METABOLIC RESPONSE DURING HIGH-INTENSITY INTERVAL EXERCISE AND RESTING VASCULAR AND MITOCHONDRIAL FUNCTION IN CROSSFIT PARTICIPANTS

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Abstract:

High-intensity functional training (HIFT) can play a major role in preventing cardiometabolic disease. The majority of HIFT interventions incorporate CrossFit (CF) training. We measured aerobic capacity, metabolic response during high-intensity interval exercise (HIIE), resting mitochondrial oxidative capacity, and resting vascular function in adults who participated in CF training (> one year) vs. a sedentary group completing <2 h·wk^{-1} of structure exercise for > one year (SED). Twenty-one participants were recruited (CF n = 13 vs. SED n = 8). CF participants had a 33.0% greater relative VO_2 peak ($p<.001$) and lower body fat percentage (CF = 18.6 [3.8] vs. SED = 30.3 [8.4]; $p<.001$). CF participants had higher exercising substrate oxidation when expressed as absolute and body weight relative values ($p<.013$), but not when expressed relative to lean mass ($p>.200$). CF participants had greater mitochondrial oxidative capacity ($p=.014$). There were no differences in large artery function, but CF participants had greater baseline arterial diameter ($p=.004$) and faster reperfusion following arterial occlusion ($p<.05$). These data support HIFT programs' effectiveness in improving fitness, weight status, and metabolic, mitochondrial, and vascular function.

Key words: *high-intensity functional training, CrossFit, metabolism, vascular function*

Introduction

High-intensity exercise increases cardiorespiratory fitness, skeletal muscle quality and function (Burgomaster, et al., 2008; Gibala, et al., 2006), mitochondrial capacity (Bishop, et al., 2019) and vascular function (Ramos, Dalleck, Tjonna, Beetham, & Coombes, 2015). Exercise also provides a potent stimulus influencing metabolic health, specifically metabolic flexibility. Metabolic flexibility is the ability to efficiently adapt metabolism by substrate sensing, trafficking, storage, and utilization, dependent on availability and requirement (Smith, Soeters, Wust, & Houtkooper, 2018). This regulation relies on a systemic control between muscular tissue and the mitochondria, and disruptions in this communication can impact metabolic health and exercise performance (Astrup, 2011; Muoio, 2014; Smith, et al., 2018). A robust indication of metabolic flexibility is the onset of exercise, as it requires the appropriate shifting of metabolic pathways to support energetic demand (Olenick, Pearson, & Jenkins, 2023). A prescribed high-intensity exercise regimen can play a major role in physiological adaptations to prevent cardiometabolic disease through increasing cardiorespiratory fitness (Kong, et al., 2016; Skutnik, Smith, Johson, Kurti, & Harms, 2016).

High-intensity functional training (HIFT) is an exercise regimen emphasizing functional, multi-joint movements at aerobic and anaerobic intensities designed to improve general physical fitness and performance (Feito, Heinrich, et al., 2018). Most HIFT interventions incorporate a CrossFit (CF) training methodology, which aims to increase work capacity over time while using mono-structural, body weight, and weightlifting movements (Glassman, 2007). Exercise regimens that target functional capacity minimize disease development (Cartee, Hepple, Bamman, & Zierath, 2016). An acute and chronic implementation of CF exercise demonstrates its efficacy for improving body composition, aerobic capacity, muscular strength, and reducing resting heart rate and blood pressure (Brisebois, Rigby, & Nichols, 2018; Feito, Hoffstetter, et al., 2018; Heinrich, et al., 2015). Several studies have elucidated unique hormonal and metabolic responses after acute CF exercise (Kliszczewicz, et al., 2021; Maté-Muñoz, et al., 2017). While CF seems to be a viable intervention to elicit positive metabolic adaptations, there is limited know-

ledge of how chronic exposure to CF affects metabolic, skeletal muscles', and vascular function in adults.

In the present study, we measured aerobic capacity, metabolic response during high-intensity interval exercise, mitochondrial oxidative capacity, large vessel function, and microvascular reactivity in adults who had been participating in a CF exercise for longer than one year vs. a sedentary group. We hypothesized that individuals who had been chronically participating in CF would have a more efficient exercising metabolic flexibility, higher mitochondrial oxidative capacity, and increased vascular function compared to sedentary counterparts.

Methods

Participants. Potential participants were recruited via word-of-mouth and by flyers from the Athens, University of Georgia community who completed a questionnaire to initially screen participants based on the below inclusion and exclusion criteria. Inclusion criteria included adults that chronically participated in a CrossFit regimen (3-4 times·wk^{-1} for >1 y) or did not participate in an exercise regimen (<2 h·wk^{-1} for >1 y), were aged between 18-30 y, and had a body mass index between 18.5-34.9 kg/m^2. Participants must be free of any history of cardiovascular, metabolic, musculoskeletal disease or illness requiring the ingestion of medications that affect metabolism or vascular function. Exclusion criteria included weight loss or gain exceeding 5% in the past 3-months, any disease or medication usage known to alter metabolic, skeletal muscle, or vascular function, and tobacco use. Female participants were allowed to take contraceptives if they reported no medically diagnosed hormonal issues and had a regular menstrual cycle. No participants reported supplementation use; thus, no washout period was warranted. Participants were assigned to one of two groups: 1) recreational CrossFit participants (CF) or 2) sedentary adults (SED).

Ethical approval. The study was approved by the University of Georgia Institutional Review Board (study no. 3250), with written informed consent being obtained prior to any experimental procedures. The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database.

Design. Participants completed three trials consisting of 1) baseline testing, 2) high-intensity interval exercise (HIIE), and 3) tests of mitochondrial oxidative capacity (mVO_2) and vascular function. Baseline testing consisted of anthropometrics, body composition, and VO_{2peak} testing. All sessions are described in detail below. Sessions two and three were randomized. All sessions were separated by at least 48 h and completed within 4-months. Participants refrained from exercise for 48 h and caffeine and alcohol ingestion for 24 h before each trial and fasted overnight for 10-12 h prior to the second and third trial. Prior to all trials, participants were instructed to consume a standard pre-trial dinner meal, consisting of 30% predicted resting energy expenditure (REE) (50% carbohydrate, 30% fat, and 20% protein) (Mifflin, et al., 1990). Prior to the first visit, participants were instructed to consume a standard pre-visit breakfast meal consisting of 25% predicted REE (50% carbohydrate, 30% fat, and 20% protein) (Mifflin, et al., 1990). For CF, REE was multiplied by 1.5 to factor for the influence of exercise metabolism. For SED, REE was multiped by 1.3 to factor for the influence of daily living on metabolism. Participants were given guidance on food selection to meet the prescribed energy content and macronutrient composition for recommended meals. All participants were instructed to follow normal dietary and exercise routines, other than the above guidance, for the duration of the study. All female participants completed all trials within 2-10 days following the self-reported onset of their menstrual cycle or during the non-active pill weeks if taking oral contraceptives (days 22-28) (Sims & Heather, 2018).

Baseline trial. On the first visit, participants' body height, body weight, body composition (via dual-energy X-ray absorptiometry, Horizon® DXA System, Hologic, Inc., Marlborough, MA, USA), and VO_{2peak} were measured. After resting anthropometrics, participants were fitted with a mask to collect respiratory gasses via indirect calorimetry (TrueOne 2400, Parvo Medics, Sandy, UT, USA), a heart rate monitor (Polar, Polar Electro Inc., Lake Success, NY, USA), and a muscle oxygen monitor (Moxy Monitor, Hutchinson, MN, USA) on their right *m. vastus lateralis*, approximately 2/3 of the way down from the greater trochanter to the patella and secured using elastic pre-wrap and an elastic bandage to reduce transient light. VO_{2peak} was assessed via ramp protocol on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Participants completed a warmup by cycling for 3-min at 50 W, after which the work rate increased 20 W·min^{-1} until volitional fatigue or participants could no longer sustain a cadence of ≥60 RPM. VO_{2peak} was confirmed by a respiratory exchange ratio of ≥1.10, rating of perceived exertion (RPE) ≥16 on a 6-20 RPE scale (Borg, 1982), and blood lactate ≥7 mmol assessed via finger stick (Lactate Plus, Nova Biomedical, Waltham, MA, USA). Breath-by-breath data were reduced to 10 s averages prior to analysis. The V-slope method was used to determine the gas exchange threshold and independently verified by RCP and AAO (Schneider, Phillips & Stoffolano, 1993). The workload for the high-intensity intervals during the HIIE trial was determined by iden-

tifying the halfway point between the gas exchange threshold and VO_{2peak}.

High-intensity interval exercise trial. Participants arrived at the laboratory in the morning after an overnight fast (10-12 h). Participants were weighed and then fitted with a mask to collect respiratory gasses via indirect calorimetry, a heart rate monitor, and a muscle oxygen monitor, as previously described. Exercise was performed on an electromagnetically braked cycle ergometer pre-programmed to the previously determined power output (Lode Excalibur Sport, Groningen, The Netherlands). Five minutes of seated resting respiratory gases were recorded. A 3-min warmup at a self-selected intensity of 8 on a 6-20 RPE scale (Borg, 1982) was provided (Scherr, et al., 2013). Then participants completed four 4-min high-intensity intervals, each separated by a 3-min passive rest. Participants were asked to maintain a cadence of ≥60 RPM during the high-intensity intervals. If a participant could not sustain ≥60 RPM during the high-intensity intervals, the workload was decreased in 5 W increments until the participant could complete the work requirement (this adjustment was required for three CF participants and five SED participants). Post-exercise resting respiratory gases were then collected for 10 minutes. Blood lactate via finger stick (Lactate Plus, Nova Biomedical, Waltham, MA, USA) and RPE were collected following each interval, at 3-min post-exercise and the end of the 10-min recovery period.

Mitochondrial oxidative capacity and vascular function trial. Participants arrived at the laboratory as previously described and were weighed. Then mVO_2 was determined by measuring changes in continuous-wave near-infrared spectroscopy (CW-NIRS; PortaMon, Artinis Medical Systems, Einsteinweg, The Netherlands) signals during periods of ischemia (Sumner, Beard, Pryor, Das, & McCully, 2020). Each participant laid supine on a padded table with both legs fully extended (0° of flexion) with a CW-NIRS optode placed on the right *m. vastus lateralis*, approximately 2/3 of the way down from the greater trochanter to the patella and secured using elastic pre-wrap and an elastic bandage to reduce transient light. The knee extensors were stimulated percutaneously by two rectangular electrodes (2 x 4 in) placed over the belly of the *m. vastus lateralis* (Theratouch 4.7, Richmar, Inola, OK, USA) proximal and distal to the CW-NIRS probe. A rapid inflating pneumatic cuff (Delfi V34, Medical Innovations Inc., Vancouver, BC, CA and D.E. Hokanson Inc., Bellevue, WA, USA) was placed proximal to the CW-NIRS optode with enough separation to prevent mechanical influence from the inflation. CW-NIRS signals were sampled at 10 Hz and laser diodes at three wavelengths (905, 850, and 760 nm) corresponding to the absorption wavelengths of oxygenated hemoglobin. Resting measurements of mVO_2 were assessed by inflation (250-300 mmHg) for 30 seconds. To assess exercise mVO_2, 30 s of twitch neuromuscular electrical stimulation (NMES; biphasic pulse, duration/interval = 200/50 μs) was administered at 6.0 Hz. The intensity was adjusted for each subject to produce twitch contractions at the maximal tolerable level. To measure the rate of recovery of muscle oxygen uptake back to resting levels, four mitochondrial oxidative metabolism tests were performed consisting of a series of six brief occlusions (5-s on/ 5-s off of 250-300 mmHg) following 30 s of twitch NMES.

Following mitochondrial oxidative capacity testing, brachial artery flow-mediated dilation (FMD) was measured in accordance with current guidelines (Thijssen, et al., 2019). The arm was extended and positioned at an angle of ~80° from the torso. A rapid inflation pneumatic cuff (Hokanson SC5, D.E; Hokanson Inc., Bellevue, WA, USA) was positioned immediately proximal to the olecranon process to provide a stimulus of forearm ischemia. A 12-MHz high-resolution Doppler ultrasound (Logiq E; GE Medical Systems, Chicago, IL, USA) was used to image the brachial artery in the distal half of the upper arm. When an optimal image was obtained, the probe was secured with a probe holder, and ultrasound parameters were set to optimize the longitudinal, B-mode image of the lumen-arterial wall interface. Continuous Doppler velocity assessments were obtained simultaneously and were collected using the lowest possible insonation angle (< 60°). Following 2-min of continuous baseline recording, the forearm cuff was inflated (~220 mmHg) for 5-min. Diameter and flow recording resumed 30 s prior to cuff deflation and continued for 3-min after the deflation.

Microvascular reactivity was measured in the forearm muscles using CW-NIRS (Willingham, et al., 2016). Briefly, the CW-NIRS probe was placed distal to the occlusion cuff on the forearm, whereas the ultrasound images the brachial artery proximal to the cuff, allowing for simultaneous measurements of large artery and microvascular reactivity. CW-NIRS signals were sampled at 10 Hz and laser diodes at three wavelengths (905, 850, and 760 nm) corresponding to the absorption wavelengths of oxygenated hemoglobin.

Data analysis. Exercising muscle oxygen monitor signals provided the following: muscle oxygen saturation percent (SmO_2%) and total hemoglobin (tHb). Macronutrient oxidation rate was assessed for the entire HIIE trial using equations developed by Frayn (Frayn, 1983):

fat ($g \cdot min^{-1}$) = [1.67*VO_2 ($L \cdot min^{-1}$)] – [1.67*VCO_2 ($L \cdot min^{-1}$)] and

carbohydrate ($g \cdot min^{-1}$) = [4.55*VCO_2 ($L \cdot min^{-1}$)] – [3.21*VO_2 ($L \cdot min^{-1}$)].

Oxidation values calculated as a negative value were replaced with a zero. Macronutrient oxidation rate was averaged for the duration of each interval. Total grams of substrate oxidized was calculated by multiplying the rate of substrate oxidation by time duration and summed for the full exercise bout, exercise (high-intensity intervals 1-4), and rest (low-intensity intervals 1-3, and recovery). Metabolic flexibility (MetFlex) was calculated as the relative percent change from the previous stage, e.g., (warmup - baseline / baseline) * 100). For calculations of MetFlex, oxidation values previously replaced with a zero were replaced with 0.0000000001 to allow for appropriate calculations. Excess post-oxygen consumption (EPOC) was assessed by calculating the iAUC for the recovery period for absolute and relative oxygen consumption via indirect calorimetry.

CW-NIRS signals provided included optic density (OD) of oxygenated hemoglobin (O_2Hb), deoxygenated hemoglobin (HHb), $Hb_{difference}$ ($Hb_{difference} = O_2Hb - HHb$), and total hemoglobin ($tHb = O_2Hb + HHb$). Tissue saturation index (TSI%) was calculated as the ratio of absorbance at 850 nm – (850 nm + 760 nm) x 100 to produce a percentage value (Sanni & McCully, 2019). mVO_2 CW-NIRS signals were analyzed using Matlab-based analysis software (MATLAB R2021a, MathWorks Inc., Natick, MA, USA), and a rate constant for the return of muscle oxygen uptake to resting levels was calculated (Sumner, et al., 2020). Microvascular reactivity CW-NIRS signals were analyzed using an electronic spreadsheet (Excel, Microsoft Corp., Redmon, WA, USA) and were assessed by comparing changes in O_2Hb, HHb, and TSI% at rest, during occlusion, and during reactive hyperemia (RH) phases (Willingham, Southern, & McCully, 2016).

Flow-mediated dilation was recorded and analyzed by automated tracking software (Cardiovascular Suite, Quipu, Pisa, Italy) by a blinded researcher. Flow-mediated dilation percent change was calculated from three cardiac cycles averaged around the highest peak diameter using the following equation:

FMD = (peak diameter – baseline diameter) / baseline diameter *100.

Velocity was calculated from the fastest moving blood cells in the center of the vessel. Shear rate was calculated as (4 x mean blood velocity) / diameter of the vessel.

Statistical analysis. Student's *t*-tests were conducted to assess the statistical significance between the groups (CF vs. SED) on demographic, VO_{2peak}, mitochondria oxidative capacity, and vascular data. A one-way repeated-measures ANOVA was conducted to assess the statistical significance between the groups (CF vs. SED) for HIIE data with Tukey HSD *post-hoc* analysis. Effect size was determined by partial eta squared (η_p^2), where a value of 0.01 represented a small effect, 0.06 represented a medium effect and >0.14 represented a large effect. The area under the curve (AUC) was calculated using the trapezoid rule (Matthews, Altman, Campbell, & Royston, 1990). Assumptions of normality and homogeneity of variance were verified for all outcome measures using Shapiro-Wilk's tests ($p>.05$), visually examining Q-Q plots, and Levene's Tests ($p>.05$). Statistical significance was accepted at $p\leq.05$. Data are presented as means ± SD. All statistical analyses were performed with SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA).

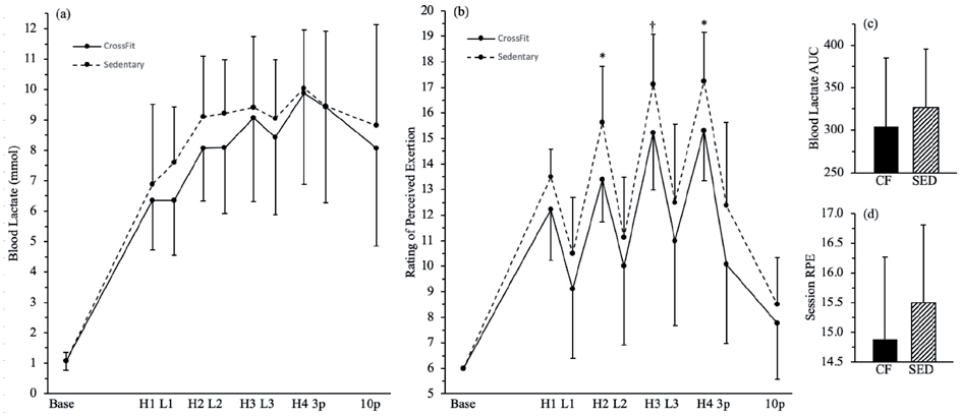
Results

Participant characteristics. Twenty-one healthy, non-smoking participants were recruited (Table 1). Participants' weight did not significantly change during their time in the study (p=.728). All

Table 1. Participants' demographics

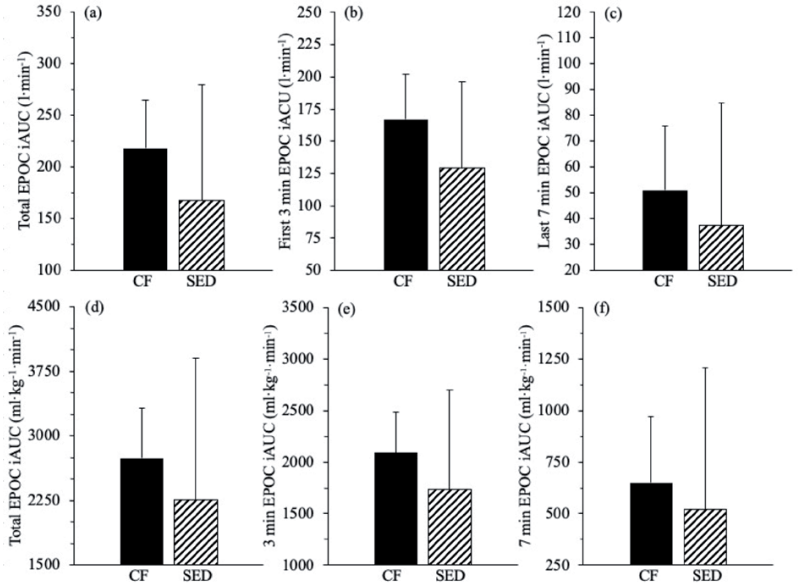
	CrossFit	Sedentary	p-value
N (M/F)	13 (9/4)	8 (4/4)	
Age (y)	25.85 (3.18)	23.25 (4.27)	p=.127
Height (cm)	174.56 (8.74)	173.20 (10.18)	p=.748
Weight (kg)	80.50 (13.06)	75.83 (12.48)	p=.429
BMI ($kg \cdot m^2$)	26.34 (2.59)	25.37 (4.48)	p=.532
Body composition (%)	18.60 (3.80)	30.29 (8.36)	p<.001
Lean mass (kg)	64.57 (11.68)	50.72 (6.33)	p=.006
Fatty mass (kg)	15.19 (2.94)	23.91 (9.14)	p=.004
Bone mineral density ($g \cdot cm^2$)	1.28 (0.09)	1.13 (0.08)	p=.001
BMD *t*-score	1.05 (0.74)	-0.04 (0.78)	p=.005
BMD z-score	1.02 (0.71)	-0.01 (0.79)	p=.006
Exercise frequency ($h \cdot wk^{-1}$)	11.25 (3.39)	1.59 (0.57)	p<.001
CrossFit participation (y)	2.90 (1.37)	0.00 (0.00)	p<.001
CrossFit frequency ($h \cdot wk^{-1}$)	4.54 (1.41)	0.00 (0.00)	p<.001

Note. M – male; F – female; y – years; cm – centimeter; m – meter; kg – kilogram; g – gram; % – percent; BMD – bone mineral density; h – hours; wk – week.



Note. (a) blood lactate response during HIIE. (b) rating of perceived exertion during HIIE. (c) blood lactate AUC. (d) session RPE for HIIE. CF – CrossFit; SED – sedentary; mmol – millimole; WU – warmup; 3p – 3 min post exercise; 10p – 10 min post exercise. * – significant difference between CF and SED (p<.05); † – approaching significant difference between CF and SED (p=.064).

Figure 1. Blood lactate response and rating of perceived exertion during HIIE.



Note. (a) Total EPOC iAUC. (b) First 3-min post EPOC iAUC. (c) Last 7-min post EPOC iAUC. (d) Total EPOC iAUC. (e) First 3-min post EPOC iAUC. (f) Last 7-min post EPOC iAUC. CF – CrossFit; SED – sedentary; EPOC – excess post-exercise oxygen consumption; iAUC – incremental area under the curve; l – liter; ml – millimeter; kg – kilogram; min – minute.

Supplemental Figure 1. EPOC response during HIIE.

female participants reported taking oral contraceptives. One participant from the CF cohort did not complete all testing visits due to scheduling problems; therefore, mitochondria oxidative capacity and vascular function measurements are represented as CF n = 12 vs. SED n = 8.

VO_{2peak} test. The CF participants had higher absolute EPOC (l, CF *n* = 12 vs SED *n* = 4), relative EPOC (ml·kg^{-1}, CF *n* = 12 vs SED *n* = 4), absolute VO_{2peak} (l·min^{-1}), relative VO_{2peak} (ml·kg^{-1}·min^{-1}), ventilatory threshold (watts, l·min^{-1}, and ml·kg^{-1}·min^{-1}), and watts for high-intensity bout during HIIE compared to the SED participants (p<.005, Table 2). Additionally, the CF participants had lower maximum heart rate (bpm) when compared to the SED participants (p=.023, Table 2).

HIIE trial. *Lactate response and RPE.* There was not a significant group main effect of the blood lactate response during HIIE (F(1, 18) = 0.470, p=.501, η_p^2 = 0.024, Figure 1a), but there was a significant time main effect of the blood lactate response during HIIE (F(1, 18) = 35.050, p<.001, η_p^2 = 0.967, Figure 1a). There were no group differences in blood lactate AUC (p=.518, Figure 1c).

There was a significant group and time main effect of RPE during HIIE (group: F(1, 18) = 6.254, p=.022, η_p^2 = 0.248; time: F(1, 18) = 216.397, p<.001, η_p^2 = 0.994; Figure 1b). RPE was significantly lower in CF during HI1 (p=.016) and HI4 (p=.039) and approached significance during HI3 (p=.064) compared to the SED participants, Figure 1b. Addi-

Table 2. Group differences in the VO_{2peak} test

	CrossFit	Sedentary	p-value
Immediate post lactate (mmol)	10.45 (1.77)	9.80 (1.99)	p=.443
Max RPE	16.77 (1.83)	17.38 (1.30)	p=.426
$VO_{2\ peak}$ ($l \cdot min^{-1}$)	3.51 (0.72)	2.33 (0.33)	p<.001
$VO_{2\ peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	43.32 (3.89)	31.05 (3.81)	p<.001
Max heart rate ($beats \cdot min^{-1}$)	178.46 (8.12)	187.88 (9.01)	p=.023
Max RER	1.18 (0.09)	1.22 (0.74)	p=.307
EPOC (l)	0.62 (0.18)	0.35 (0.10)	p=.013
EPOC ($ml \cdot kg^{-1}$)	7.53 (1.77)	4.64 (1.25)	p=.010
VT (W)	156.08 (37.84)	115.88 (22.33)	p=.014
VT ($VO_2\ l \cdot min^{-1}$)	2.08 (0.47)	1.55 (0.24)	p=.008
VT ($VO_2\ ml \cdot kg^{-1} \cdot min^{-1}$)	25.77 (4.18)	20.74 (3.80)	p=.012
VT (% VO_{2peak})	59.52 (8.74)	66.76 (8.82)	p=.082
Watts for high bout	220.92 (39.38)	155.50 (20.26)	p<.001

Note. mmol – millimole; RPE – Borg rate of perceived exertion, EPOC – excess post-exercise oxygen consumption; l – liters; min – minute; kg – kilogram; RER – respiratory exchange ratio; VT – ventilatory threshold; W – watts; VO_2 – volume of oxygen; % – percent.

tionally, there were no group differences in session RPE (p=.326, Figure 1d).

Respiratory and heart rate response. There was a significant group and time main effect of absolute VO_2 ($l \cdot min^{-1}$) during HIIE (group: F(1, 18) = 19.745, p<.001, $\eta_p^2 = 0.510$; time: F(1, 18) = 108.601, p<.001, $\eta_p^2 = 0.991$; Figure 2a). Absolute VO_2 ($l \cdot min^{-1}$) was significantly higher in CF during warmup (p=.007), HI1 (p<.001), LOW1 (p=.033), HI2 (p<.001), LOW2 (p=.039), HI3 (p<.001), LOW3 (p=.038), HI4 (p<.001), 3-min post (p=.036), and 10-min post (p=.022) compared to the SED participants, Figure 2a.

There was a significant group and time main effect for relative VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) during HIIE (group: F(1, 18) = 26.564, p<.001, $\eta_p^2 = 0.583$; time: F(1, 18) = 127.409, p<.001, $\eta_p^2 = 0.992$; Figure 2b). Relative VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) was significantly higher in CF during HI1 (p<.001), HI2 (p<.001), HI3 (p<.001), and HI4 (p<.001) compared to the SED participants, Figure 2b.

There was a significant group and time main effect for absolute VCO_2 ($l \cdot min^{-1}$) during HIIE (group: F(1, 18) = 8.893, p<.001, $\eta_p^2 = 0.495$; time: F(1, 18) = 83.408, p<.001, $\eta_p^2 = 0.998$; Figure 2c). Absolute VCO_2 ($l \cdot min^{-1}$) was significantly higher in CF during HI1 (p=.003), LOW1 (p=.012), HI2 (p=.001), LOW2 (p=.014), HI3 (p<.001), LOW3 (p=.012), HI4 (p<.001), and 3-min post (p=.009) and approached significance during warmup (p=.059) compared to the SED participants, Figure 2c.

There was a significant group and time main effect for ventilation (VE; $l \cdot min^{-1}$) during HIIE (group: F(1, 18) = 5.881, p=.025, $\eta_p^2 = 0.236$; time: F(1, 18) = 132.520, p<.001, $\eta_p^2 = 0.993$; Figure 2d). VE ($l \cdot min^{-1}$) was significantly higher in CF during HI1 (p=.037), HI3 (p=.025), and HI4 (p=.010) and approached significance during HI2 (p=.054) compared to the SED participants, Figure 2d.

There was not a significant group main effect of respiratory exchange ratio (RER) during HIIE (F(1, 18) = 0.130, $P = 0.722$, $\eta_p^2 = 0.007$, Figure 2e), but there was a significant time main effect of RER during HIIE (F(1, 18) = 42.691, p<.001, $\eta_p^2 = 0.977$, Figure 2e).

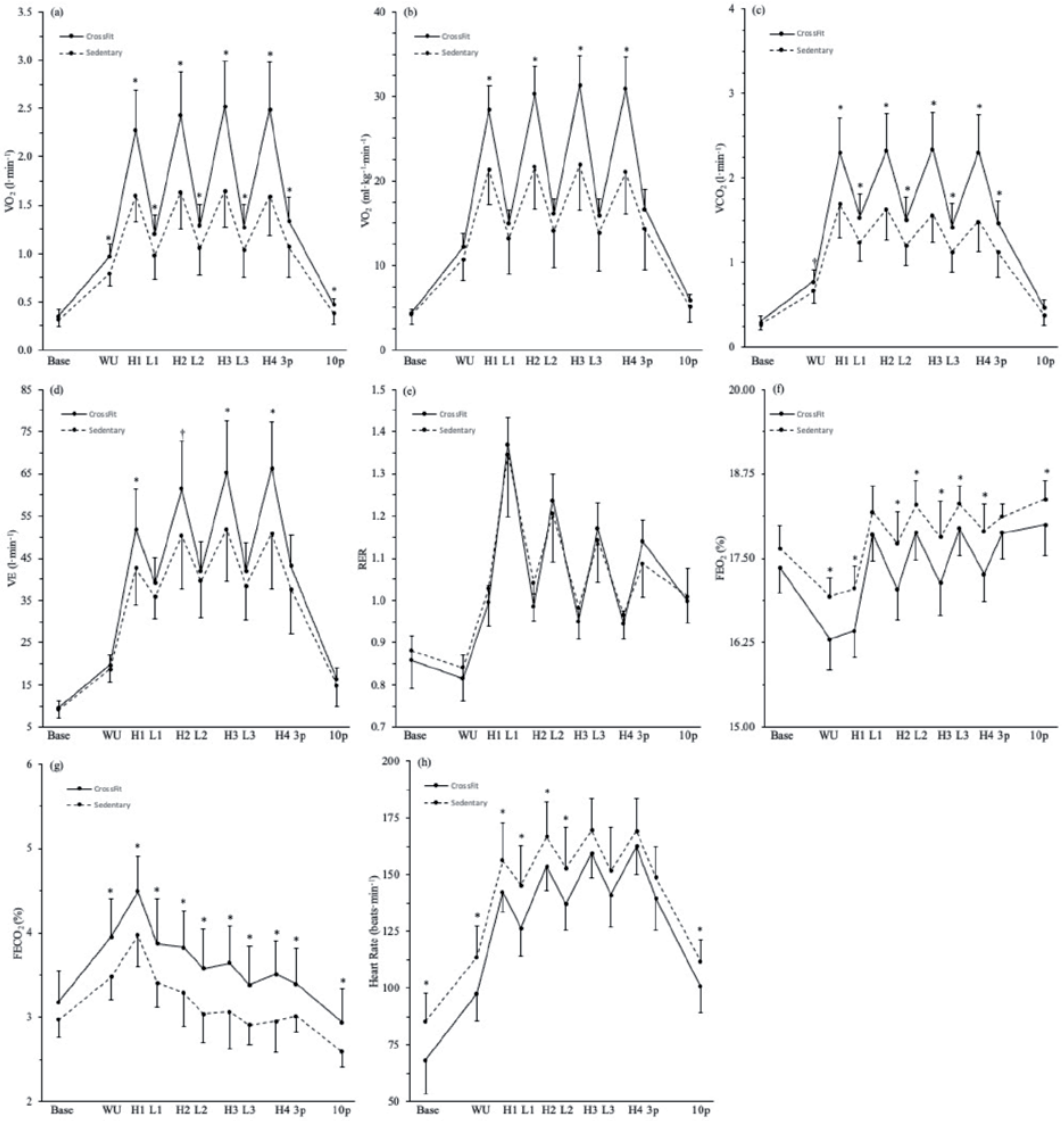
There was a significant group and time main effect for fractional oxygen percent (FEO_2%) during HIIE (group: F(1, 18) = 11.992, p=.003, $\eta_p^2 = 0.387$; time: F(1, 18) = 49.160, p<.001, $\eta_p^2 = 0.980$; Figure 2f). FEO_2% was significantly lower in CF during warmup (p=.002), HI1 (p=.001), HI2 (p=.003), LOW2 (p=.030), HI3 (p=.008), LOW3 (p=.033), HI4 (p=.003), and 10-min post (p=.048) compared to the SED participants, Figure 2f.

There was a significant group and time main effect for fractional expired carbon dioxide percent ($FECO_2$%) during HIIE (group: F(1, 18) = 11.589, p=.008, $\eta_p^2 = 0.318$; time: F(1, 18) = 38.882, p<.001, $\eta_p^2 = 0.975$; Figure 2g). $FECO_2$% was significantly higher in CF during warmup (p=.014), HI1 (p=.010), LOW1 (p=.030), HI2 (p=.009), LOW2 (p=.011), HI3 (p=.009), LOW3 (p=.013), HI4 (p=.004), 3-min post (p=.030), and 10-min post (p=.048) compared to the SED participants, Figure 2g.

There was a significant group and time main effect for heart rate (bpm) during HIIE (group: F(1, 18) = 6.561, p=.024, $\eta_p^2 = 0.252$; time: F(1, 18) = 38.82, p<.001, $\eta_p^2 = 0.975$; Figure 2h). Heart rate (bpm) was significantly lower in CF during baseline (p=.019), warmup (p=.014), HI1 (p=.021), LOW1 (p=.012), HI2 (p=.037), LOW2 (p=.028), and 10-min post (p=.046) compared to the SED participants, Figure 2h.

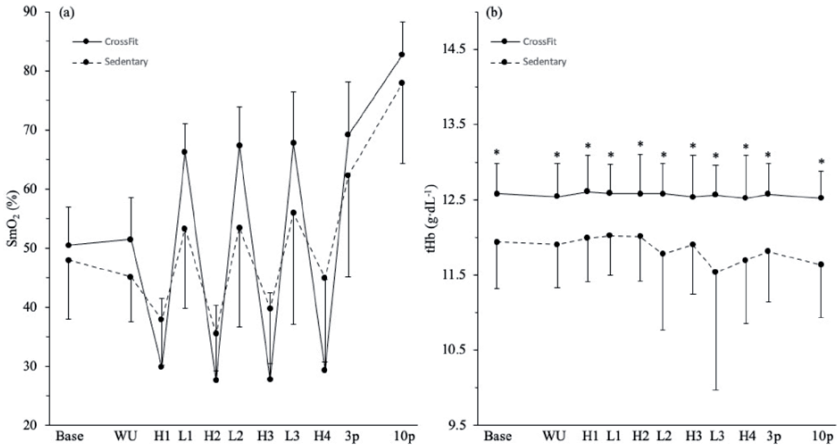
EPOC. There were no significant differences between the groups for total EPOC ($l \cdot min^{-1}$ and $ml \cdot kg^{-1} \cdot min^{-1}$), the first 3-min post EPOC (l and $ml \cdot kg^{-1}$), or the last 7-min post EPOC (l and $ml \cdot kg^{-1}$; p>.05, Supplemental Figure 1).

SmO_2% and tHb. There was not a significant group main effect for the SmO_2% during HIIE (F(1, 18) = 0.118, p=.735, $\eta_p^2 = 0.006$, Supplemental Figure 2a), but there was a significant group time



Note. (a) VO_2 l·min^{-1} response during HIIE. (b) VO_2 ml·kg^{-1}·min^{-1} response during HIIE. (c) VCO_2 l·min^{-1} response during HIIE (†, p=.059). (d) VE l·min^{-1} response during HIIE (†, p=.054). (e) RER response during HIIE. (f) FEO_2% response during HIIE. (g) $FECO_2$% response during HIIE. (h) heart rate response during HIIE. V – volume; O_2 – oxygen; ml – milliliter; l – liter; kg – kilogram; min – minute; VE – ventilation; RER – respiratory exchange ratio; FE – fractional expiratory; % – percent; WU – warmup; 3p – 3 min post exercise; 10p – 10 min post exercise; * – significant difference between CF and SED (p<.05).

Figure 2. Respiratory and heart rate response during HIIE.



Note: (a) SmO_2% response during HIIE. (b) tHb response during HIIE. SmO_2, muscle oxygen saturation; tHb, total hemoglobin; %, percent; WU, warmup; 3p, 3 min post exercise; 10p, 10 min post exercise. *, significant difference between CF and SED (P < 0.05).

Supplemental Figure 2. SmO_2% and tHb response during HIIE.

effect for the SmO_2% during HIIE (F(1, 18) = 90.256, p<.001, η_p^2 = 0.989, Supplemental Figure 2a). There was a significant group and time effect for the tHb response during HIIE (HIIE (group: F(1, 18) = 8.643, p=.008, η_p^2 = 0.313; time: F(1, 18) = 9.879, p<.001, η_p^2 = 0.908; Supplemental Figure 2b). tHb was significantly higher in CF during baseline (p=.008), warmup (p=.010), HI1 (p=.016), LOW1 (p=.011), HI2 (p=.034), LOW2 (p=.019), HI3 (p=.029), LOW3 (p=.033), HI4 (p=.015), 3-min post (p=.004), and 10-min post (p=.001), Supplemental Figure 2b.

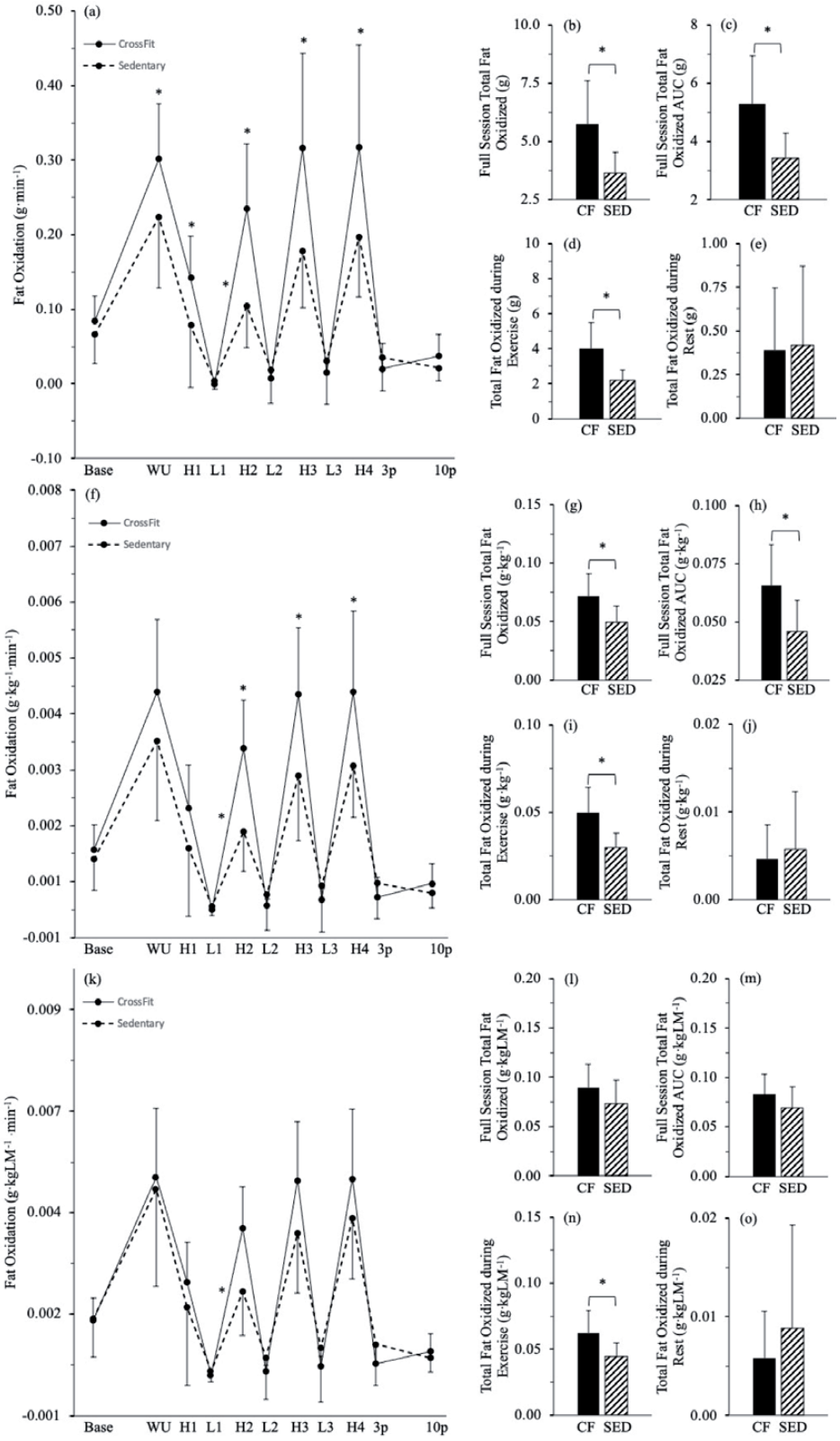
Fat oxidation. There was a significant group and time main effect for absolute fat oxidation ($g \cdot min^{-1}$) during HIIE (group: F(1, 18) = 8.375, p=.009, η_p^2 = 0.306; time: F(1, 18) = 27.963, p<.001, η_p^2 = 0.965; Figure 3a). Absolute fat oxidation ($g \cdot min^{-1}$) was significantly higher in CF during warmup (p=.048), HI1 (p=.050), HI2 (p=.001), HI3 (p=.013), and HI4 (p=.039) compared to the SED participants, Figure 3a. CF had significantly higher total fat oxidized (g) for the full session (p=.008, Figure 3b), full session AUC (p=.009, Figure 3c) and exercise portions of HIIE (p=.003, Figure 3d), but no significance was observed for resting portions of HIIE (p=.879, Figure 3e). There was significant group and time main effect for absolute fat oxidation MetFlex during HIIE (group: F(1, 18) = 8.035, p=.011, η_p^2 = 0.297; time: F(1, 18) = 36.576, p<.001, η_p^2 = 0.901; Figure 3a). Absolute fat oxidation MetFlex was significantly higher in CF from LOW to HI1 (p=.001) compared to the SED participants, Figure 3a.

There was a significant group and time main effect for body weight relative fat oxidation ($g \cdot kg^{-1} \cdot min^{-1}$) during HIIE (group: F(1, 18) = 7.471, p=.013, η_p^2 = 0.282; time: F(1, 18) = 28.054, p<.001, η_p^2 = 0.966; Figure 3f). Body weight relative fat oxidation ($g \cdot kg^{-1} \cdot min^{-1}$) was significantly higher in CF during HI2 (p<.001), HI3 (p=.013), and HI4 (p=.033) compared to the SED participants, Figure 3f. CF had significantly higher total body weight relative fat oxidized ($g \cdot kg^{-1}$) for the full session (p=.010, Figure 3g), full session AUC (p=.013, Figure 3h) and exercise portions of HIIE (p=.002, Figure 3i), but no significance was observed for resting portions of HIIE (p=.634, Figure 3j). There was significant group and time main effect for relative fat oxidation MetFlex during HIIE (group: F(1, 18) = 5.837, p=.026, η_p^2 = 0.235; time: F(1, 18) = 36.040, p<.001, η_p^2 = 0.900; Figure 3f). Relative fat oxidation MetFlex was significantly higher in CF from LOW to HI1 (p<.001) compared to the SED participants, Figure 3f.

There was not a significant group main effect for lean mass relative fat oxidation ($g \cdot kgLM^{-1} \cdot min^{-1}$) during HIIE (F(1, 18) = 1.741, p=.203, η_p^2 = 0.084, Figure 3k), but there was a significant time main effect for fat oxidation ($g \cdot kgLM^{-1} \cdot min^{-1}$) during HIIE (F(1, 18) = 28.512, p<.001, η_p^2 = 0.966, Figure 3k). CF had significantly higher total body weight relative fat oxidized ($g \cdot kgLM^{-1}$) for the exercise portions of HIIE (p=.017, Figure 3n), but no significance was observed for the full session (p=.149, Figure 3l), full session AUC (p=.198, Figure 3m), and resting portions of HIIE (p=.374, Figure 3o). There was significant time main effect (F(1, 18) = 37.785, p<.001, η_p^2 = 0.900; Figure 3k) and an approaching significant group main effect (F(1, 18) = 3.991, p=.060, η_p^2 = 0.174; Figure 3f) for lean mass relative fat oxidation MetFlex during HIIE. Lean mass relative fat oxidation MetFlex was significantly higher in CF from LOW to HI1 (p=.004) compared to the SED participants, Figure 3f.

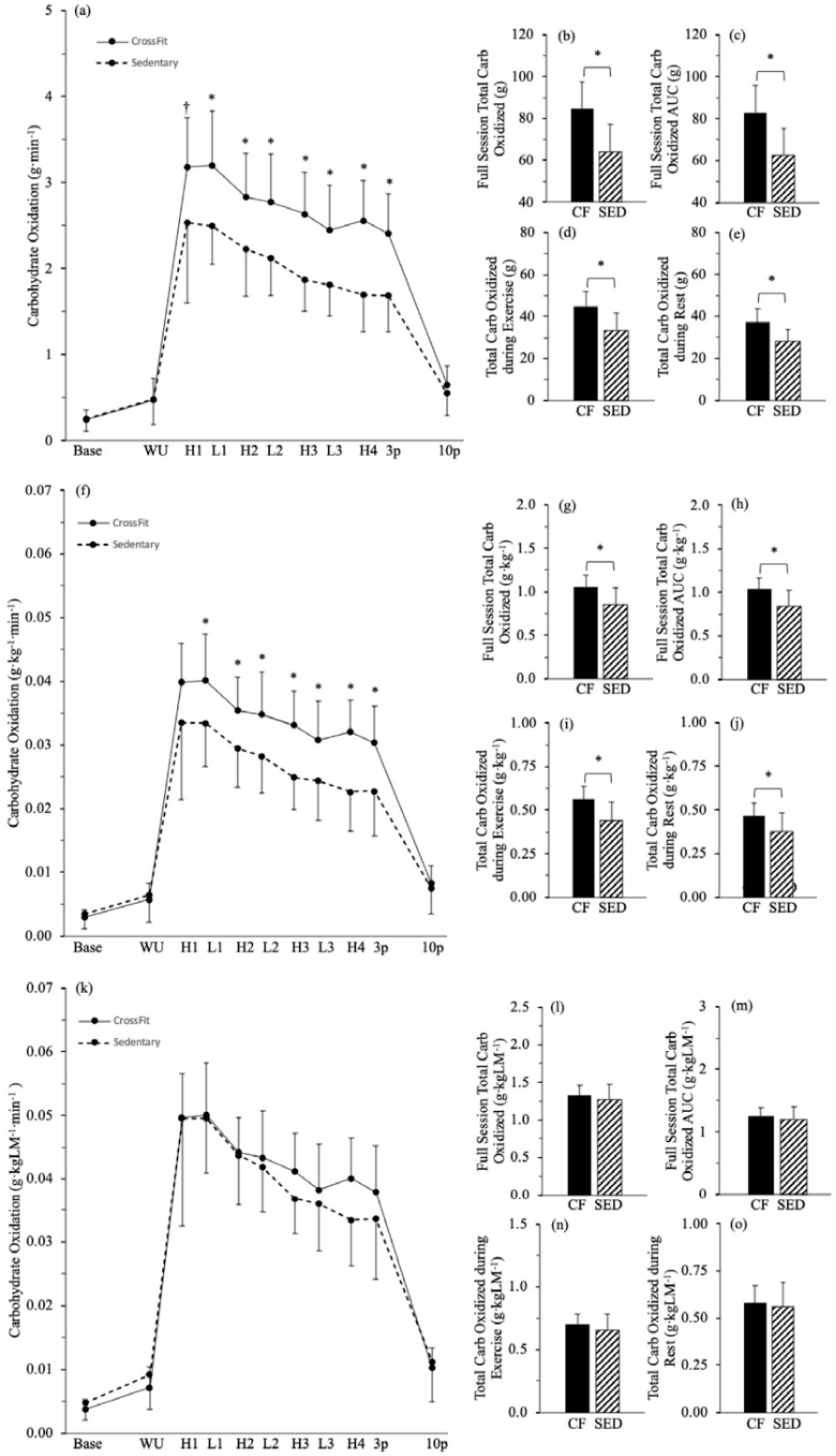
Carbohydrate oxidation. There was a significant group and time main effect for absolute carbohydrate oxidation ($g \cdot min^{-1}$) during HIIE (group: F(1, 18) = 12.239, p=.002, η_p^2 = 0.392; time: F(1, 18) = 46.366, p<.001, η_p^2 = 0.979; Figure 4a). Absolute carbohydrate oxidation ($g \cdot min^{-1}$) was significantly higher in CF during LOW1 (p=.012), HI2 (p=.019), LOW2 (p=.011), HI3 (p=.001), LOW3 (p=.007), HI4 (p<.001) and 3-min post (p=.002) and approached significance at HI1 (p=.065) compared to the SED participants, Figure 4a. CF had significantly higher total carbohydrate oxidized (g) for the full session (p=.003, Figure 4b), full session AUC (=.002, Figure 4c), exercise portions of HIIE (p=.003, Figure 4d), and resting portions of HIIE (p=.005, Figure 4e). There was not a significant group main effect for absolute carbohydrate oxidation MetFlex during HIIE (F(1, 18) = 0.043, p=.838, η_p^2 = 0.002, Figure 4a), but there was a significant time main effect for absolute carbohydrate oxidation MetFlex during HIIE (F(1, 18) = 144.382, p<.001, η_p^2 = 0.992, Figure 4a).

There was a significant group and time main effect for body weight relative carbohydrate oxidation ($g \cdot kg^{-1} \cdot min^{-1}$) during HIIE (group: F(1, 18) = 8.731, p=.008, η_p^2 = 0.315; time: F(1, 18) = 54.947, p<.001, η_p^2 = 0.982; Figure 4f). Body weight relative carbohydrate oxidation ($g \cdot kg^{-1} \cdot min^{-1}$) was significantly higher in CF during LOW1 (p=.048), HI2 (p=.026), LOW2 (p=.033), HI3 (p=.003), LOW3 (p=.037), HI4 (p=.001), and 3-min post (p=.014) compared to the SED participants, Figure 4f. CF had significantly higher total body weight relative carbohydrate oxidized ($g \cdot kg^{-1}$) for the full session (p=.009, Figure 4g), full session AUC (p=.009, Figure 4h), exercise portions of HIIE (p=.006, Figure 4i), and resting portions of HIIE (p=.035, Figure 4j). There was not a significant group main effect for relative carbohydrate oxidation MetFlex during HIIE (F(1, 18) = 0.043, p=.838, η_p^2 = 0.002, Figure 4f), but there was a significant time main effect for relative carbohydrate oxidation MetFlex during HIIE (F(1, 18) = 144.382, p<.001, η_p^2 = 0.992, Figure 4f).



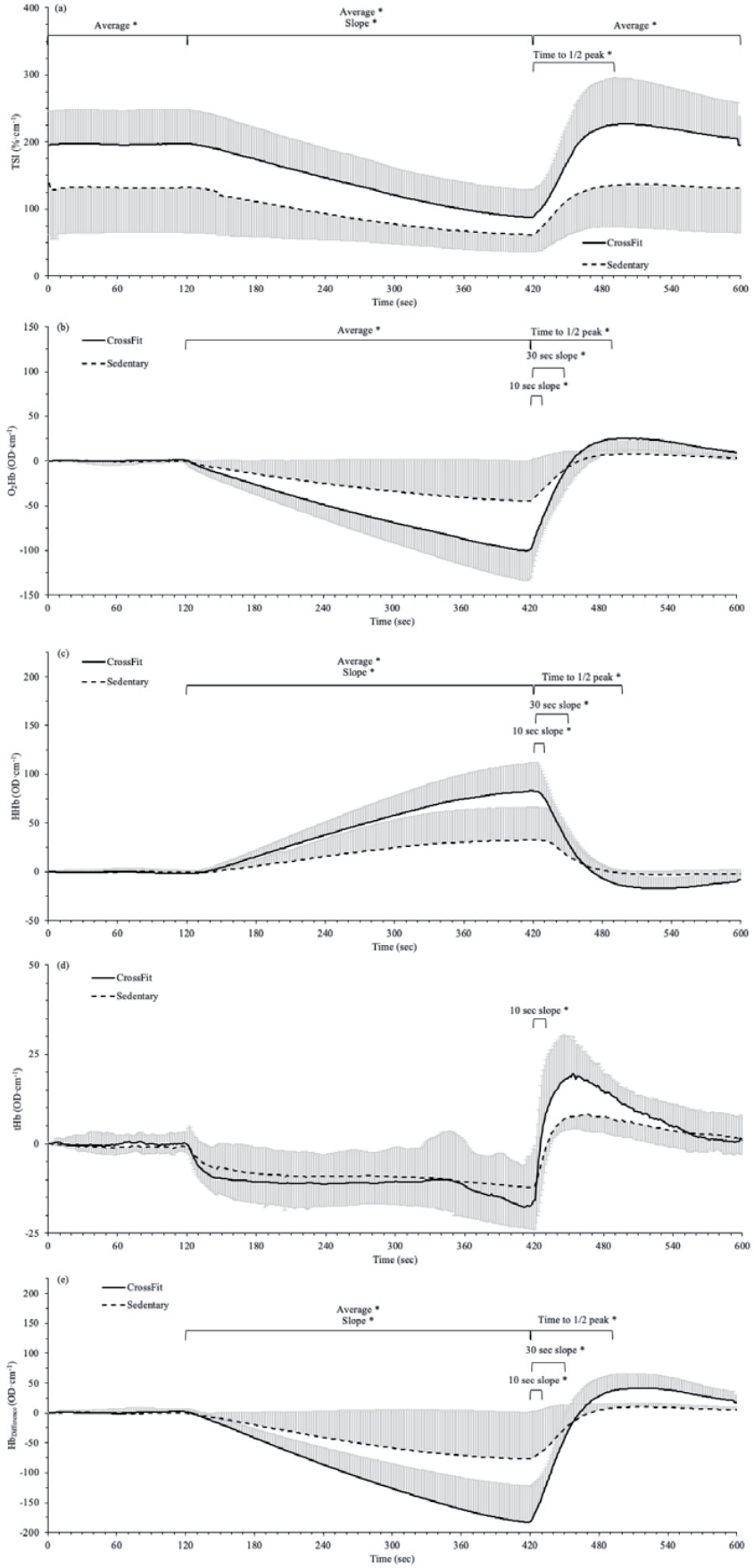
Note. (a) Fat oxidation (g·min^{-1}) response during HIIE and metabolic flexibility during HIIE. (b) Total fat oxidized (g) during full session HIIE. (c) Total fat oxidized AUC (g) during full session HIIE. (d) Total fat oxidized (g) during exercise portions of HIIE. (e) Total fat oxidized (g) during rest portions of HIIE. (f) Fat oxidation (g·kg^{-1}·min^{-1}) response during HIIE and metabolic flexibility during HIIE. (g) Total fat oxidized (g·kg^{-1}) during full session HIIE. (h) Total fat oxidized AUC (g·kg^{-1}) during full session HIIE. (i) Total fat oxidized (g·kg^{-1}) during exercise portions of HIIE. (j) Total fat oxidized (g·kg^{-1}) during rest portions of HIIE. (k) Fat oxidation (g·LM^{-1}·min^{-1}) response during HIIE and metabolic flexibility during HIIE. (l) Total fat oxidized (g·LM^{-1}) during full session HIIE. (m) Total fat oxidized AUC (g·LM^{-1}) during full session HIIE. (n) Total fat oxidized (g·LM^{-1}) during exercise portions of HIIE. (o) Total fat oxidized (g·LM^{-1}) during rest portions of HIIE. g – gram; kg – kilogram; kgLM – kilograms of lean mass; min – min; AUC – area under the curve; CF – CrossFit; SED – sedentary; WU – warmup; 3p – 3 min post; 10p – 10 min post. * – significant difference between CF and SED (p<.05).

Figure 3. Fat oxidation response during HIIE.



Note. (a) Carbohydrate oxidation (g·min^{-1}) response during HIIE and MetFlex response during HIIE. (b) Total carbohydrate oxidized (g) during full session HIIE. (c) Total carbohydrate oxidized AUC (g) during full session HIIE. (d) Total carbohydrate oxidized (g) during exercise portions of HIIE. (e) Total carbohydrate oxidized (g) during rest portions of HIIE. (f) Carbohydrate oxidation (g·kg^{-1}·min^{-1}) response during HIIE and MetFlex response during HIIE. (g) Total carbohydrate oxidized (g·kg^{-1}) during full session HIIE. (h) Total carbohydrate oxidized AUC (g·kg^{-1}) during full session HIIE. (i) Total carbohydrate oxidized (g·kg^{-1}) during exercise portions of HIIE. (j) Total carbohydrate oxidized (g·kg^{-1}) during rest portions of HIIE. (k) Carbohydrate oxidation (g·LM^{-1}·min^{-1}) response during HIIE and MetFlex response during HIIE. (l) Total carbohydrate oxidized (g·LM^{-1}) during full session HIIE. (m) Total carbohydrate oxidized AUC (g·LM^{-1}) during full session HIIE. (n) Total carbohydrate oxidized (g·LM^{-1}) during exercise portions of HIIE. (o) Total carbohydrate oxidized (g·LM^{-1}) during rest portions of HIIE. g – gram; kg – kilogram; kgLM – kilograms of lean mass; min – min; AUC – area under the curve; CF – CrossFit; SED – sedentary; WU – warmup; 3p – 3 min post; 10p – 10 min post. * – significant difference between CF and SED (p<.05). † – approaching significant difference between CF and SED (p=.065).

Figure 4. Carbohydrate oxidation response during HIIE.



Note. CF n = 12 vs. SED n = 8. O_2Hb – oxygenated hemoglobin; HHb – deoxygenated hemoglobin; $Hb_{difference}$ – hemoglobin difference (O_2Hb – HHb); tHb – total hemoglobin (O_2Hb + HHb); TSI – tissue saturation index; OD – optic density; cm – centimeter. * – significant difference between CF and SED ($p<.05$).

Figure 5. Microvascular data.

Table 3. Flow mediated dilation

	CrossFit (n = 12)	Sedentary (n = 8)	p-value
FMD (%)	6.95 (3.38)	6.45 (2.67)	p=.733
"Corrected" FMD (equivalent %)	13.60 (3.21)	12.15 (3.02)	p=.326
Baseline			
Diameter (mm)	4.26 (0.60)	3.48 (0.36)	p=.004
Shear rate (sec^{-1})	56.24 (19.48)	160.30 (77.33)	p<.001
Shear rate AUC	6355.60 (1996.20)	18608.61 (9186.07)	p<.001
Velocity ($cm \cdot sec^{-1}$)	5.92 (2.10)	13.93 (6.98)	p=.001
Reactive hyperemia			
Peak dilation (mm)	4.54 (0.56)	3.70 (0.42)	p=.002
Shear rate (sec^{-1})	143.20 (54.96)	299.48 (100.59)	p<.001
Total shear rate iAUC	24388.53 (9289.89)	50995.48 (16992.13)	p<.001
Total shear rate iAUC above peak diameter	14222.71 (5470.29)	29499.73 (11876.16)	p=.001
Velocity ($cm \cdot sec^{-1}$)	15.58 (5.95)	26.38 (8.97)	p=.004
Halftime to peak (sec)	25.50 (7.06)	26.81 (6.23)	p=.675

Note. FMD – flow mediated dilation; "Corrected" FMD – allometrically scaled; mm – millimeter; cm – centimeter; sec – second; AUC – area under the curve.

There was not a significant group main effect for lean mass relative carbohydrate oxidation ($g \cdot kgLM^{-1} \cdot min^{-1}$) during HIIE ($F(1, 18) = 0.583$, p=.455, $\eta_p^2 = 0.030$, Figure 4k), but there was a significant time main effect for carbohydrate oxidation ($g \cdot kgLM^{-1} \cdot min^{-1}$) during HIIE ($F(1, 18) = 68.244$, p<.001, $\eta_p^2 = 0.986$, Figure 4k). There were no significant group differences for total body weight relative carbohydrate oxidized ($g \cdot kgLM^{-1}$) for the full session (p =.486, Figure 4l), full session AUC (p=.480, Figure 4m), exercise portions of HIIE (p=.322, Figure 4n), and resting portions of HIIE (p=.696, Figure 4o). There was not a significant group main effect for lean mass relative carbohydrate oxidation MetFlex during HIIE ($F(1, 18) = 0.043$, p=.838, $\eta_p^2 = 0.002$, Figure 4k), but there was a significant time main effect for lean mass relative carbohydrate oxidation MetFlex during HIIE ($F(1, 18) = 144.382$, p<.001, $\eta_p^2 = 0.992$, Figure 4k).

Mitochondria oxidative capacity. CF had significantly higher mVO_2 (OD) compared to the SED participants (CF = 1.74 (0.51) vs. SED = 0.98 (0.17); p<.001). CF had significantly lower amounts of adipose tissue thickness (cm) of the upper leg compared to the SED participants (CF = 0.63 (0.20) vs. SED = 1.09 (0.37), p=.002). When mVO_2 was controlled for adipose tissue thickness, CF still had a significantly higher mVO_2 ($OD \cdot cm^{-1}$) compared to the SED participants (CF = 3.15 (1.56) vs. SED = 1.11 (0.68); p=.003). Additionally, CF had significantly higher amounts of lean mass (kgLM) in the right leg, via DEXA (CF = 10.90 (1.99) vs. SED = 8.37 (1.20); p=.004). When mVO_2 was controlled for the right leg lean mass, CF still had a significantly higher mVO_2 ($OD \cdot kgLM^{-1}$) compared to the SED participants (CF = 0.16 (0.04) vs. SED = 0.12 (0.02); p=.014).

Vascular function

Flow-mediated dilation. There were no significant differences in FMD% or half time to RH between the CF and SED participants (p=.733 and p=.675, respectively, Table 3). CF had significantly higher baseline diameter and peak diameter, and higher baseline shear rate, baseline shear rate AUC, baseline velocity, RH shear rate, RH total shear rate iAUC, RH total shear rate iAUC above the peak diameter, and RH velocity compared to the SED participants (p<.01, Table 3). Due to the differences in baseline diameter and shear rate, we scaled FMD% to a "corrected" FMD% with an allometric exponent of 0.958 as described in Atkinson and Batterham (2013). There were no significant differences in "corrected" FMD% between the CF and SED participants (p=.326, Table 3).

Microvascular function. CF had significantly lower amounts of adipose tissue thickness (cm) of the forearm (CF = 0.31 (0.08) vs. SED = 0.64 (0.27), p=.002) compared to the SED participants. Thus, we chose to control CW-NIRS data for forearm adipose tissue thickness. CF had higher TSI% during baseline, occlusion and RH (p=.005, p=.006, and p=.004, respectively, Figure 5a) and a greater occlusion slope and RH half time to peak (p=.033 and p=.034, respectively, Figure 5a) compared to the SED participants. CF had lower O_2Hb during occlusion (p=.012, Figure 5b) and a greater RH half time to peak, RH first 10sec slope, and RH first 30sec slope (p=.033, p=.013, and p=.009, respectively, Figure 5b). CF had higher HHb during occlusion (p=.003, Figure 5c) and a greater occlusion slope, RH half time to peak, RH first 10sec slope, and RH first 30sec slope (p=.002, p=.039, p=.029, and p=.009, respectively, Figure 5c) compared to the SED participants. CF had a greater tHb RH

first 10sec slope compared to the SED participants (p=.033, Figure 5d). CF had lower $Hb_{Difference}$ during occlusion (p=.006, Figure 5e) and a greater occlusion slope, RH half time to peak, RH first 10 sec slope, and RH first 30 sec slope (p=.003, p=.038, p=.014, and p=.008, respectively, Figure 5e).

Discussion and conclusions

In the current study, we assessed the metabolic response to HIIE in adults who had been participating in a CF regimen for over one year and adults who had been participating in less than two $h \cdot wk^{-1}$ for over one year of structure exercise. Additionally, we evaluated resting vascular function and mitochondrial oxidative capacity between these groups. Major findings include: (i) CF participants had higher cardiovascular fitness and lower body fat percentage, (ii) CF participants had lower ratings of perceived exertion with higher respiratory response and a lower heart rate response during HIIE, (iii) CF participants had higher fat and carbohydrate oxidation when expressed as absolute and body weight relative values but not when expressed as lean mass relative values indicating that skeletal muscle mass is a primary driver in substrate oxidation, (iv) CF participants had greater mitochondria oxidative capacity independent of the amount of lean mass, and (v) there were no differences in macrovascular function, but CF participants had greater baseline arterial diameter and faster reperfusion suggesting some influence of exercise habits on microvascular function. These data suggest that chronic participation in CF could improve fitness and weight status as well as metabolic, mitochondrial, and vascular function.

Increased adiposity significantly elevates the risk for chronic disease (Ballantyne, et al., 2008; Wilson & Meigs, 2008). HIFT is reported to improve body composition (Choi, So, & Jeong, 2017; Feito, Hoffstetter, et al., 2018; Heinrich, et al., 2015). The present study confirmed improvements in body composition, possibly leading to reduced risks of chronic disease. Physical activity is the only intervention to increase bone mass and strength (Kohrt, et al., 2004) with HIFT improving bone mineral content in women (Feito, Hoffstetter, et al., 2018). The present study confirmed higher bone mineral density in individuals participating in the CF regimen. Lastly, cardiorespiratory fitness has a strong and inverse association with metabolic syndrome (Hassinen, et al., 2008) and HIFT has been found to increase cardiorespiratory fitness (Brisebois, et al., 2018; Greenlee, et al., 2017; Heinrich, et al., 2015). The present study confirmed higher cardiorespiratory fitness of the CF participants compared to the sedentary counterparts, potentially reducing the risk of developing metabolic syndrome and chronic disease. These findings support the notion that CF is an effective strategy for stimulating changes in body composition, bone health, and cardiorespiratory fitness.

Improved cardiorespiratory fitness plays an important role in acute responses to exercise, but the influence of fitness status on this response to HIIE is less known (Buchheit & Laursen, 2013). While it has been previously reported there is a similar acute cardiorespiratory response to HIIE across fitness levels, it is important to note that the cohorts used were all of higher fitness status, $>47\ ml \cdot kg^{-1} \cdot min^{-1}\ VO_{2max}$ (Cipryan, 2018). In the present study, we found that individuals participating in the CF exercise had higher cardiorespiratory responses indicated by VO_2, VCO_2, and VE. This is not surprising due to the autonomic and cardiovascular adaptations to increase the response to exercise demand in trained individuals and it is in agreement with research utilizing groups with large differences in fitness status (Hetlelid, Plews, Herold, Laursen, & Seiler, 2015). Additionally, we found that individuals participating in the CF exercise had lower ratings of perceived exertion and heart rate responses during HIIE compared to the sedentary counterparts. This was also not surprising due to the increased stroke volume allowing for a lower heart rate at a given power output (Hellsten & Nyberg, 2015) and the relationship between heart rate and perceived exertion (Zinoubi, Zbidi, Vandewalle, Chamari, & Driss, 2018).

Trained individuals exhibit lower RER during steady-state exercise when compared to untrained individuals (Bergman & Brooks, 1999; Jeukendrup, Mensink, Saris, & Wagenmakers, 1997). More recently, Ramos-Jimenez et al. (2008) confirmed these results but used a sedentary cohort with high cardiorespiratory fitness ($51.7\ ml \cdot kg^{-1} \cdot min^{-1}\ VO_{2max}$). In the current study, we found no differences in RER between the groups. We speculate this is due to the healthy cohorts compared; specifically, neither cohort was diagnosed with any cardiometabolic disease. Additionally, there is a large interindividual variability in resting and exercise RER in healthy individuals with muscle glycogen content, training volume, free fatty acids, blood lactate, and dietary fat intake contributing to the RER response (Goedecke, et al., 2000). These data could indicate that the metabolic stimulus provided was similar between the groups.

Individuals with greater cardiorespiratory fitness oxidized greater amounts of lipids during high-intensity exercise, representing a hallmark adaptation of exercise training (Aslankeser & Balcı, 2017; Hetlelid, et al., 2015) potentially due to increased skeletal muscle mass and function (Goodpaster & Sparks, 2017). Herein, we found similar results to previously reported raw and body weight-controlled data. These differences were negated when data were controlled for lean mass, supporting the understanding that skeletal muscle mass influ-

ences metabolic responses to exercise. Exercise training regimens that improve skeletal muscle mass could be a powerful solution to increasing the ability to respond to a metabolic demand, thus potentially reducing levels of adiposity through a long-term participation.

Mitochondria dysfunction is a contributing factor in the progression of cardiovascular and metabolic disease (Chistiakov, Shkurat, Melnichenko, Grechko, & Orekhov, 2018) and is strongly associated with a higher Framingham Risk Score independent of age, body composition, and physical activity (Zampino, Spencer, Fishbein, Simonsick, & Ferrucci, 2021). CW-NIRS has been used as a non-invasive approach to measuring muscle mitochondrial oxidative capacity and agrees with similar measures of phosphocreatine recovery rates (Ryan, Southern, Reynolds, & McCully, 2013) and mitochondrial respiration rates of biopsy (Ryan, Brophy, Lin, Hickner, & Neufer, 2014). This is the first study to incorporate this technique to examine mitochondrial function differences in individuals who participate in CrossFit. We found that individuals who participate in CF had greater mVO_2, absolute, normalized for ATT, and normalized for the right leg lean mass compared to the sedentary counterparts. Thus, participation in CF could aid in preventing metabolic disease through improved mitochondrial oxidative capacity via increased skeletal muscle mitochondrial function.

High-intensity interval training improves FMD to a greater extent when compared to moderate-intensity continuous training (Sawyer, et al., 2016), potentially improving endothelial dysfunction. To our knowledge, our study is the first to report vascular function in individuals participating in CrossFit. While there were no differences in FMD% or a "corrected" FMD%, the CF participants had larger baseline and peak vessel diameters. Measures of endothelial function may not be enhanced in an athletic population when compared to a young, healthy controls due to the difficulty of improving artery function in an already healthy population and the impact of compensatory structural remodeling, in that the arterial diameter and wall thickness both affect function (Green, Spence, Rowley, Thijssen, & Naylor, 2012). Still, structural vascular adaptations occur with exercise training related to body composition and type of training (Naylor, Spence, Donker, Thijssen, & Green, 2021). Additionally, there is an age-dependent decrease in artery function (Thijssen, et al., 2008). The current study suggests that adaptations of vessel diameter could protect future age-related decrements in vascular function.

Although FMD provides insight into large conduit arterial function, it does not directly assess microcirculatory hyperemia (McLay, et al., 2016). The reperfusion slope of O_2Hb is correlated with FMD% indicating CW-NIRS-derived saturation levels can be used as an additional measure of vascular function (McLay, et al., 2016). In the present study, the individuals participating in CF were characterized by having higher TSI% and greater desaturation and reperfusion slopes when compared to the sedentary counterparts. The individuals participating in CF exercise experience greater microvascular function in response to a vascular occlusion test with the ability to recover faster than the sedentary individuals. These data, coupled with large artery characteristics, demonstrate the efficacy of CF participation to improve vascular structure and function compared to sedentary counterparts. Participation in CF could provide a protective effect to age-related vascular detriments.

In summary, we found that the individuals participating in CF for at least one year had higher cardiovascular fitness, better body composition, lower ratings of perceived exertion and lower heart rate during HIIE, higher fat and carbohydrate oxidation during HIIE when expressed as absolute and body weight relative values but not when expressed as lean mass relative values, greater mitochondria oxidative capacity independent of the amount of lean mass, greater baseline arterial diameter, and faster reperfusion compared to the sedentary cohort. Overall, these data support the effectiveness of CF to improve fitness, weight status, and metabolic, mitochondrial, and vascular function. Chronic participation in the CF exercise seems to induce physiological adaptations possibly leading to a reduced risk of developing cardiovascular and metabolic disease later in life.

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Data availability statement

The datasets generated during and/or analyzed during the current work are not publicly available but are available from the corresponding author on reasonable request.

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