mechanisms that lead to better adaptation.

Conclusion

This study provides some novel information for sport scientists and coaches involved in the training of athletes. We have demonstrated that performing sprint interval training in a fasted state enhances actual performance to a greater extent than the same training performed under an ample carbohydrate condition. These results could be considered a novel ergogenic aid that has

the potential to elevate both team and individual sport performance. Future replication of these results will affirm the understanding of how energy status can influence adaptation in training programs.

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Table titles

Table 1. Baseline demographic and aerobic characteristics

Table 2. Pre- and post-training substrate oxidation, energy substrate concentration and hormone concentrations before, during and after time-to-exhaustion at a workload corresponding to 85% of baseline V̇O2peak (T85%)

Figure legends

Figure 1. Schematic presentation of study flow.

SIT: sprint interval training

SIT_{CHO}: sprint interval training with breakfast prior to training and carbohydrate during exercise; SIT_{Fast}: sprint interval training following an overnight fast. $\dot{V}O_{2peak}$: peak oxygen consumption; $T_{85\%}$: time-to-exhaustion test at 85% $\dot{V}O_{2peak}$.

*The same protocols as baseline tests were used.

Figure 2. **a)** Mechanical work, **b)** peak power output, and **c)** fatigue index over the 4-week training period.

SIT_{CHO}: sprint interval training performed under ample carbohydrate; SIT_{Fast}: sprint interval training following an overnight fast. Mechanical work is sum of mechanical work during SIT only. Peak power output was calculated as the sum of peak power output over number of SIT performed. *Repeated measures ANOVA showed a significant difference between SIT_{CHO} and SIT_{Fast} (main effects: p=0.021 and p=0.010, respectively). [a]signficantly different from week 1; [b]signficantly different from week 1 and 2; [c]signficantly different from week 1,2, and 3 (all $p<0.001$).

Figure 3. Peak oxygen consumption in response to SIT_{Fast} and SIT_{CHO}.

SIT_{CHO}: sprint interval training with breakfast prior to training and carbohydrate during exercise; SIT_{Fast}: sprint interval training following an overnight fast. $\dot{V}O_{2peak}$: peak oxygen consumption Post-training $\dot{V}O_{2peak}$ was adjusted for baseline differences.

Figure 4. High-intensity aerobic endurance in response to SIT_{Fast} and SIT_{CHO}.

*significantly different from SIT_{CHO} (ANCOVA adjusting for baseline values, p=0.038).

SIT_{CHO}: sprint interval training with breakfast prior to training and carbohydrate during exercise; SIT_{Fast}: sprint interval training following an overnight fast. $T_{85\%}$: time-to-exhaustion test at 85% peak oxygen consumption. Post-training $T_{85\%}$ was adjusted for baseline differences.

Table 1. Baseline demographic and aerobic characteristics

	SIT_{Fast}, n=11	SIT_{CHO}, n=9	t-test
	Mean ± SD	Mean ± SD	p-value
Age, years	33.3 ± 7.2	34.0 ± 8.2	0.837
Height, cm	180.4 ± 7.9	177.7 ± 6.6	0.416
Weight, kg	81.1 ± 13.3	78.9 ± 11.7	0.701
BMI, kg/m^2	24.8 ± 2.7	25.0 ± 3.1	0.899
$\dot{V}O_{2peak}$, $L \cdot O_2/min$	4.3 ± 0.6	4.5 ± 0.4	0.419
$\dot{V}O_{2peak}$, $mL \cdot O_2/kg/min$	53.1 ± 7.8	57.3 ± 7.8	0.248
VT_1, $\%\dot{V}O_{2peak}$	55.5 ± 8.7	58.0 ± 10.8	0.577
VT_2, $\%\dot{V}O_{2peak}$	78.1 ± 8.6	80.0 ± 7.2	0.597
$T_{85\%}$, min	12.6 ± 8.0	17.4 ± 7.5	0.184

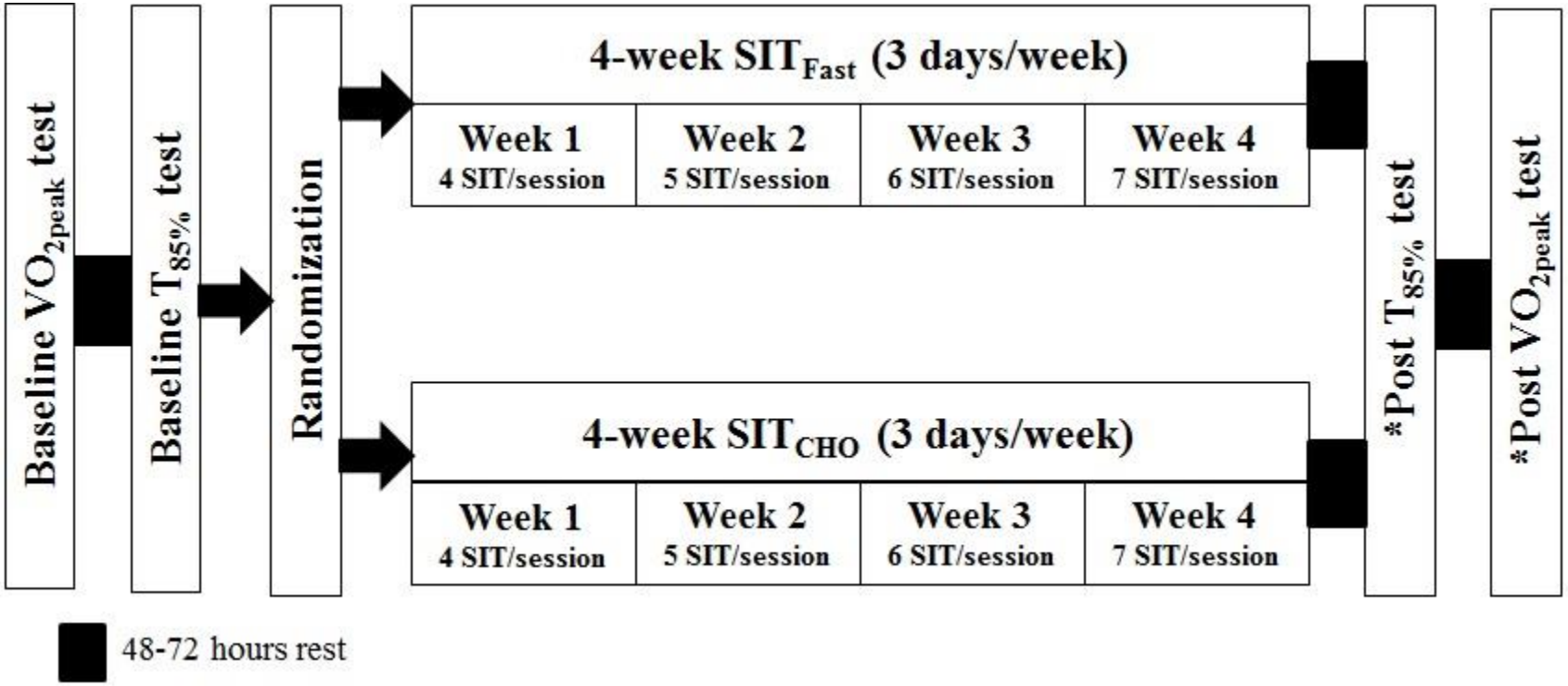
BMI: body mass index; $\dot{V}O_{2peak}$: peak oxygen consumption; VT_1: first ventilatory threshold; VT_2; second ventilatory threshold; SIT_{CHO}: sprint interval training with ample carbohydrate; SIT_{Fast}: sprint interval training after overnight fast; $T_{85\%}$: Time-to-exhaustion at 85% of individually determined $\dot{V}O_{2peak}$.

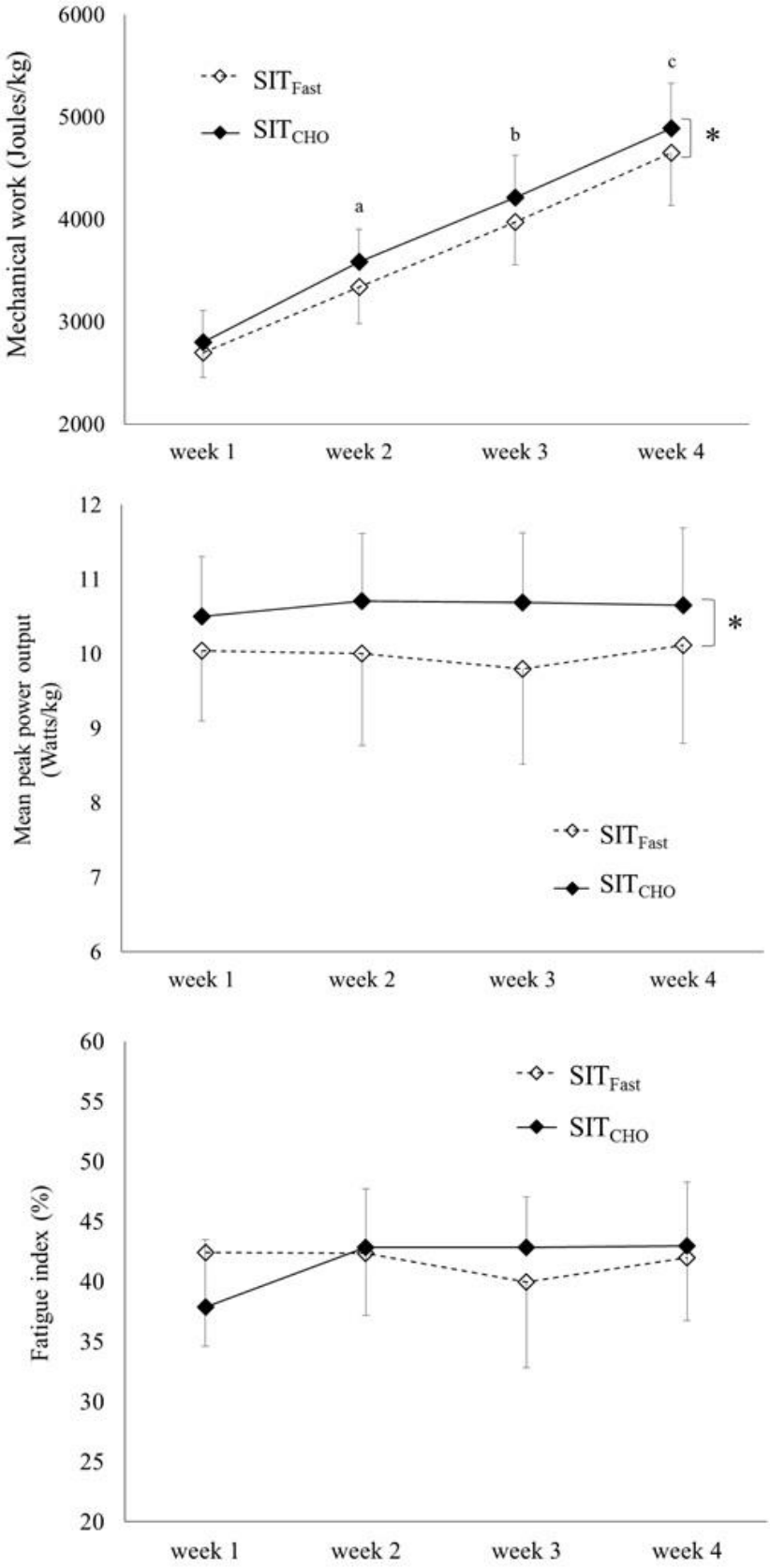
Table 2. Pre- and post-training substrate oxidation, energy substrate concentration and hormone concentrations before, during and after time-to-exhaustion at a workload corresponding to 85% of baseline $\dot{V}O_{2peak}$ ($T_{85\%}$)

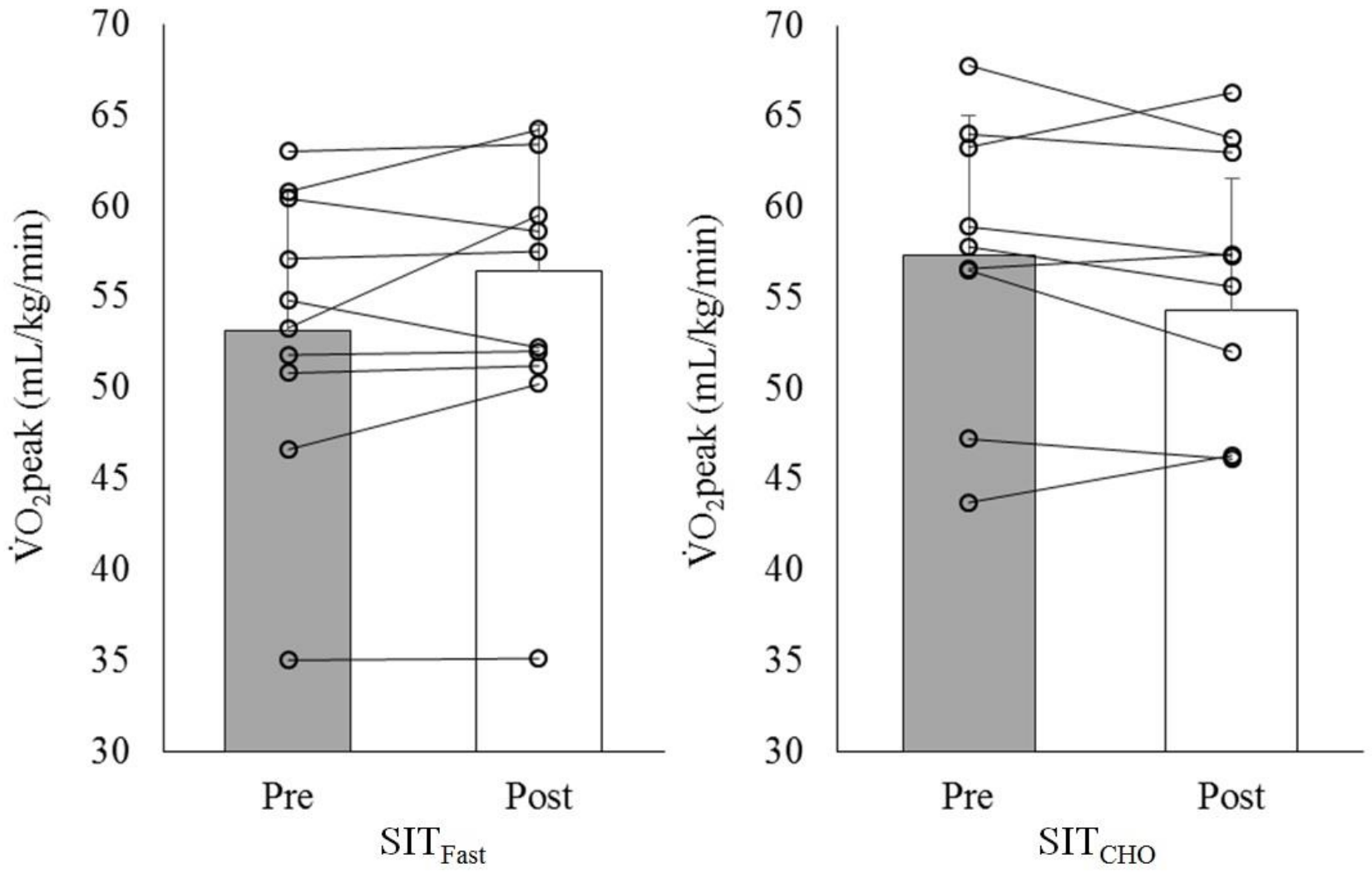
		SIT_{Fast}, n=11			SIT_{CHO}, n=9		
		Before $T_{85\%}$	During $T_{85\%}$	After $T_{85\%}$	Before $T_{85\%}$	During $T_{85\%}$	After $T_{85\%}$
RER	pre		0.99 ± 0.04			0 .98 ± 0.03	
	post		0.99 ± 0.03			0.99 ± 0.04	
RPE	pre		14 ± 2			14 ± 2	
	post		14 ± 1			13 ± 1	
Heart rate (bpm)	pre		176 ± 9			171 ± 12	
	post		175 ± 7			175 ± 14	
Glucose, mmol/L	pre	5.3 ± 0.9	4.1 ± 0.4	4.8 ± 1.0	4.5 ± 0.9	3.9 ± 1.5	5.1 ± 0.9
	post	4.2 ± 0.8*	3.8 ± 0.6	5.1 ± 1.1*	5.1 ± 0.3	3.8 ± 0.7	6.0 ± 1.0
Lactate, mmol/L	pre	1.1 ± 0.5	8.9 ± 2.4	8.8 ± 2.2	1.4 ± 0.5	7.2 ± 3.0	6.9 ± 1.9
	post	1.3 ± 0.5	8.0 ± 2.5	7.8 ± 2.1	1.2 ± 0.5	7.5 ± 2.6	8.7 ± 2.0
NEFA, mmol/L	pre	0.24 ± 0.11	0.12 ± 0.05	0.17 ± 0.12	0.21 ± 0.12	0.11 ± 0.03	0.11 ± 0.03
	post	0.19 ± 0.11	0.13 ± 0.05	0.20 ± 0.10	0.12 ± 0.08	0.10 ± 0.03	0.10 ± 0.03

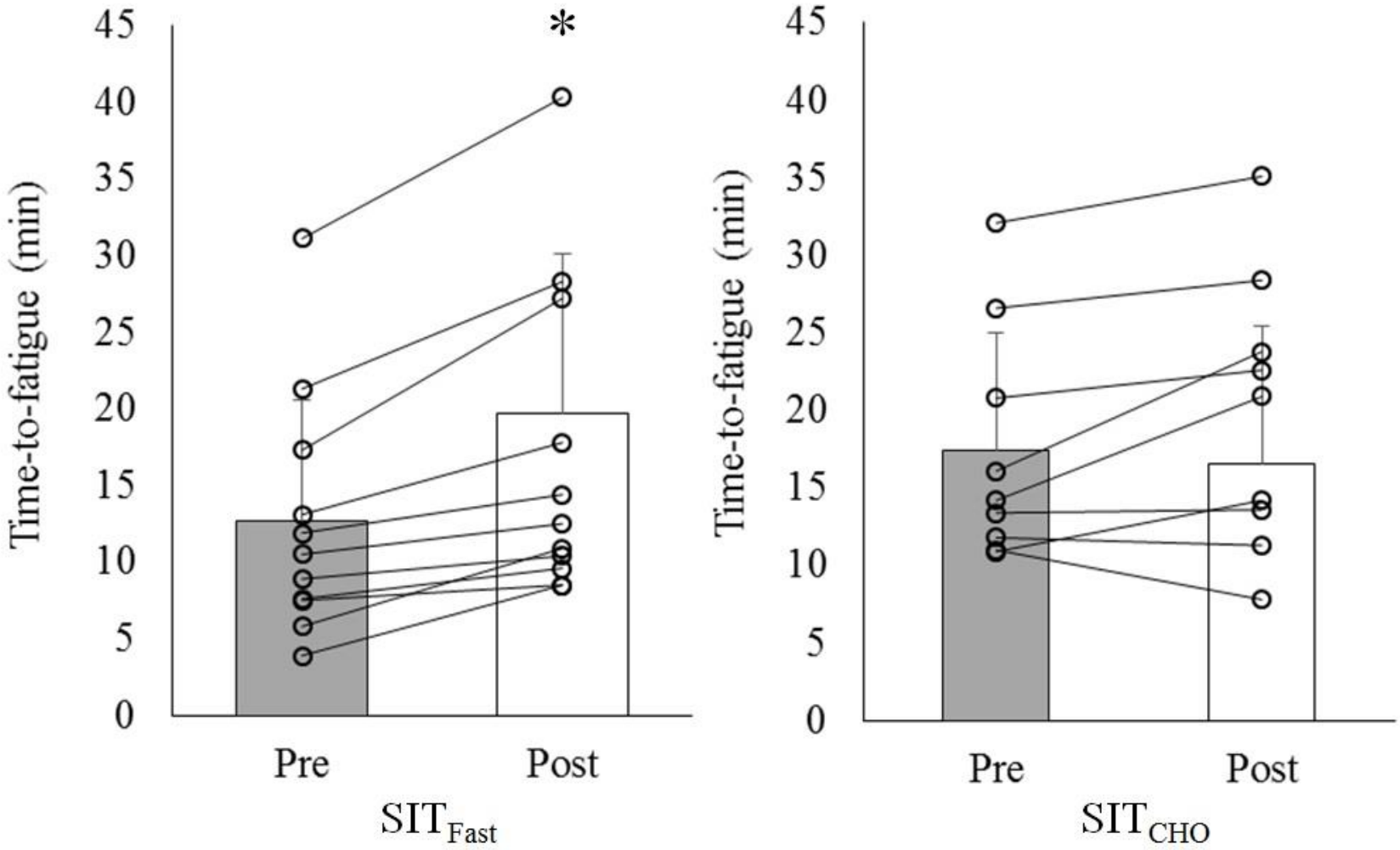
Insulin, pmol/L	pre	777 ± 1069	612 ± 622	580 ± 324	868 ± 572	438 ± 327	979 ± 1025
	post	698 ± 509	545 ± 686	254 ± 161	763 ± 909	1159 ± 914	391 ± 176
Glucagon, pmol/L	pre	49.3 ± 20.4	32.7 ± 14.2	44.3 ± 20.3	40.7 ± 11.8	44.6 ± 28.9	42.8 ± 14.4
	post	29.5 ± 12.7*	49.7 ± 11.6	46.4 ± 22.9	53.2 ± 25.7	40.9 ± 23.7	41.2 ± 11.7

RER: respiratory exchange ratio; CHO: carbohydrate; RPE: rate of perceived exertion; NEFA: non-esterified fatty acids; SIT_{CHO}: sprint interval training with ample carbohydrate; SIT_{Fast}: sprint interval training after overnight fast; Before $T_{85\%}$: blood sample was collected immediately before exercise following 5 minutes of sitting on cycle ergometer without pedaling; During $T_{85\%}$:calorimetry measures were taken during the 6-9 minute interval and blood sample was collected at minute 10; After $T_{85\%}$: blood sample was collected immediately after cool-down. Adjusted means are presented for post-training values. *Significantly different from SIT_{CHO} ($p<0.05$). For circulating substrates and hormones during $T_{85\%}$, n=6 for SIT_{Fast} and n=8 for SIT_{CHO}. For RER and RPE during $T_{85\%}$, n=9 for SIT_{Fast} and n=9 for SIT_{CHO}.


Baseline VO_{2peak} test
Baseline $T_{85\%}$ test
Randomization
4-week SIT_{Fast} (3 days/week)
Week 1
4 SIT/session
Week 2
5 SIT/session
Week 3
6 SIT/session
Week 4
7 SIT/session
4-week SIT_{CHO} (3 days/week)
Week 1
4 SIT/session
Week 2
5 SIT/session
Week 3
6 SIT/session
Week 4
7 SIT/session
*Post $T_{85\%}$ test
*Post VO_{2peak} test
48-72 hours rest


Mechanical work (Joules/kg)
6000
5000
4000
3000
2000
SIT Fast
SIT CHO
a
b
c
*
week 1
week 2
week 3
week 4
Mean peak power output (Watts/kg)
12
11
10
9
8
7
6
*
SIT Fast
SIT CHO
week 1
week 2
week 3
week 4
Fatigue index (%)
60
55
50
45
40
35
30
25
20
SIT Fast
SIT CHO
week 1
week 2
week 3
week 4


70
65
60
55
50
45
40
35
30
$\dot{V}O_2$peak (mL/kg/min)
Pre
Post
SIT_{Fast}
70
65
60
55
50
45
40
35
30
$\dot{V}O_2$peak (mL/kg/min)
Pre
Post
SIT_{CHO}


45
40
35
30
25
20
15
10
5
0
Time-to-fatigue (min)
*
Pre
Post
SIT Fast
45
40
35
30
25
20
15
10
5
0
Time-to-fatigue (min)
Pre
Post
SIT CHO