

Original article

Six high-intensity interval training sessions over 5 days increases maximal oxygen uptake, endurance capacity, and sub-maximal exercise fat oxidation as much as 6 high-intensity interval training sessions over 2 weeks

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Abstract

Background: High-intensity interval training (HIIT) induces similar or even superior adaptations compared to continuous endurance training. Indeed, just 6 HIIT sessions over 2 weeks significantly improves maximal oxygen uptake (VO_{2max}), submaximal exercise fat oxidation, and endurance performance. Whether even faster adaptations can be achieved with HIIT is not known. Thus, we aimed to determine whether 2 sessions of HIIT per day, separated by 3 h, every other day for 5 days (double HIIT (HIIT-D), $n = 15$) could increase VO_{2max} , submaximal exercise fat oxidation, and endurance capacity as effectively as 6 sessions of HIIT over 2 weeks (single HIIT (HIIT-S), $n = 13$).

Methods: Each training session consisted of 10×60 s of cycling at 100% of VO_{2max} interspersed with 75 s of low-intensity cycling at 60 watt (W). Pre- and post-training assessments included VO_{2max} , time to exhaustion at $\sim 80\%$ of VO_{2max} , and 60-min cycling trials at $\sim 67\%$ of VO_{2max} .

Results: Similar increases ($p < 0.05$) in VO_{2max} (HIIT-D: 7.7% vs. HIIT-S: 6.0%, $p > 0.05$) and endurance capacity (HIIT-D: 80.1% vs. HIIT-S: 79.2%, $p > 0.05$) were observed. Submaximal exercise carbohydrate oxidation was reduced in the 2 groups after exercise training (HIIT-D: 9.2%, $p = 0.014$ vs. HIIT-S: 18.8%, $p = 0.012$) while submaximal exercise fat oxidation was significantly increased in HIIT-D (15.5%, $p = 0.048$) but not in HIIT-S (9.3%, $p = 0.290$).

Conclusion: Six HIIT sessions over 5 days was as effective in increasing VO_{2max} and endurance capacity and was more effective in improving submaximal exercise fat oxidation than 6 HIIT sessions over 2 weeks.

Keywords: Double interval training; Exercise metabolism; Performance; Substrate oxidation

1. Introduction

The World Health Organization recommends at least 150 min of moderate-intensity physical activity per week or at least 75 min of vigorous-intensity physical activity per week for healthy adults.¹ Unfortunately, over a quarter of all adults, approximately 1.4 billion people, do not reach these recommended physical activity levels.² Given that a lack of time is the most commonly cited barrier to exercise adherence, recent studies have focused on exercise training models that can be effectively undertaken in a short period of time. There is a

growing body of research suggesting that high-intensity interval training (HIIT) is such a time-saving exercise model.³

HIIT refers to an intermittent style of exercise training involving short bursts of high-intensity exercise, for example, intermittent exercise that achieves $\geq 90\%$ of maximal heart rate (HR) and maximal oxygen uptake (VO_{2max}), separated by periods of recovery or rest.⁴ This exercise model has been ranked number 1 in the health and fitness trends in 2018 due to its time-saving nature.⁵ Although HIIT has been used by athletes in their training since the beginning of the 20th century, its use has recently gained momentum⁶ and the number of published studies relevant to HIIT has increased exponentially over the past decade.⁴

Research indicates that an HIIT training program ranging from 2 weeks to 8 months in length is a time-efficient exercise

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strategy to confer wide-ranging health benefits, including changes in body composition and decreases in visceral adiposity.^{7–11} HIIT also increases cardiorespiratory fitness, exercise performance,^{7–9,12} and fat oxidation during sub-maximal exercise.¹¹ The underlying mechanisms behind these improvements may include, at least partly, increases in skeletal muscle mitochondrial biogenesis and mitochondrial function, which manifests itself as greater oxidative phosphorylation capacity.¹² The briefest time that HIIT has been used thus far, as reported in published research studies, has been 2 weeks, with 6 HIIT sessions over that time, and these studies found increases in VO_{2max} , fat oxidation during sub-maximal exercise and endurance capacity, as well as skeletal muscle metabolic adaptations.^{8–11} It is also worth noting that some studies in the published literature have found that physiological adaptations took place when a non-HIIT program involved even less than 2 weeks of exercise training. For example, Green et al.¹³ found increased levels of skeletal muscle glucose and lactate transporters after 3 consecutive days of cycling at 60% of VO_{2max} . Phillips et al.,¹⁴ who reported on the shortest regimen of training that produced exercise-induced improvements in substrate oxidation, also documented an increased rate of intramuscular lipolysis and a decreased rate of glycogen oxidation after 2 h cycling at 60% of peak oxygen uptake (VO_{2peak}) daily over 5 days. To our knowledge, no study has examined whether these types of beneficial effects can be achieved when HIIT is used over a time frame of less than 2 weeks. It has been found that 2 sessions of HIIT per day increases cardiorespiratory fitness¹⁵ and cycling time trial performance,¹² most likely because HIIT increases skeletal muscle oxidative phosphorylation capacity and markers of exercise-induced mitochondrial biogenesis.¹⁶ This may be because when exercise training is done while muscle glycogen is depleted (e.g., when exercise has taken place earlier on the same day), greater adaptations occur than when the same type and level of exercise is undertaken when muscle glycogen is at normal levels.¹⁷ Indeed, HIIT conducted with reduced carbohydrate (CHO) availability increases whole-body fat oxidation during a steady-state ride and time trial performance compared to when HIIT is conducted with normal CHO availability.^{17,18} Similarly, Hansen and colleagues¹⁹ found that knee extensor exercise conducted twice per day every second day for 10 weeks resulted in increased resting muscle glycogen levels and citrate synthase activity compared with training only once per day for 10 weeks. Hansen et al.¹⁹ hypothesized that this was because the lower muscle glycogen content available for the second bout of exercise on the same day acted as a greater stimulus for exercise adaptations. Furthermore, HIIT interventions comprising total 60 min of intense cycling over 5 days may result in health benefits that are similar or superior to the benefits derived from 2 weeks of HIIT, especially as it relates to the effects on VO_{2max} , a powerful independent predictor of cardiovascular morbidity and mortality.²⁰ Confirming that 5 days of HIIT intervention is as effective as 2 weeks of HIIT intervention will enable a new HIIT model to be developed that takes advantage of the time-saving nature of HIIT for those who

want to experience both health and athletic benefits of training over a shorter period of time. Thus, we examined whether twice-daily HIIT sessions conducted every other day over 5 days improves VO_{2max} , fat oxidation during sub-maximal exercise, and time to exhaustion (TTE) in a way that is similar to once-daily HIIT conducted every other day (on weekdays) over 2 weeks. We hypothesized that the 6 HIIT sessions performed over 5 days would provide these health benefits at a level that was similar or superior to once-daily HIIT sessions conducted every other day on weekdays over 2 weeks.

2. Methods

2.1. Participants

Twenty-eight healthy recreationally active males (age: 25 ± 4 years; height: 176.3 ± 6.0 cm; weight: 72.7 ± 8.1 kg; mean \pm SD) who participated in exercise (e.g., cycling, running, soccer) 2–3 times per week volunteered for the study. Participants were randomly assigned to either the double high-intensity interval group (HIIT-D) ($n = 15$) or the single high-intensity interval group (HIIT-S) ($n = 13$). Inclusion criteria were: recreationally active males, non-smokers, aged 20–30 years, who did not engage in any systematic training program, including endurance, sprint, and resistance. Participants were excluded if they had a known exercise-limiting cardiovascular, respiratory or metabolic illness or were taking any medication or supplements known to affect metabolism. Participants were informed of the benefits and risks of the investigation prior to signing an institutionally approved informed written consent document for participation in the study. Participants also completed the International Physical Activity Questionnaire prior to their participation. The Hacettepe University Clinical Researches Ethics Boards approved the study, and the study conformed to the Declaration of Helsinki.

2.2. Experimental approach to the problem

The experimental protocol included a familiarization procedure, pre-tests, 6 HIIT sessions, and post-tests. The pre-tests consisted of, in the following order, on separate days, with at least 48 h between each test, (a) VO_{2max} test, (b) steady-state sub-maximal exercise test, and (c) TTE test (Fig. 1). The post-tests, which were identical to the pre-tests, were then conducted beginning 72 h after the last HIIT training session (Fig. 1). The study duration, inclusive of pre- and post-training testing, was 3 weeks for the HIIT-D group and 4 weeks for the HIIT-S group. During the HIIT phase, the HIIT-D group performed 2 consecutive training sessions on the same day, separated by a rest period of 3 h, during which participants were provided with a meal containing 10 kcal/kg composed of 58% CHO, 27% fat, and 15% protein. The participants were asked to consume the meal provided during the first hour of the rest time; they were allowed water *ad libitum*. The HIIT-S group participated in the HIIT sessions on weekdays once per

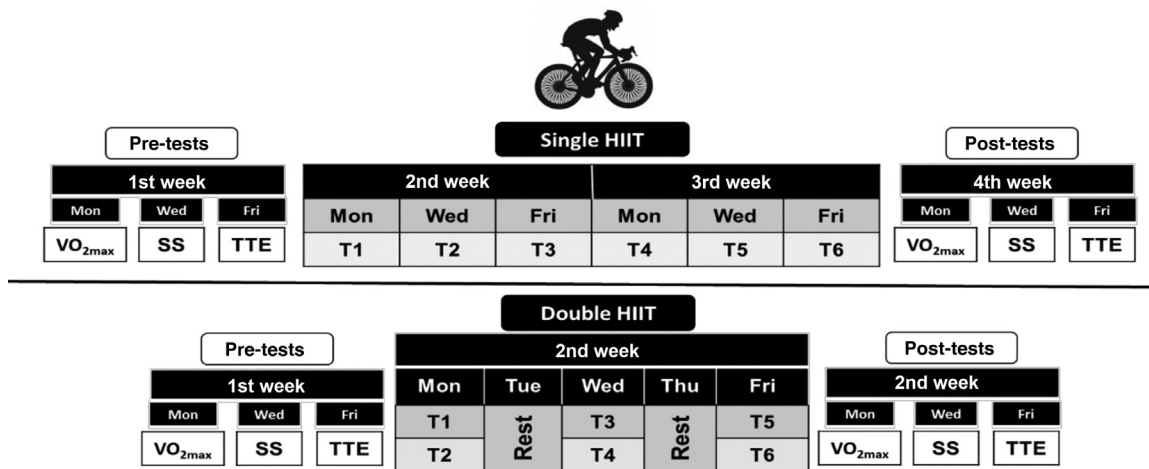


Fig. 1. Study design. Fri = Friday; HIIT = high-intensity interval training; Mon = Monday; SS = steady-state submaximal test; T = training; Tue = Tuesday; TTE = time to exhaustion; VO_{2max} = maximal oxygen uptake; Wed = Wednesday.

day (3 times/week) for 2 weeks. The HIIT-D and the HIIT-S groups were matched for total work. The participants were required to refrain from any strenuous physical activity for the 48 h before the pre- and post-tests, and from alcohol and any exercise for 24 h before each training day. To avoid performance fluctuations because of circadian rhythms, the first daily exercise sessions of individuals in the HIIT-D group and all exercise sessions of the HIIT-S group were performed in the morning (9:00 a.m.–noon), while individuals in the HIIT-D group completed their second daily sessions in the afternoon (noon–3:00 p.m.)

2.3. Procedures

2.3.1. Familiarization procedures

At least 2 weeks prior to baseline measurements, all participants performed familiarization trials to become oriented with all the testing procedures. The familiarization tests consisted of VO_{2max}, the TTE test, and an HIIT session involving 10 × 60 s of exercise at VO_{2max} interspersed by 75 s rest at 60 watt (W). These familiarization tests were separated by 24 h.

2.3.2. Reproducibility of exercise performance tests

Twelve individuals (age: 27.1 ± 3.2 years; height: 180.3 ± 3.1 cm; weight: 70.1 ± 4.4 kg) who weren't participants in our main study performed 2 VO_{2max} tests and 2 TTE tests on separate days at least 1 week apart in order to gauge the reproducibility of the exercise performance tests. The coefficient of variation for the VO_{2max} test and TTE test was 1.3% and 9.2%, respectively, which is similar to that reported in other studies.⁹ The intraclass correlation coefficient (ICC), calculated using the equation of Weir,²¹ was 0.91 and 0.95 for VO_{2max} and TTE, respectively. The SE of the measurement, calculated by SE of the measurement = SD√1–ICC, was 0.16 and 1.89 for VO_{2max} and TTE, respectively, where the SD is the SD of the scores from all participants.

2.3.3. VO_{2max} tests

The participants in our study performed an incremental exercise test to exhaustion on a cycle ergometer (Monark Ergonomic 894 E a; Pike Bike, Varberg, Sweden) to determine VO_{2max}. The initial 3 stages of the test consisted of 2 min of cycling at 60 W, 120 W, and 150 W, respectively, and then the workload was increased by 30 W every minute until volitional exhaustion. HR was recorded continuously during the test using an HR monitor (Heart Rate Monitor; Garmin International Inc., Olathe, KS, USA). Breath-by-breath expired air measurements were performed throughout the test using an on-line gas analysis system (Quark CPET; COSMED Cardio-Pulmonary Exercise Testing, Rome, Italy). Prior to each test, O₂, CO₂, and volume calibrations were made according to procedures prescribed by the manufacturer. The value used for VO_{2max} corresponded to the highest value achieved over a 30-s sampling period. Peak power output was calculated according to the following formula: Peak power output = $W_{com} + (t/60) \times 30$, where W_{com} is the last completed power output W , t is the time completed in the final unfinished workload (in seconds), 60 is the increment duration(s) in each workload and 30 is the workload increment.²² The test was considered valid when at least two of the following criteria were met: (a) plateauing of VO₂ while increasing work rate (increase of no more than 2 mL/kg/min),²³ (b) a respiratory exchange ratio (RER) greater than 1.1, and (c) an HR within 10 beats/min of the predicted maximum (220 – age (beat/min)).²⁴

2.3.4. Steady-state submaximal exercise test

The participants cycled for 60 min on an ergometer at a workload calculated to elicit ~67% of pre-training VO_{2max} at a cadence of 60 repetition per minute (rpm) before and after the 6 sessions of HIIT. Three to 4 h before the pre-training and post-training 60-min ride, the participants consumed a 10-kcal/kg standard meal composed of 58% CHO, 27% fat, and 15% protein as recommended by the Institute of Medicine of National Academies²⁵ (a normal eating pattern should

derive from about 55%–65% CHO, 30% fat, and 10%–20% protein). The volume of water consumed during the test was recorded and then replicated during the post-training test. Ratings of perceived exertion (RPE) were recorded every 15 min during exercise. Respiratory gases were collected at 13–18 min, 28–33 min, 43–48 min, and 55–60 min during exercise in order to measure VO_2 and CO_2 production and volume of RER.¹¹ RER was calculated as the ratio between CO_2 production and VO_2 , while fat oxidation (FOX) and CHO oxidation (CHOX) were calculated using stoichiometric equations,²⁶ with the assumption that the urinary nitrogen excretion rate was negligible:

$$\text{FOX}(\text{g}/\text{min}) = 1.67 \times \text{VO}_2(\text{L}/\text{min}) - 1.67 \times \text{VCO}_2(\text{L}/\text{min})$$

$$\text{CHOX}(\text{g}/\text{min}) = 4.55 \times \text{VCO}_2(\text{L}/\text{min}) - 3.21 \times \text{VO}_2(\text{L}/\text{min})$$

2.3.5. TTE test

The effect of HIIT intervention on endurance capacity was assessed as TTE. After a 3-min warm-up at 60 W, participants cycled to volitional exhaustion on a cycle ergometer at a workload eliciting $\sim 80\%$ of $\text{VO}_{2\text{max}}$ (at 80 rpm). No temporal, verbal or physiological feedback was applied during the test.⁹ The test was terminated when the pedal cadence dropped below 75 rpm for more than 5 s or when the participants voluntarily stopped cycling. Exercise duration and distance covered were recorded. Expired breath samples for the determination of ventilation volume, O_2 uptake, CO_2 production, and RER were collected in the 6–10-min period after the TTE began.⁹ HR was monitored continuously throughout the test.

2.3.6. HIIT

Seventy-two hours following the TTE test, participants started the exercise training protocol, which consisted of either 6 HIIT sessions over 5 days for the HIIT-D group or 6 sessions over 2 weeks for the HIIT-S group (Fig. 1). To determine the most feasible protocol for the study design (HIIT administered twice daily), we conducted a pre-study involving different HIIT and sprint interval training protocols, including the all-out Wingate test, 10×4 -min cycling at 85%–90% of $\text{VO}_{2\text{max}}$ and 10×60 s cycling at 100% of $\text{VO}_{2\text{max}}$, interspersed by 75 s of cycling at 60 W. Following the tests, the recruited participants ($n = 12$; age: 25.6 ± 3.4 years) were asked if they could do the same exercise within 3 h. Based on the responses from the participants, we decided that the 10×60 s protocol was feasible for our training model. The protocol consisted of repeated 60 s efforts of high-intensity cycling at a workload that corresponded to the peak power output reached by the participants at the end of the $\text{VO}_{2\text{max}}$ test (264 ± 48 W). These intervals were interspersed by 75 s of cycling at low intensity (60 W) for recovery.¹⁰ Each session was started and finished with a 3-min warm-up/warm-down at 60 W. All participants completed 10 high-intensity intervals during each training session.

2.3.7. Nutritional monitoring

To minimize any diet-induced variability in post-tests, food diaries were given to each participant to record food and fluid

consumption for the 24 h prior to each pre-test, and the participants were asked to replicate this during post-training testing. The diaries were photocopied and given back to the participants, and a dietician contacted them regularly to ensure fidelity. Household units such as cups, pieces, or plates were used to express portion size. Ingredients of mixed dishes were specified. Serving sizes were calculated by product name and standard weights of food items. All the results were calculated and analyzed by the same experienced dietician using BeBIS 6.1 software (Nutrition Information System, Dr J. Erhardt, Stuttgart, Hohenheim, Germany).

2.3.8. Statistical analyses

The sample size was calculated using the statistical program G*Power software (Version 3.1.9.5.; Institut der Universität Bonn, Bonn, Germany),²⁷ which revealed that 13 participants per group were needed, with an effect size of 0.25, an α of 0.05, and a power of 0.85 as reported by Schubert et al.²⁸ Cohen's effect sizes (d) were classified as small (0.20), moderate (0.60), and large (0.80)²⁹ and were calculated from data on the means, the number of participants, and the pooled SDs for each trial. A two-way repeated measures analysis of variance (time \times group) was used to determine whether main effects existed between the groups and over time, followed by a Bonferroni *post hoc* test. Independent sample t tests were also used to determine if differences between groups existed at baseline across outcome variables. Statistical analyses were computed using SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA), and the level of significance was set at $p < 0.05$. All data are presented as mean \pm SD.

3. Results

The characteristics of the participants are presented in Table 1. There were no differences ($p > 0.05$) in age, anthropometric measures, $\text{VO}_{2\text{max}}$, peak power output, or maximal HR between the 2 groups at baseline. The mean percentage of maximal HR for overall HIIT sessions was $91.5\% \pm 1.0\%$ in the HIIT-S group and $90.8\% \pm 1.6\%$ in the HIIT-D group ($p > 0.05$). Dietary analyses revealed no differences in the

Table 1
Participant characteristics at baseline (mean \pm SD).

Variable	HIIT-S ($n = 13$)	HIIT-D ($n = 15$)	p
Age (year)	26 ± 4	24 ± 4	0.32
Body weight (kg)	74.7 ± 8.0	70.2 ± 5.8	0.10
Height (cm)	178 ± 6	175 ± 3	0.12
$\text{VO}_{2\text{max}}$ (L/min)	3.09 ± 0.41	3.17 ± 0.51	0.66
$\text{VO}_{2\text{max}}$ (mL/kg/min)	41.8 ± 6.9	45.2 ± 6.1	0.19
Peak power output (W)	259 ± 26	264 ± 50	0.74
Maximal HR (beat/min)	178 ± 10	181 ± 12	0.57
Workload during submaximal test (W)	137 ± 26	134 ± 18	0.36

Abbreviations: HIIT-D = double high-intensity interval training; HIIT-S = single high-intensity interval training; HR = heart rate; $\text{VO}_{2\text{max}}$ = maximal oxygen uptake; W = watt.

total energy or macronutrient content of diets before exercise training compared to after exercise training (Table 2).

3.1. VO_{2max}

VO_{2max} (mL/kg/min) increased by $6.1\% \pm 5.9\%$ (41.8 ± 6.9 mL/kg/min to 44.5 ± 6.0 mL/kg/min, $p = 0.001$) in HIIT-S and $7.7\% \pm 4.1\%$ (45.2 ± 6.6 mL/kg/min to 48.7 ± 6.1 mL/kg/min; $p < 0.001$) in HIIT-D ($d = 0.68$), with no significant difference between the 2 exercise protocols (Fig. 2A). Peak power output elicited by the participants at the end of the VO_{2max} test also increased ($p < 0.001$; $d = 0.09$) to a similar extent in both exercise protocols (by $13.4\% \pm 4.7\%$, 259.8 ± 26.8 W to 294.9 ± 24.6 W in the HIIT-S group and by $10.4\% \pm 4.4\%$, 264.9 ± 50.2 W to 292.5 ± 53.3 W in the HIIT-D group; Fig. 2B). Both groups showed similar increases ($p > 0.05$) in maximal expired ventilation (VE) with the HIIT training (HIIT-S: 120.6 ± 25.1 L/min vs. 145.4 ± 22.1 L/min; HIIT-D: 131.7 ± 30.2 L/min vs. 156.6 ± 30.1 L/min, $p < 0.001$; $d = 0.43$).

3.2. Steady-state submaximal exercise and substrate oxidation

Given that exercise training increased VO_{2max} in both groups and the absolute workload was the same before and after the training, the percentage of pre-training VO_{2max} was lower ($p < 0.05$; Table 3) after exercise training in both groups, with no difference between groups (HIIT-S: decreased by 10.7% from 72.4% to 61.7%; HIIT-D decreased by 6.4% from 67.2% to 60.8% pre-training VO_{2max} ; $d = 0.13$). The RER during the steady-state submaximal exercise test was significantly lower at 15 min in the HIIT-S group ($d = 0.01$) and at 30 min ($d = 0.33$) and 45 min ($d = 0.28$) in the HIIT-D group ($p < 0.05$). Lowering of VE was significant at 15 min ($d = 0.26$), 30 min ($d = 0.04$), and 60 min ($d = 0.01$) in the HIIT-S group and at 30 min ($d = 0.22$) and 60 min ($d = 0.01$) in the HIIT-D group ($p < 0.05$), with no differences between groups ($p > 0.05$). There was a significant decrease in absolute

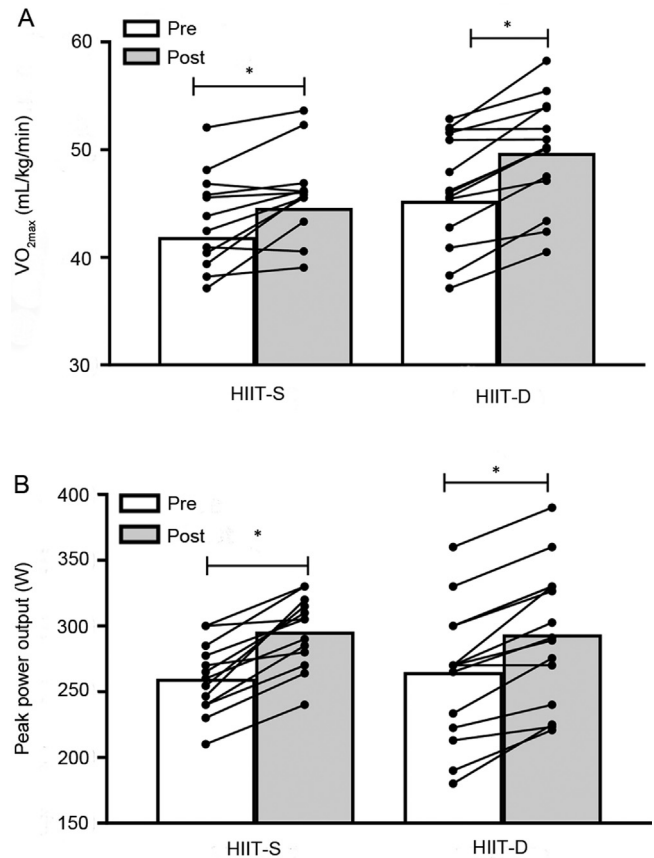


Fig. 2. Changes in VO_{2max} and peak power output pre- and post-intervention for the HIIT-S and HIIT-D groups. * $p < 0.05$, significant difference within group. Values are means and individual changes. HIIT-D = double high-intensity interval training; HIIT-S = single high-intensity interval training; VO_{2max} = maximal oxygen uptake; W = watt.

oxygen uptake during 60 min of cycling at 15 min ($d = 0.57$) and 60 min ($d = 0.35$) after training in the HIIT-S group ($p < 0.05$), yet no significant change in the HIIT-D group was observed ($p > 0.05$). The relative percentage of the VO_{2max}

Table 2
Nutritional data preceding the pre- and post-tests for HIIT-S and HIIT-D groups (mean \pm SD).

	VO_{2max} test		Submaximal exercise test		Time to exhaustion test	
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training
CHO (g/kg)						
HIIT-S	2.93 ± 0.6	2.91 ± 1.0	2.42 ± 0.9	2.96 ± 0.9	3.32 ± 1.6	3.21 ± 1.4
HIIT-D	3.21 ± 0.9	3.15 ± 0.7	3.24 ± 1.3	3.53 ± 0.9	3.22 ± 1.1	3.13 ± 1.1
Protein (g/kg)						
HIIT-S	1.36 ± 0.6	1.42 ± 0.5	1.26 ± 0.6	1.34 ± 0.6	1.23 ± 0.5	1.25 ± 0.5
HIIT-D	1.26 ± 0.4	1.23 ± 0.4	1.36 ± 0.6	1.36 ± 0.5	1.41 ± 0.5	1.45 ± 0.6
Fat (g/kg)						
HIIT-S	1.43 ± 0.5	1.41 ± 0.4	1.30 ± 0.5	1.37 ± 0.5	1.28 ± 0.5	1.31 ± 0.4
HIIT-D	1.39 ± 0.3	1.36 ± 0.3	1.38 ± 0.6	1.39 ± 0.6	1.39 ± 0.4	1.38 ± 0.4
TEI (kcal/day)						
HIIT-S	2213 ± 671	2246 ± 701	2114 ± 745	2178 ± 742	2184 ± 768	2214 ± 761
HIIT-D	2144 ± 441	2126 ± 487	2320 ± 666	2284 ± 600	2195 ± 597	2173 ± 587

Abbreviations: CHO = carbohydrate; HIIT-D = double high-intensity interval training; HIIT-S = single high-intensity interval training; VO_{2max} = maximal oxygen uptake; TEI = total energy intake.

Table 3
Effects of 2 HIIT regimes on VO_2 , RER, and VE during steady-state submaximal exercise test (mean \pm SD).

	15 min	30 min	45 min	60 min
VO_2 (L/min)				
<i>HIIT-S</i>				
Pre-training	2.11 \pm 0.25	2.18 \pm 0.35	2.22 \pm 0.26	2.32 \pm 0.30
Post-training	1.90 \pm 0.36*	2.00 \pm 0.30	2.06 \pm 0.31	2.08 \pm 0.34*
<i>HIIT-D</i>				
Pre-training	2.06 \pm 0.34	2.09 \pm 0.31	2.12 \pm 0.30	2.19 \pm 0.30
Post-training	2.04 \pm 0.33	2.07 \pm 0.39	2.09 \pm 0.41	2.13 \pm 0.38
%$\text{VO}_{2\text{max}}$				
<i>HIIT-S</i>				
Pre-training	69.3 \pm 10.2	71.5 \pm 11.3	72.8 \pm 10.2	75.9 \pm 10.2
Post-training	58.4 \pm 10.1*	61.4 \pm 7.1*	63.1 \pm 8.1*	63.7 \pm 9.1*
<i>HIIT-D</i>				
Pre-training	65.3 \pm 6.2	66.3 \pm 5.3	67.5 \pm 5.1	69.9 \pm 6.2
Post-training	59.6 \pm 4.1*	60.5 \pm 6.3*	61.1 \pm 6.1*	62.2 \pm 6.1*
RER				
<i>HIIT-S</i>				
Pre-training	0.93 \pm 0.03	0.91 \pm 0.03	0.90 \pm 0.04	0.89 \pm 0.03
Post-training	0.91 \pm 0.03*	0.89 \pm 0.03	0.89 \pm 0.04	0.87 \pm 0.03
<i>HIIT-D</i>				
Pre-training	0.92 \pm 0.02	0.91 \pm 0.02	0.89 \pm 0.02	0.89 \pm 0.02
Post-training	0.91 \pm 0.02	0.89 \pm 0.03*	0.88 \pm 0.02*	0.87 \pm 0.02
VE (L/min)				
<i>HIIT-S</i>				
Pre-training	62.1 \pm 14.2	65.4 \pm 15.8	67.1 \pm 13.7	69.9 \pm 13.2
Post-training	54.4 \pm 12.4*	56.2 \pm 11.4*	60.1 \pm 12.2	60.2 \pm 14.1*
<i>HIIT-D</i>				
Pre-training	58.8 \pm 9.9	61.3 \pm 10.1	60.9 \pm 10.4	64.6 \pm 9.9
Post-training	57.4 \pm 10.2	58.8 \pm 11.1*	59.6 \pm 11.2	60.4 \pm 11.9*

* $p < 0.05$, significantly different from pre-training.

Abbreviations: HIIT-D = double high-intensity interval training; HIIT-S = single high-intensity interval training; RER = respiratory exchange ratio; VE = expired ventilation; VO_2 = oxygen consumption; $\text{VO}_{2\text{max}}$ = maximal oxygen uptake.

at 15 min ($d=0.15$), 30 min ($d=0.13$), 45 min ($d=0.26$), and 60 min ($d=0.20$) during the steady-state submaximal exercise test was significantly decreased after training in both groups ($p < 0.05$), while no significant difference between groups was present (main effect for condition, $p > 0.05$; Table 3).

Total fat oxidation during submaximal exercise increased by 15.5% in HIIT-D (19.6 \pm 7.5 g to 22.6 \pm 7.1 g; 15.4%; $p=0.048$), with a non-significant increase in HIIT-S (18.6 \pm 6.8 g to 20.4 \pm 6.8 g; 9.2%; $p=0.290$; $d=0.32$). Total CHO oxidation significantly decreased by 18.8% (132.6 \pm 33.5 g to 107.6 \pm 30.7 g; $p=0.014$) and 9.2% (118.4 \pm 18.6 g to 107.4 \pm 23.2 g; $p=0.012$; $d=0.01$) in the HIIT-S group and in the HIIT-D group, respectively, with no difference between groups (main effect for condition, $p > 0.05$).

There was also a reciprocal decrease in CHO oxidation at 15 min ($d=0.12$), 30 min ($d=0.03$), 45 min ($d=0.11$), and 60 min ($d=0.02$) after training in the HIIT-S group and at 30 min ($d=0.03$) and 60 min ($d=0.02$) in the HIIT-D group ($p < 0.05$; Figs. 3A and 3B). Fat oxidation increased at 15 min in the HIIT-S group ($p < 0.05$; $d=0.36$; Fig. 3C) and at 30 min in the HIIT-D group ($p < 0.05$; $d=0.39$; Fig. 3D). HR was significantly lower after training at 15 min ($d=0.48$), 30 min ($d=0.31$), 45 min ($d=0.27$), and 60 min ($d=0.35$) of cycling at ~67% of

pre-training $\text{VO}_{2\text{max}}$ in both groups ($p < 0.05$; Figs. 3E and 3F), with no differences between groups.

3.3. TTE

TTE increased by 79.2% in the HIIT-S group (13.2 \pm 5.1 min vs. 23.8 \pm 10.7 min, $p < 0.003$; $d=1.2$) and 80.1% in the HIIT-D group (17.6 \pm 6.9 min vs. 31.7 \pm 17.9 min, $p < 0.001$; $d=0.56$; Fig. 4), with no difference between groups.

Oxygen uptake during exercise was not affected by exercise training in either group ($p > 0.05$). There was also a similar ($p > 0.05$) significant decrease ($p < 0.005$) for both groups in HR recorded from the 6th to 10th min of the TTE tests ($d=0.39$). Also, VE was lower in both groups after training (HIIT-S: 114.1 \pm 14.7 L/min vs. 102.5 \pm 14.1 L/min; $p=0.008$; HIIT-D group: 105.4 \pm 20.9 L/min vs. 99.7 \pm 19.2 L/min; $p=0.026$; $d=0.16$), with no differences between the groups. The average absolute VO_2 during the TTE test did not differ ($p > 0.05$) between groups. During the 6th to 10th min of the TTE tests, the HIIT-S group reached 97.0% of pre-training $\text{VO}_{2\text{max}}$ and the HIIT-D group reached 92.2% of pre-training $\text{VO}_{2\text{max}}$. The same power outputs represented 94.3% and 88.4% of pre-training $\text{VO}_{2\text{max}}$ in the HIIT-S and HIIT-D groups, respectively, during the TTE exercise

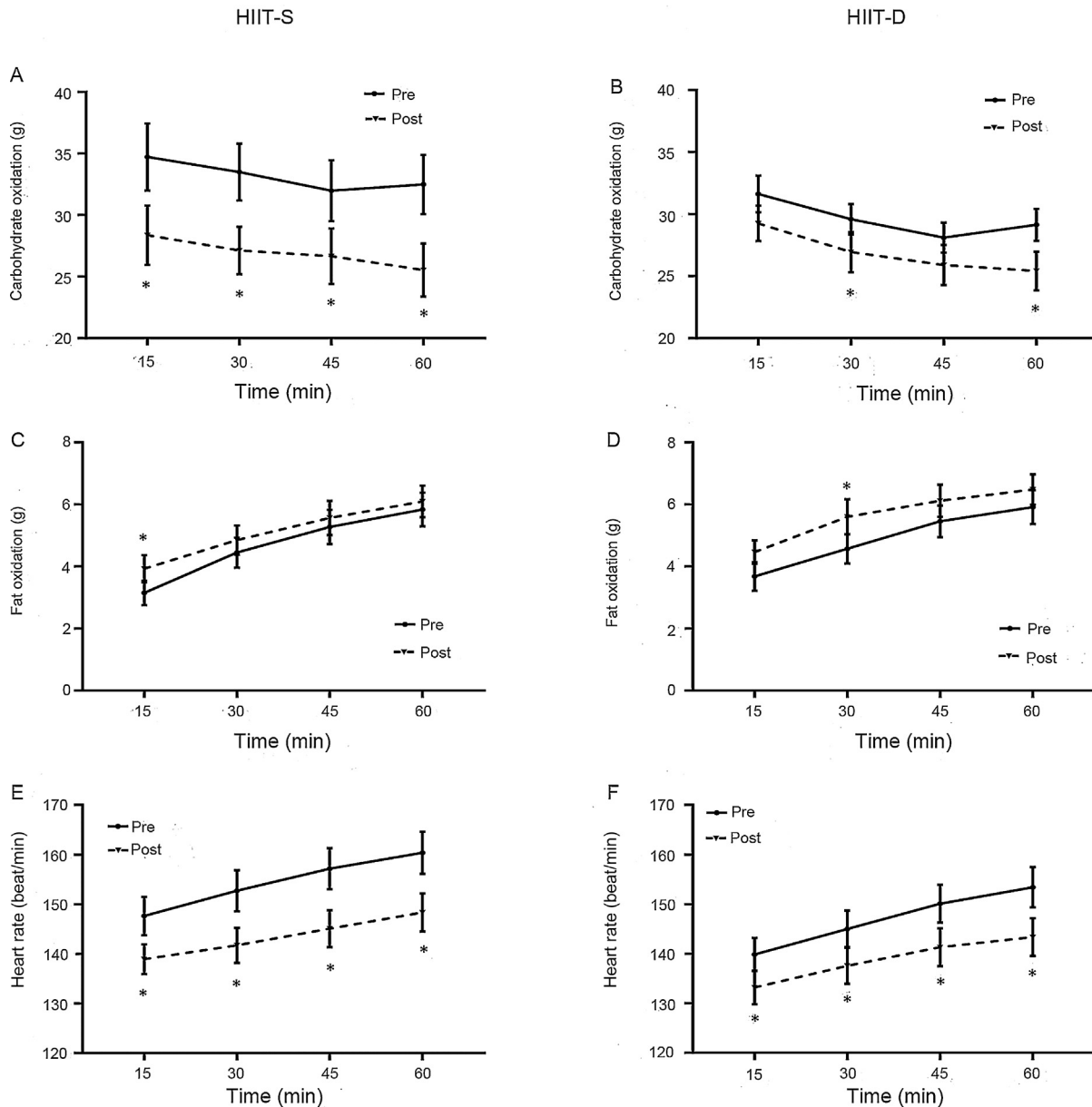


Fig. 3. Changes in carbohydrate oxidation (A) in the HIIT-S group and (B) in the HIIT-D group; fat oxidation (C) in the HIIT-S group and (D) in the HIIT-D group; and heart rate (E) in the HIIT-S group and (F) in the HIIT-D group, during steady-state submaximal exercise test at pre- and post-intervention for the HIIT-S group and HIIT-D group. Values are presented as mean \pm SD. * $p < 0.05$, significant difference within group.

test at the same absolute workload post-training, which is similar for both groups when compared with pre-training ($p > 0.05$).

4. Discussion

The beneficial effects of HIIT on health, body composition, skeletal muscle oxidative capacity, and exercise metabolism have been well documented.^{4,30} Prior to our study, the shortest HIIT study consisted of 6 sessions of HIIT over 2 weeks, with 1 session of HIIT conducted 3 days per week.^{8–11,31} The main findings of this study indicated that only 5 days of HIIT with 2 sessions conducted every other day resulted in improvements in

VO_{2max} , submaximal exercise fat and CHO oxidation and cycle endurance capacity that were similar to those achieved with 6 HIIT sessions conducted over 2 weeks. Ours is the shortest HIIT study that has shown such improvements.

4.1. HIIT and VO_{2max}

We found that 6 sessions of HIIT completed in 5 days resulted in increases in VO_{2max} similar in magnitude to those that were observed for 6 sessions of HIIT administered over 2 weeks.^{8–11} It should be noted that although HIIT was conducted over 5 days in our study, the post-training VO_{2max} test was conducted 72 h after the last HIIT session, or 7 days after

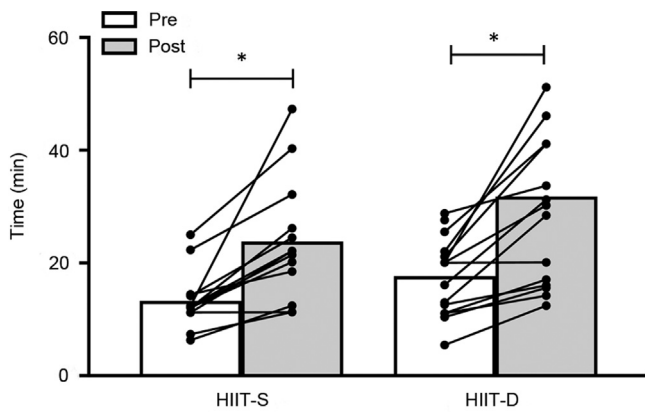


Fig. 4. Changes in time to exhaustion at pre- and post-intervention for the HIIT-S and HIIT-D groups. Values are means and individual changes. * $p < 0.05$, significant within group. HIIT-D = double high-intensity interval training; HIIT-S = single high-intensity interval training.

the first HIIT session. Therefore, even though there were 5 days of HIIT training, there were 8 days during which adaptations to exercise could manifest themselves.

We are aware of very few studies using any type of exercise that have examined whether a 5-day duration of exercise training increases VO_{2max} . One group of researchers found that 2 h of cycling at $\sim 60\%$ of VO_{2peak} over 3–4 consecutive days did not increase VO_{2peak} ,³² but the same group found that 10 consecutive days of such exercise increased VO_{2max} by 4.3%.³³ A study by Hickson et al.³⁴ is the only study that we are aware of that reported an increase in VO_{2max} as quickly as our HIIT-D protocol did. Hickson et al.³⁴ found that 6 consecutive days of strenuous endurance exercise consisting of six 5-min cycling intervals corresponded to the increases seen in VO_{2max} when participants cycled for 3 days and ran as far as they could for 40 min over 3 days, which increased VO_{2max} by 5%.

It is quite remarkable that VO_{2max} increases under these circumstances after about a week of exercise training. One would not expect that there would be time for increases given the classic limiting factors for determining VO_{2max} , specifically, maximum cardiac output.^{7,35} Indeed, even 2 weeks of HIIT⁸ or 6 weeks of sprint interval training³⁶ does not increase maximum cardiac output despite increasing VO_{2max} , as pointed out in a recently published review.³⁷

Although VO_{2max} is usually considered to be limited by maximum cardiac output,³⁵ it is possible that skeletal muscle mitochondrial adaptations may have occurred quickly enough to have contributed to the increase in VO_{2max} after 5 days (HIIT-D) or 2 weeks (HIIT-S) by increasing maximum arteriovenous oxygen difference.³⁵ Moreover, the training-induced improvements in VO_{2max} in such a short time are also associated with the augmented red blood cell volume, resulting in higher O_2 -carrying capacity and stroke volume and, hence, O_2 transport capacity.³⁸ Increases in skeletal muscle peroxisome-proliferator activated receptor γ coactivator-1 α (PGC-1 α), which is a major regulator of exercise-induced mitochondrial biogenesis and substrate utilization in the muscle, have been observed after 2 weeks of HIIT.^{10,31} Indeed, increases in skeletal muscle mitochondrial biogenesis and mitochondrial enzyme activity (e.g., citrate

synthase activity) have been observed after 2 weeks of HIIT in normal healthy individuals,^{7–10,39} unhealthy populations,³¹ and even highly trained athletes.⁴⁰ Considering that a single session of HIIT activates adenosine monophosphate-activated protein kinase and p38 mitogen-activated protein kinase,⁴¹ which phosphorylate and activate PGC-1 α and turns on mitochondrial biogenesis,^{16,41} it will be important to determine if there are increases in mitochondrial biogenesis and mitochondrial enzyme activities even after the second day of 5 days of HIIT-D.

In summary, 5 days of HIIT training increases VO_{2max} , and previous literature on studies involving 2 weeks of HIIT suggests that these increases occur without increases in maximum cardiac output. Further studies are required to determine the ways in which 5 days of HIIT increases VO_{2max} .

4.2. HIIT and substrate oxidation during submaximal exercise

Previous studies have found that 2 or more weeks of HIIT results in more efficient energy utilization during submaximal exercise. More efficient energy use is indicated by less skeletal muscle glycogen use,^{11,17} less muscle lactate production,¹¹ lower blood lactate levels,⁴² lower RER,¹¹ and attenuated hormonal responses such as insulin, epinephrine, norepinephrine, and glucose.^{11,31} The mechanism for this improved aerobic profile during submaximal exercise is likely to be the observed increase, after HIIT, in skeletal muscle oxidative enzymes,^{8,10,12,31} increases in enzymes related to fat oxidation such as cluster of differentiation 36, carnitine palmitoyltransferase I, and beta-hydroxyacyl-CoA-dehydrogenase^{17,39} and reduced lactate dehydrogenase activity,³⁹ which is likely due to the observed increase in PGC-1 α and other regulatory factors.^{4,30} Remarkably, we found that just 5 days of HIIT-D was as effective as 2 weeks of HIIT-S in increasing fat oxidation and decreasing CHO oxidation during submaximal exercise. No other study has shown such rapid beneficial effects on submaximal exercise metabolism of HIIT, and it will be important for follow-up studies to determine if skeletal muscle adaptations and/or hormonal changes are involved. It is possible that the lower skeletal muscle glycogen levels during the second bout of HIIT (only 3 h after the first HIIT bout) may be a stimulus that results in more rapid skeletal muscle adaptations. It is very unlikely that the meal provided to the HIIT-D group following the first HIIT session would have replaced a substantial amount of the glycogen.¹⁷

Although no sprint interval training or HIIT studies have examined whether skeletal muscle metabolic or hormonal changes occur after only 5–7 days of training, we are able to gain some insight from studies that involved endurance training. Indeed, it has been reported that 3 consecutive days of exercise eliciting $\sim 60\%$ of VO_{2max} increases skeletal muscle glucose and lactate transporters.¹³ Phillips et al.¹⁴ found that 5 days of cycling 2 h/day at 60% of VO_{2peak} increased total fat oxidation by 10% and decreased glycogen oxidation by 16% during 90 min of submaximal exercise.¹⁴

4.3. HIIT and endurance capacity

We found that TTE was increased to a similar extent by either 6 sessions of HIIT over 2 weeks (HIIT-S) or by 6

sessions of HIIT over 5 days (HIIT-D). These results are in line with what others have found for improvements in TTE⁹ and time trial performance^{9,10,17,18} after 2 weeks of 4–6 repeated 30 s “all-out” Wingate cycling exercise bouts. Also, studies using the same HIIT protocol as used in our study (10 × 60 s at VO_{2max} , interspersed by 75 s rest) have reported improvements in endurance capacity measured by TTE or time trial performance after 6 sessions of HIIT conducted over 2 weeks.^{8,10} Remarkably, we found such improvements in exercise capacity after 6 sessions of HIIT over only 5 days (HIIT-D).

Studies with different HIIT protocols applied over 2 weeks have shown that such short-term, training-induced changes in endurance capacity are highly associated with peripheral adaptations.^{8,39} This notion has been supported by evidence that no change in maximum cardiac output occurred despite increases in VO_{2max} and endurance capacity after 6 sessions of HIIT carried out over 2 weeks.⁸ It has also been recently reported that a single day of HIIT administered twice in 1 day along with aerobic exercise is a potent stimulus for skeletal muscle signaling responses associated with mitochondrial biogenesis.¹⁶ We are not aware of any study that reported that previously demonstrated improvements in endurance capacity induced by 6 sessions of HIIT over 2 weeks can be observed when the 6 sessions are administered over 5 days. As mentioned earlier, it is possible that the higher cellular stress of the twice-a-day approach (HIIT-D) used in our study may induce faster mitochondrial biogenesis adaptations than those induced by the standard HIIT approach of 6 sessions over 2 weeks (HIIT-S).^{12,16} Indeed, Andrade-Souza et al.¹⁶ recently showed that acute twice-a-day training can amplify the increase in genes associated with mitochondrial biogenesis. Additional studies are needed to reveal the molecular mechanisms driving the improvements in skeletal muscle oxidative capacity in the HIIT-D protocol. Given that CHO oxidation was reduced during prolonged submaximal exercise in both the HIIT-D and HIIT-S groups, it is likely that, compared to pre-training, exercise TTE was increased at least in part due to less glycogen use and lower lactate production during exercise.⁴³

Our study had some limitations. For example, given that the data were collected from 28 recreational healthy males, it is not clear if similar results would occur if trained athletes were recruited. Moreover, our study involved males only, and recruiting both male and female participants would enable a comparison of whether there are gender differences in responses to the HIIT-D model. Our study was a proof of concept and did not involve mechanistic examinations that could be carried out with muscle biopsies. Therefore, it will be important for follow-up studies to examine skeletal muscle molecular adaptations to determine if similar mechanisms are at play in HIIT-D and HIIT-S.

5. Conclusion

We demonstrated for the first time that 6 sessions of HIIT over only 5 days (HIIT-D) increased VO_{2max} , endurance capacity, and submaximal exercise fat oxidation similar to those achieved by 6 sessions of HIIT over 2 weeks (HIIT-S).

Our findings make this study the shortest HIIT study (5 days) that we are aware of. This demonstrates the remarkable adaptability of the human body to exercise stress. Therefore, coaches and athletes may wish to apply the HIIT-D model to improve endurance and substrate oxidation during exercise.

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Authors' contributions

MMA designed the study, collected the data, and wrote the manuscript; HHT was involved in developing the study idea, constructing the methods, reviewing the data analyses, and writing the manuscript; YG was involved in the data collection and constructing the methods; SB contributed to the study design and data collection; ŞNK contributed to the development of the study idea, data collection, and manuscript preparation; GKM critically reviewed the data and contributed to manuscript preparation. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

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