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The acute effect of time-restricted feeding (12 & 16 h) and varying exercise intensities on fat-oxidation rate in inactive young adults – a randomized control trial

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Abstract

Background and purpose Time-restricted feeding (TRF) is a dietary pattern that alternates between periods of fasting and feeding, which has gained significant attention in recent years. The 16/8 approach consists of fasting for 16 h and feeding for an 8-h window, while the 12/12 method consists of fasting for 12 h and a 12-h feeding window. Limited research exists comparing the effects of these methods coupled with physical activity (PA). The aim of this investigation was to examine the acute effects between conditions of varying TRF durations (12 and 16 h) and PA intensities on the fat oxidation rate (FOR). It was hypothesized that i) the TRF16 conditions would exhibit higher FOR_{max} and that PA would enhance these effects, and ii) High Intensity Interval Training (HIIT) would result in greater effects on FOR_{max} compared to Low-Moderate Intensity Steady State Continuous Training (MICT) PA.

Methods and results Eighteen young adults (age: 23 ± 2.0 yrs., body mass index: 23.5 ± 2.8 kg·m⁻²) were recruited and participated in the supervised intervention. The discrete component open circuit spirometry system was used to measure oxygen consumption (VO₂), and Frayne's equation was used to determine the FOR plus FOR_{max}. ANOVA was used to determine pre/post-intervention differences in FOR_{max}. The FORmax for the TRF16+HIIT intervention was significantly higher than the TRF12 (mean difference=0.099 g·min-1, *p*=0.011, 95% CI 0.017 to 0.180) and TRF16 fast alone (mean difference=0.093 g·min-1, *p*=0.002, 95% CI 0.027 to 0.159). The FOR_{max} for TRF12+HIIT intervention was significantly higher than the TRF12 fast alone (mean difference=0.070 g·min⁻¹, *p*=0.023, 95% CI 0.007 to 0.134). The TRF16+HIIT intervention was also significantly higher than the TRF12 fast alone (mean difference=0.099 g·min⁻¹, *p*=0.011, 95% CI 0.017 to 0.180).

Conclusion This study contributes to the ever-growing body of literature on the acute effects of TRF and PA on young adult males and females. The findings suggest that the TRF16 + HIIT PA intervention results in the highest FOR_{max}.

Trial registration Retrospective Registration ISRCTN # 10076373 (October 6, 2023).

Keywords Intermittent Fasting, Physical Activity Intervention, High Intensity Interval Training, Obesity, Metabolic Conditioning

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Introduction

Exercise and time-restricted feeding (TRF) are two lifestyle choices that have gained significant attention in recent years. TRF is a dietary pattern that alternates between periods of fasting and eating. The 16/8 approach consists of fasting for 16 h and feeding for an 8-h window, while the 12/12 method consists of fasting for 12 h and feeding for a 12-h window. The 16/8 method has been studied more extensively than the 12/12 method. Moro et al. (2016) found that the 16/8 method can lead to significant reductions in body fat mass, insulin resistance, and blood pressure in resistance-trained men [1]. Another randomized controlled trial (RCT) by Gabel et al. (2018) found that the 16/8 method can lead to significant reductions in body mass, waist circumference, and blood pressure in obese adults [2]. However, limited research exists comparing the effects of the 16/8 and 12/12 methods on health outcomes. A recent study by Wilkinson et al. (2020) investigated the effects of these two methods on weight loss and glycemic control in adults living with obesity and overweightness. The study found that both methods can lead to significant improvements in body mass and glycemic control, but there were no significant differences between the two fasting methods [3]. Another study by Hutchison et al. (2019) investigated the effects of the 16/8 and 12/12 methods on appetite and energy intake in overweight and obese men. The study found that both methods can lead to significant reductions in appetite and energy intake, but there were no significant differences between the two methods [4].

A systematic review and meta-analysis of 40 studies examined various TRF strategies and found that TRF can lead to significant improvements in body mass, blood pressure, insulin resistance, and oxidative stress markers [5]. Another systematic review and meta-analysis of 23 studies also reported that TRF can improve glycemic control and lipid profiles and reduce inflammation [6]. Furthermore, some studies suggest that TRF may have potential benefits on cognitive function and longevity [7]. However, the acute effects of combining TRF and physical activity (PA) are less known.

PA, including exercise, is an essential lifestyle factor that can have significant health benefits, including improvements in cardiovascular, metabolic and mental health [8]. Various forms of exercise intensities exist; typically, low-moderate intensity steady-state continuous training (MICT) and high-intensity interval (HIIT) or sprint interval training (SIT) are commonly cited. MICT involves performing exercise at a low- to- moderate- intensity continuously for an extended period, such as jogging or cycling at a steady pace for 30–60 min. HIIT involves performing short bouts of high-intensity exercise followed by brief periods of low-intensity exercise, such as jogging or running for 30 s followed by a 30-s walk period (i.e., 1:1 ratio, but may vary). According to the ACSM, HIIT programming is typically prescribed relative to HR or VO₂ (>75% max) with lower-intensity complementary intervals (~50% max). HIIT has gained in popularity as it is considered a time-efficient training modality, which has been shown to improve cardiorespiratory fitness in various populations [9]. The SIT involves performing a maximum-effort sprint activity bout, greater than that associated with VO₂peakEAK_{PEAK}, maximal workload, or maximal running speed, followed by an extended period of rest/recovery (i.e., 1:8 ratio, but may vary) [10, 11].

A systematic review and meta-analysis citing several studies investigated the effects of MICT and HIIT on various health outcomes, including improvements in cardiorespiratory fitness, insulin sensitivity, and body composition. Another systematic review and meta-analysis of 13 studies found that HIIT can lead to greater improvements in these outcomes compared to MICT [10, 11]. Another systematic review and meta-analysis of 31 studies found that both HIIT and MICT can improve glycemic control and lipid profiles, but HIIT may provide additional benefits, such as metabolic adaptations [12]. Further, in overweight and obese adult populations, HIIT has been associated with psychological adaptations linked to increased exercise adherence and enjoyment and improved cardiometabolic health through various interventions [13–16].

It is important to note that chronic exercise training can lead to significant adaptations and improvements in health outcomes. A systematic review and meta-analysis of 65 studies found that chronic exercise training can lead to improvements in cardiovascular fitness, glucose regulation, lipid metabolism, and inflammation [17]. The type and intensity of exercise can also influence the magnitude and type of adaptations. A study by Ruffino et al. (2017) found that chronic HIIT can lead to greater improvements in cardiorespiratory fitness, insulin sensitivity, and muscle mitochondrial content compared to chronic MICT [18].

Despite the established benefits of TRF and exercise on health outcomes, limited research is available comparing the effects of different types of TRF and exercise on health outcomes, particularly in combination. Therefore, the aim of this study was to investigate the effects of TRF12 vs. TRF16, combined with either MICT or HIIT, on various health outcomes, including acute changes in cardiovascular fitness and established maximum fat oxidation rate (FOR_{max}). It was hypothesized that i) the TRF16 group would exhibit higher FOR_{max} and that PA would enhance these effects, and ii) High Intensity Interval Training (HIIT) would result in greater effects on FOR_{max} compared to Low-to-Moderate- Intensity Steady State Continuous Training (MICT) PA.

Methods

All protocols were reviewed and approved by the Human Participants Review Sub-Committee at York University's Office of Research Ethics (certificate # e2019-311), and the experimental protocol conformed to the standards set by the Declaration of Helsinki. The study adheres to CONSORT guidelines. Participants reported to the York University Human Performance Laboratory. All the study participants provided written informed consent for voluntary participation in the study prior to performing any study-related procedures. A copy of the consent form was provided to the study participants, and another copy was added to the study master file. Following completion of the informed consent, the PAR-Q+(www.eparmedx. com) [19] and the physical activity (PA) guestionnaire [20], study participants underwent a laboratory assessment to ensure that they met the inclusion criteria [19–21]. Pre-exercise screening took place on the first experimental day. The first visit consisted of prescreening plus an incremental-to-maximal effort treadmill test for the determination of maximum aerobic fitness or power (VO₂max) using the criterion discrete open circuit spirometry system. On the second experimental day, the study participants underwent the validated maximum fat-oxidation rate (FOR_{max}) protocol following either a 12- or 16-h overnight fast and having consumed 500 mL of water [22]. The FOR_{max} protocol in this investigation was a modified version of the Achten, Venables and Jeukendrup treadmill protocol [23]. All workloads were equivalent in duration and length, and the increase in intensity at each new stage was consistent with the Achten et al. protocol. The VO2max and FORmax protocols utilized are the same and detailed in a previous investigation [22]. A further description of the visits and the types of exercise interventions are detailed below. The fasting and PA intervention order was determined based on a simple randomization program in Excel.

Study participants and requirements

Eighteen young adult males and females aged 23 ± 2.0 years were recruited to participate in the study. The study participants' mean body mass index (BMI) was 23.5 ± 2.8 kg·m⁻². Study participants did not have any physical ailments contraindicating participation in the study (e.g., cardiomyopathies, neuropathy, other diabetes-related complications). Menstrual cycle status was not controlled for or documented by the researchers. Study participants were screened by certified exercise physiologists using resting blood pressure in conjunction

with the evidence-based screening tools, including the current PAR-Q+ and, if needed, the ePARmed-X + (www. eparmedx.com) for exercise contraindications and risk stratification [21]. Eligibility was further confirmed once the data on participants' BMI plus Physical Activity and Sedentarism Score had been assessed. If the participant fulfilled the inclusion criteria and agreed to participate, they were randomized using Excel's simple randomization technique and informed of the requirements of the study and the TRF regime.

Inclusion criteria

The inclusion criteria indicated that the study participants must be between the ages of 18–65 years, from York University to ensure that the location of testing was convenient and avoid participants from dropping out, classified as normal weight (BMI=18.5–24.9), overweight (BMI=25.0–29.9) or obese class I (30.0–34.9), absent of injuries that would diminish their ability to complete an exercise session, having a VO₂max \geq 30 mL·kg⁻¹·min⁻¹, and resting blood pressure < 160/90 mmHg.

Exclusion criteria

Study participants were not considered for the trial if they did not meet all inclusion criteria. Those who were classified as normal weight, overweight or obese class I using BMI but were currently considered regular exercisers (being active for the past 3 months, more than twice a week) were not considered for the trials.

All study participants were asked to maintain the same lifestyle prior to each experimental day, including no change in their diet and/or nonstructured or structured PA. Anthropometric data, including height, body mass, BMI and body fat percentage (%BF), were collected using standardized laboratory protocols [19, 20]. Pre-exercise blood pressure and heart rate (HR) measurements were determined in the seated position in a private room using an automated device (BpTRU Medical Devices Ltd. BC Canada). Following a five-minute seated rest period, the BpTRU[™] recorded six sequential measurements, one minute apart. The BpTRU[™] device generated an average value for the pre-exercise systolic plus diastolic blood pressures and pulse rate using the last five of the six measurements. Although not required, all hypertensive values were re-evaluated using the auscultatory blood pressure method. Body mass was measured upon each visit using the Seca Alpha Scale (Model 770, Germany). %BF was measured, without shoes, using bioelectrical impedance analysis (Tanita scale, model TBF-612, Arlington Heights, IL). Height was measured without footwear using a wall-mounted stadiometer.

Study participants were then outfitted with a chestmounted heart rate monitor (Polar Electro, Kempele, Finland) and briefed on the VO₂max test. The incremental-to-maximal effort treadmill test for the determination of VO₂max followed the same loading sequence for all participants. The VO₂ was determined from measurements obtained during the last 30 s of each workload via analysis of mixed expired gases using the discrete open circuit spirometry system. A continuous modified Bruce protocol was utilized for the determination of VO₂peak. VO₂max was determined by one or more verification workloads. The VO₂max test was terminated if the study participant could no longer complete the workload or if the VO₂max plateau criteria was attained using one or more verification workloads [22–27]. For full details of the equipment and protocol refer to Yavelberg et.al 2022 [22].

Visit 2: FOR_{max} assessment

Once the study participants met the preliminary inclusion criteria, they were then randomized into conditions based on fasting durations TRF12 (12-h fast) or TRF16 (16-h fast) and exercise intensities (MICT or HITT). Following randomization, the study participants returned to the laboratory following either a 12- or 16-h overnight fast to complete the FOR_{max} test. This initial FOR_{max} test was used as the study participants' own control for the purposes of statistical analysis.

The FOR_{max} test protocol was a validated version of the Achten, Venables and Jeukendrup walking FOR treadmill protocol [23]. The discrete open circuit spirometry system was utilized for the determination of FOR_{max}. For full details of the FOR_{max} protocol refer to Yavelberg et.al 2022 [22].

Frayn's equation for the calculation of FOR was employed to measure substrate oxidation at each workload using indirect calorimetry. Urinary nitrogen excretion rate was assumed to be negligible for the purpose of the calculations. The equation employed is as follows:

Fat
$$(g \min^{-1}) = 1.67 * VO_2(L \min^{-1}) - 1.67 * VCO_2(L \min^{-1}))$$

FOR was expressed relative to body mass for each participant at each workload.

Visits 3 to 8: Start of Intervention TRF, PA & FORmax

Based on their randomly selected exposure, study participants arrived at the laboratory following a 12- or 16-h overnight fast. A FOR_{max} test was conducted following the fasted PA session that was either MICT PA or HIIT. The continuous MICT PA intervention consisted of treadmill-based activity at a targeted heart rate equal to the heart rate that corresponded to the study participants FOR_{max}, for a duration of approximately 60 min (based on ~ 300 kcal per session expenditure). The HIIT protocol consisted of treadmill-based activity, starting with a MICT bout at ~ 50% HRmax for 60 s, followed by a 60-s maximal intensity bout (~ 100% HRmax) repeated ~ 10 times until 300 kcals were expended. Furthermore, both PA sessions were volume equivalent, meaning that study participants were required to expend 300 kcals during the PA session to complete the session successfully. During these visits, Kcals were derived from the measured absolute VO₂ and by using the conversion factor of 1 L of $O_2 = 4.86$ kcal, which assumes a mixed diet [28].

The FOR_{max} test was repeated at the end of each acute intervention for a total of 5 times throughout the course of the study: Baseline, TRF12+MICT PA, TRF12+HIIT PA, TRF16+MICT PA, and TRF16+HIIT PA.

Statistical analysis

The study participant characteristics are expressed as the means \pm standard deviations (mean \pm SD). A total of n=18 study participants were recruited: 8 females and 10 males. Statistical analyses conducted using a standard statistical software program, SPSS 28. Repeated-measures ANOVA was used to test for significant differences between the intervention groups (varying PA intensities and fasting durations). A Mauchly's test of sphericity was conducted. The Wilks' Lambda post-hoc test was implemented to compare the means of several groups across multiple outcomes.

Results

There were no significant differences in the FOR_{max} values between the fasting-only interventions (12/16 h) (p > 0.05). ANOVA was used to determine pre/post-intervention differences in FOR_{max}.

The FORmax for the TRF16+HIIT intervention was significantly higher than the TRF12 (mean difference=0.099 g·min-1, p=0.011, 95% CI 0.017 to 0.180) and TRF16 fast alone (mean difference=0.093 g·min-1, p=0.002, 95% CI 0.027 to 0.159). The FOR_{max} for TRF12+HIIT intervention was significantly higher than the TRF12 fast alone (mean difference=0.070 g·min⁻¹, p=0.023, 95% CI 0.007 to 0.134). The TRF16+HIIT intervention was also significantly higher than the TRF12 fast alone (mean difference=0.099 g·min⁻¹, p=0.011, 95% CI 0.017 to 0.180).

The Wilks' Lambda post-hoc test was implemented (p=0.388, F=4.098, η^2 =0.612) with 0.826 observed power. Table 1 contains the anthropometric and physical plus physiological fitness profiles of all study participants (8 males, 10 females). As expected, statistical significance was observed between the sexes in height, body mass and VO₂max. Given the normal expected sex differences and innate individual FOR variability, the males and females were combined for the analyses.

Figure 1 contains the group mean (mean \pm SD) FOR_{max} (g·min⁻¹) values for each of the acute interventions:

Characteristic	Combined n = 18 (mean ± SD)	Males n=8 (mean ± SD)	Females n = 10 (mean ± SD)	<i>P</i> -value
Height (cm)	170.1 ± 9.8	176.9±7.9	164.7±7.6	0.05
Body mass (kg)	68.6±13.2	76.5 ± 12.7	62.3 ± 10.2	0.018
BMI (kg·m ^{−2})	23.5 ± 2.8	24.4 ± 2.8	22.9 ± 2.8	0.283
$VO_2max (mLO_2 \cdot kg^{-1} \cdot min^{-1})$	41.2±6.1	44.8±7.3	38.2 ± 2.9	0.018

Table 1 Anthropometric, physical plus physiological fitness profiles of all of the study participants

TRF12, TRF12 + MICT, TRF12 + HIIT, TRF16, TRF16+MICT and TRF16+HIIT. A repeated-measures ANOVA was completed with SPSS 28.0. A statistically significant difference (*p*=0.011, 95% CI 0.017–0.180) was found when comparing the group mean FOR_{max} value between the TRF16+HIIT and 12-h fast-alone interventions. The FOR_{max} in the TRF16+HIIT was 28.3% higher than the TRF12 alone. A statistically significant difference (*p* = 0.023, 95% CI 0.007 – 0.134) was also found when comparing the group mean $\ensuremath{\mathsf{FOR}}_{max}$ value between the TRF12+HIIT and TRF12 fast-only interventions. The FOR_{max} in the TRF12+HIIT was 20.3% higher than the TRF12 alone. A statistically significant difference (p=0.002, 95% CI 0.027 - 0.159) was also found when comparing the group mean $\ensuremath{\mathsf{FOR}}_{\ensuremath{\mathsf{max}}}$ value between the TRF16+HIIT and TRF16 fast-only interventions, as shown in Fig. 1. The FOR_{max} in the TRF16+HIIT was 26.1% higher than the TRF16 alone. The Wilks' Lambda post-hoc test was implemented (p = 0.388, F = 4.098, $\eta^2 = 0.612$) with 0.826 observed power.

Given the variability in FOR_{max} (g·min⁻¹) group responses above, it is important to also examine the individual study participant FOR_{max} response to each acute intervention. Figure 2 contains the study participants' individual FOR_{max} (g·min⁻¹) responses and variability for each acute intervention: TRF12 only, TRF12+MICT, TRF12+HIIT, TRF16 only, TRF16+MICT and TRF16+HIIT.

Discussion

The purpose of this study was to examine the effects of varying TRF durations and PA intensities on FOR_{max} . It was hypothesized that the TRF16 condition would exhibit higher FOR_{max} and that PA would further



Average FOR_{max} (g·min⁻¹) for each Time Restricted Feeding (TRF) intervention

Intervention

Fig. 1 contains the group mean (mean \pm SD) FOR_{max} (g·min⁻¹) values for each of the acute interventions. Light Intensity Steady State (MICT), High Intensity Interval Training (HIIT). * p < 0.05 compared to TRF12 hour fast alone (p = 0.023, 0.011, respectively). $\pm p < 0.05$ compared to TRF16 hour fast alone (p = 0.023, 0.012, respectively).



Individual FOR_{max} (g·min⁻¹) values for each acute intervention

TRF12 TRF12 + **MICT TRF12** + **HIIT TRF16 TRF16** + **MICT TRF16** + **HIIT Fig. 2** contains the individual acute responses in FOR_{max} (g·min⁻¹) values for each acute intervention. Individual responses 1–8 are male study participants, and responses 9–18 are female study participants

enhance these effects. Furthermore, it was hypothesized that HIIT would result in greater effects on $\mathrm{FOR}_{\mathrm{max}}$ compared to MICT PA. In support of these hypotheses, statistically significant differences were observed in both TRF conditions when coupled with HIIT. As hypothesized, TRF16 + HIIT resulted in the largest mean FOR_{max} . The results of this investigation are supported by many published studies. A meta-analysis of 16,129 studies from 1,192 study participants found that combined aerobic exercise and dietary interventions are considered essential for achieving holistic cardiometabolic health benefits and are recognized as contemporary anti-obesity treatments. These findings are primarily based on their significant positive impact on patients with obesity and type 2 diabetes [29]. In slight contrast to the hypothesis, no significant differences were observed between the fasting durations without PA (fast alone), thus indicating the importance of coupling PA with TRF for acute physiological perturbations. The mechanisms for these results are likely attributable to some combination of physiological and genetic adaptations typically observed with highintensity PA. Evidence suggests that the combination of low muscle glycogen from TRF plus high-intensity PA may result in enhanced metabolic and skeletal muscle adaptations [30–33].

As a result of this research, new information was reported on the effectiveness of TRF and acute

physiological responses in FOR_{max} in young adult males and females. The current study is one of a few that utilized a treadmill-based protocol to measure and calculate FOR over a wide range of submaximal exercise intensities and provide values on FOR and FOR_{max} . Liepinsh et al. examined the effects of 60 min of low-intensity exercise on twelve overweight, overnight fasted and fed study participants and found increases in FOR [34]. Although the findings of our investigation did not reveal statistically significant improvements in FOR_{max} for the TRF + MICT PA intervention, low-intensity exercise, such as walking, has been shown to promote a greater reliance on fat as a fuel source, leading to increases in FOR [34, 35]. Highintensity exercise, such as sprinting or resistance training, has been associated with increased carbohydrate oxidation rates due to the higher energy demands [36-38]. Interestingly, the combination of TRF16+HIIT resulted in the highest $\mathrm{FOR}_{\mathrm{max}}$, indicating a potential synergistic effect when combining these interventions. Our findings suggest that combining TRF with PA may have synergistic effects on FOR and $\mathrm{FOR}_{\mathrm{max}}$, potentially enhancing fat metabolism, which could support weight management goals. However, future research is needed to confirm these results and investigate the chronic effects of this combination. Further studies are required to explore the inter-individual variability in response to combining TRF and varying PA intensities on FOR/FOR_{max}.

Despite the comprehensive nature of this study, some limitations need to be acknowledged. First, the study was acute, and the long-term/chronic effects of TRF and PA could not be evaluated. Future research should include longer follow-up periods to investigate the sustainability and persistence of the observed effects. Second, the participant cohort consisted of healthy young adults, limiting the generalizability of the findings to other age groups and populations. Although both male and female study participants were observed, future studies should include a more diverse sample to understand the effects in different populations. This study was part of a series of studies that examined the immediate acute effects of exercise on FOR_{max} post-TRF (12 vs 16 h). Although some statistical significance was found, the researchers believe that the differences between the TRF+PA strategies may have been more pronounced and significant with a larger sample size. Furthermore, the study participants' meals prior to fasting were not rigidly controlled. It is known that a heavy fat/carbohydrate meal prior to fasting may influence FOR/FOR_{max}. Although the study participants may have consumed differing macronutrients prior to the fast, the TRF durations and PA intensities were carefully controlled. In addition, the primary focus of the investigation was FOR, but there is a known inverse relationship between carbohydrate oxidation and FOR. If the investigators had analyzed carbohydrate oxidation, they may have been able to observe additional significant differences between interventions. Menstrual cycle was not controlled for or documented, but current evidence suggests that this may not affect FOR [39]. It is possible that hormonal fluctuations throughout the menstrual cycle could affect female FOR_{max}. Thus, the findings of this investigation can be cautiously generalized given the external and internal validity. In addition, this study will contribute to the gaps in the literature pertaining to varying TRF durations + PA intensities.

Conclusion

This study contributed to the ever-growing body of literature on the acute effects of varying TRF durations + PA intensities on young adults, and the findings suggest that TRF16+HIIT PA intervention results in the highest FOR_{max} .

Abbreviations

Time-restricted feeding
Maximum fat-oxidation rate
Physical activity
High-intensity interval training
Low-moderate intensity steady-state exercise
Maximum aerobic capacity
Body mass index

Authors' contributions

All authors reviewed the manuscript. L.Y, PhD Candidate, Designed the study, wrote the main manuscript, prepared figures, collected data, data analysis and interpretation V.J, PhD Graduate Supervisor, Assisted in study design, assisted in writing the main manuscript, collected data, provided laboratory space and equipment. N.G, Professor Emeritus, Assisted in study design, assisted in writing the main manuscript.

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Availability of data and materials

The dataset generated and/or analysed during the current study are not publicly available due to fact that this investigation is part of an ongoing multi-segmented investigation. The dataset can be made available from the corresponding author (ronij@yorku.ca) on reasonable request.

Declarations

Ethics approval and consent to participate

All protocols were reviewed and approved by the Human Participants Review Sub-Committee at York University's Office of Research Ethics (certificate # e2019-311), and the experimental protocol conformed to the standards set by the Declaration of Helsinki. Participants reported to the York University Human Performance Laboratory. All the study participants provided written informed consent for voluntary participation in the study prior to performing any study-related procedures. A copy of the consent form was provided to the study participants, and another copy was added to the study master file.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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