Scandinavian Journal of Medicine & Science in Sports

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Cardiorespiratory Fitness Improvements Following Low-Frequency Training Are Not Inferior to High-Frequency Training Matched for Intensity and Volume

Thomas R. Tripp[1] | Rachel S. Ghitter[1] | Hilkka Kontro[1] | Sarah J. Hargrave[1] | Martin J. Gibala[2] | S. Jalal Aboodarda[1] | Martin J. MacInnis[1]

[1]Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada | [2]Department of Kinesiology, McMaster University, Hamilton, Ontario, Canada

Correspondence: Martin J. MacInnis (martin.macinnis@ucalgary.ca)

Received: 15 October 2024 | **Revised:** 19 December 2024 | **Accepted:** 16 January 2025

Funding: This research was funded by internal funding from the University of Calgary Faculty of Kinesiology and a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to MJM (RGPIN-2018-06424). TRT was funded by an NSERC CGS-D scholarship and a University of Calgary Eyes High Doctoral Recruitment scholarship.

Keywords: exercise performance | exercise thresholds | fatigue | hemoglobin mass | muscle oxidative capacity | neuromuscular function | $\dot{V}O_2$max | Weekend Warrior

ABSTRACT

Epidemiological evidence suggests low-frequency physical activity provides health benefits, but the physiological impacts of weekly training frequency are understudied. We investigated whether "Weekend Warrior" (WW) training was inferior to traditional, high-frequency (HF) training for improving maximal oxygen uptake ($\dot{V}O_2$max). The secondary aim was to assess integrative physiological adaptations to each protocol. Twenty-eight sedentary-to-recreationally-active adults aged 18–45 years (14 males and 14 females) were randomized to perform 8-weeks of HF or WW training on a cycle ergometer (either four or two sessions weekly, respectively), consisting of continuous and interval exercise, with intensity and volume matched between groups. WW training was not inferior to HF training for improving $\dot{V}O_2$max (mean ± standard deviation; WW: 43.5 ± 6.5 vs. 47.8 ± 6.4 mL/kg/min; HF: 42.3 ± 6.2 vs. 47.3 ± 6.7; main effect of training, $p < 0.001$). Severe domain cycling time-to-task-failure also increased in both groups (WW: 3.7 ± 1.6 vs. 8.6 ± 3.2 min; HF: 3.5 ± 0.9 vs. 7.7 ± 2.8; main effect of training: $p < 0.001$). Frequency did not affect improvements in hemoglobin mass (WW: 771 ± 203 vs. 790 ± 189 g; HF: 754 ± 185 vs. 765 ± 202; main effect of training: $p = 0.043$) or skeletal muscle oxidative capacity (WW: 0.034 ± 0.008 vs. 0.045 ± 0.015 s^{-1}; HF: 0.036 ± 0.011 vs. 0.041 ± 0.010; main effect of training: $p = 0.002$), nor did it influence improvements in cardiorespiratory, substrate oxidation, voluntary muscle contractile, and perceptual responses to submaximal exercise (interaction effect: $p > 0.05$ for all outcomes). Eight weeks of training improved $\dot{V}O_2$max and a wide range of physiological outcomes with no difference between training frequencies, suggesting that the distribution of weekly exercise volume has a limited effect during short-term training.

Trial Registration: This trial was registered at ClinicalTrials.gov identifier: NCT05908578

1 | Introduction

Physiological responses to exercise training depend on the imposed exercise stress [1]. The perturbation elicited by a single exercise session is primarily determined by the duration and intensity of the exercise performed, with repeated sessions (i.e., training) leading to physiological adaptations [2]. The influence of intensity on responses to acute exercise and chronic training is relatively well described but remains debated [3, 4]. The impact of weekly training frequency has received less attention by comparison. While more frequent training sessions can augment adaptations to endurance training [5, 6], these studies are unable to disentangle the direct effects of frequency (the number of training sessions per week) from those attributable to the overall greater weekly exercise volume (the product of exercise duration and intensity). Matching training volume between protocols while comparing different frequencies of exercise requires a simultaneous adjustment in session duration. Increasing duration augments the magnitude of the exercise stress for a given intensity of exercise [7, 8], but it is unclear whether potentially greater disruptions to homeostasis sufficiently compensate for less frequent disruptions. Whether training frequency is a critical determinant of adaptations to aerobic training for a fixed volume of exercise and program duration is unclear.

Epidemiological studies provide some insight into varied physical activity distributions and their impact on overall health [9–11], but do not directly inform our understanding of cardiorespiratory fitness. Many of these studies suggest that exercise accumulated on just one or two days per week–commonly referred to as a "Weekend Warrior" (WW) pattern [9]–lowers the risk of all-cause mortality [10, 11] and adverse cardiovascular health events compared to sedentary behavior [12] but not to the same extent as more distributed activity patterns [10, 11]. These studies rely on physical activity surveys or accelerometry to assess training habits, often at a single time point, rather than prescribed training protocols, and focus on long-term clinical endpoints (e.g., mortality) over directly measured cardiorespiratory fitness. While potentially informative at a population level, these studies do not provide a physiological understanding of the effects stemming from different exercise frequencies.

Stronger evidence on the impact of training frequency on cardiorespiratory fitness comes from experimental trials, but few studies have addressed this question. The limited studies have reported low-frequency training to be inferior [13], similar [14], or superior [15] to high-frequency training for improving maximal oxygen uptake ($\dot{V}O_2$max). These studies recruited various populations (e.g., women with obesity [14], military members [13]), prescribed exercise at relatively low intensities (i.e., moderate exercise) [14, 15], and included limited measures to understand the mechanisms through which training frequency could differentially influence a broader range of cardiorespiratory fitness [13, 15]. Factors relating to oxygen delivery (e.g., hemoglobin mass) [16], oxygen consumption (e.g., muscle oxidative capacity) [17], substrate oxidation (e.g., glycogen sparing) [18], and neuromuscular function (e.g., voluntary and evoked muscle force output) [19, 20], all improve with training, but whether these adaptations are influenced by exercise frequency has not been investigated. Cardiorespiratory fitness is a multifaceted concept that extends well beyond simply the maximal rate of oxygen uptake, so studies that include additional basic and integrative physiological parameters are critical to understand the influence of exercise frequency.

The present study aimed to assess whether low-frequency training (WW) was inferior to high-frequency (HF) training for improving cardiorespiratory fitness. Non-inferiority testing sets a threshold (based on functional significance and feasibility), below which differences between protocols are deemed "not substantially worse" than the standard treatment, given other benefits of the novel treatment [21]. The primary objective was to assess this question using maximal oxygen uptake ($\dot{V}O_2$max) at a non-inferiority margin of 3.5 mL/kg/min (1 metabolic equivalent of task; MET). This margin represents the value that the lower bound of the 95% confidence interval must not reach to conclude non-inferiority. We hypothesized that WW training would not be inferior to HF training for improving $\dot{V}O_2$max when intensity and weekly exercise volume are matched. This hypothesis was based on epidemiological evidence that Weekend Warrior physical activity patterns can provide similar health benefits to more evenly distributed physical activity [10], as well as the equivocal findings of experimental studies [13–15]. The secondary objective was to contextualize findings from the first objective by examining underlying changes in hemoglobin mass, skeletal muscle oxidative capacity, fatigue resistance, and endurance exercise performance, which, in line with $\dot{V}O_2$max, we hypothesized would improve similarly across training frequencies.

2 | Materials and Methods

2.1 | Participants and Ethical Approval

We recruited 30 apparently healthy males ($n = 15$) and females ($n = 15$) between the ages of 18–45 to take part in the study. To be eligible for participation, individuals could not be pregnant, taking medications known to affect cardiovascular or metabolic responses to exercise, or endurance trained (training more than 4× per week or for > 3 h per week, including high-intensity or sprint interval training) and could not have donated blood in the previous 90 days, smoked or consumed > 21 units of alcohol per week within the past year. Health status was determined using the exclusion criteria and physical activity screening tool, described below. These criteria were selected to minimize confounding effects on training responses [6], while also increasing the generalizability of the study findings by recruiting a wider range of fitness levels than those who were completely sedentary.

Recruitment was based on a calculated required sample size of 26 for the non-inferiority comparison of improvement in relative $\dot{V}O_2$max ($\alpha = 0.05$, power = 0.9, a standard deviation for $\dot{V}O_2$max improvement of 3 mL/kg/min [22], non-inferiority margin of 3.5 mL/kg/min). Prior to commencement of the study, we selected the non-inferiority margin of 3.5 mL/kg/min (1 MET). This margin was selected based on previous research showing the benefits of a 1 MET improvement in cardiorespiratory fitness on all-cause mortality [23] and trial feasibility given the nature of the study [24].

Participants were randomly allocated to one of two 8-week training protocols (HF or WW) using a concealed allotment procedure (in blocks of $n=4$), stratified by sex and baseline $\dot{V}O_2$max. Two participants ($n=1$ male and $n=1$ female) completed baseline testing and 2–3 weeks of training prior to dropping out of the study due to scheduling conflicts. Their results are excluded from all analyzes; therefore, 28 participants ($n=14$ male, $n=14$ female; $n=14$ per group) completed training and all testing procedures. Baseline participant characteristics are shown in Table 1.

Ethical approval was received from the University of Calgary Conjoint Health Research Ethics Board (23-0467). Prior to the commencement of testing, a researcher explained all study procedures to participants, who provided written informed consent prior to beginning the study. Participants also completed a physical activity screening questionnaire (Get Active Questionnaire) to confirm their eligibility before any exercise testing began. The study conformed to the Declaration of Helsinki and was preregistered at ClinicalTrials.gov (ID: NCT05908578).

2.2 | Overview of Experimental Design

An overall study timeline is shown in Figure 1A. Participants completed a comprehensive series of tests before and after 8 weeks of training, with two tests conducted at the midpoint.

2.2.1 | Baseline Testing (0 Week)

The first testing visit included height (stadiometer) and body mass measurements (balance-beam scale), near infrared spectroscopy (NIRS)-derived skeletal muscle oxidative capacity tests, and a step-ramp-step incremental test on a cycle ergometer. The second visit included a dual-energy x-ray absorptiometry (DXA) scan and a hemoglobin mass (Hb_{mass}) measurement. The third visit involved familiarization to the performance trial that included neuromuscular function (NMF) assessments, gas exchange measurement, and a time-to-task failure (TTF) trial. The fourth visit was a repeated Hb_{mass} measurement. The fifth and final visit was the performance trial. All testing took place within 2 weeks, with at least 48 h between visits involving an exercise test and 24 h between non-exercise visits.

2.2.2 | Midpoint Testing (4 Week)

After 4 weeks of training, protocols for body mass, NIRS-derived oxidative capacity, and the step-ramp-step incremental test were repeated. To accommodate the testing session, four intervals of HIIT (one session for the HF group, ½ session for the WW group) were removed from week 4. The testing session was completed 3–5 days following the last training session of week 4 and at least 24 h prior to the first session of week 5.

TABLE 1 | Participant anthropometrics and maximal oxygen uptake.

	HF			WW			ANOVA (effect p [η_p^2]) G × T; G; T
	0 weeks	4 weeks	8 weeks	0 weeks	4 weeks	8 weeks	
Body mass (kg)	70.5 ± 11.9	70.9 ± 12.2	70.5 ± 12.1	72.3 ± 13.9	71.9 ± 14.0	71.3 ± 13.7	**0.049 [0.11];** 0.806 [0.45]; **0.050 [0.11]**
Body fat (%)	23.6 ± 7.5[a]		23.1 ± 7.4[b]	27.1 ± 6.4[a]		26.3 ± 6.3[b]	0.557 [0.01]; 0.215 [0.89]; **0.016 [0.20]**
Fat-free mass (kg)	54.4 ± 10.3		54.5 ± 10.2	52.8 ± 9.9		52.6 ± 9.7	0.356 [0.03]; 0.643 [0.77]; 0.882 [< 0.01]
$\dot{V}O_2$max (L/min)	3.07 ± 0.72[a]	3.19 ± 0.65[b]	3.37 ± 0.72[c]	3.04 ± 0.59[a]	3.18 ± 0.62[b]	3.35 ± 0.67[c]	0.921 [< 0.01]; 0.938 [0.07]; **< 0.001 [0.63]**
$\dot{V}O_2$max (mL/kg BM/min)	43.5 ± 6.4[a]	45.3 ± 6.9[b]	47.8 ± 6.4[c]	42.3 ± 6.2[a]	44.5 ± 6.3[b]	47.3 ± 6.7[c]	0.772 [0.01]; 0.734 [0.07]; **< 0.001 [0.63]**
$\dot{V}O_2$max (mL/kg FFM/min)	56.3 ± 5.4[a]		61.7 ± 6.1[b]	57.8 ± 7.5[a]		63.9 ± 7.5[b]	0.652; 0.450; **< 0.001** 0.01; 0.24; **0.75**
PPO (W)	257 ± 47[a]	278 ± 51[b]	288 ± 52[c]	266 ± 53[a]	280 ± 50[b]	295 ± 53[c]	0.159 [0.07]; 0.759 [0.25]; **< 0.001 [0.84]**
PPO (W/kg BM)	3.6 ± 0.6[a]	3.9 ± 6.9[b]	4.1 ± 0.7[c]	3.8 ± 0.5[a]	4.0 ± 0.5[b]	4.2 ± 0.4[c]	0.070 [0.10]; 0.905 [0.27]; **< 0.001 [0.82]**
PPO (W/kg FFM)	4.9 ± 0.7[a]		5.5 ± 0.8[b]	4.9 ± 0.4[a]		5.4 ± 0.4[b]	0.308 [0.04]; 0.787 [0.09]; **< 0.001 [0.90]**

Note: ANOVA results show the p-values from the interaction effect (group [G] × training [T]), the main effect of group (HF or WW), and the main effect of training. Statistically significant effects ($p < 0.05$) are bolded. Data points with different letters are significantly different from one another (main effect of time). $N=14$ (HF) and $n=14$ (WW) for all variables.

Abbreviations: η_p^2, partial eta squared; BM, body mass; FFM, fat-free mass; HF, high-frequency; PPO, peak power output; $\dot{V}O_2$max, maximal oxygen uptake; WW, Weekend Warrior.

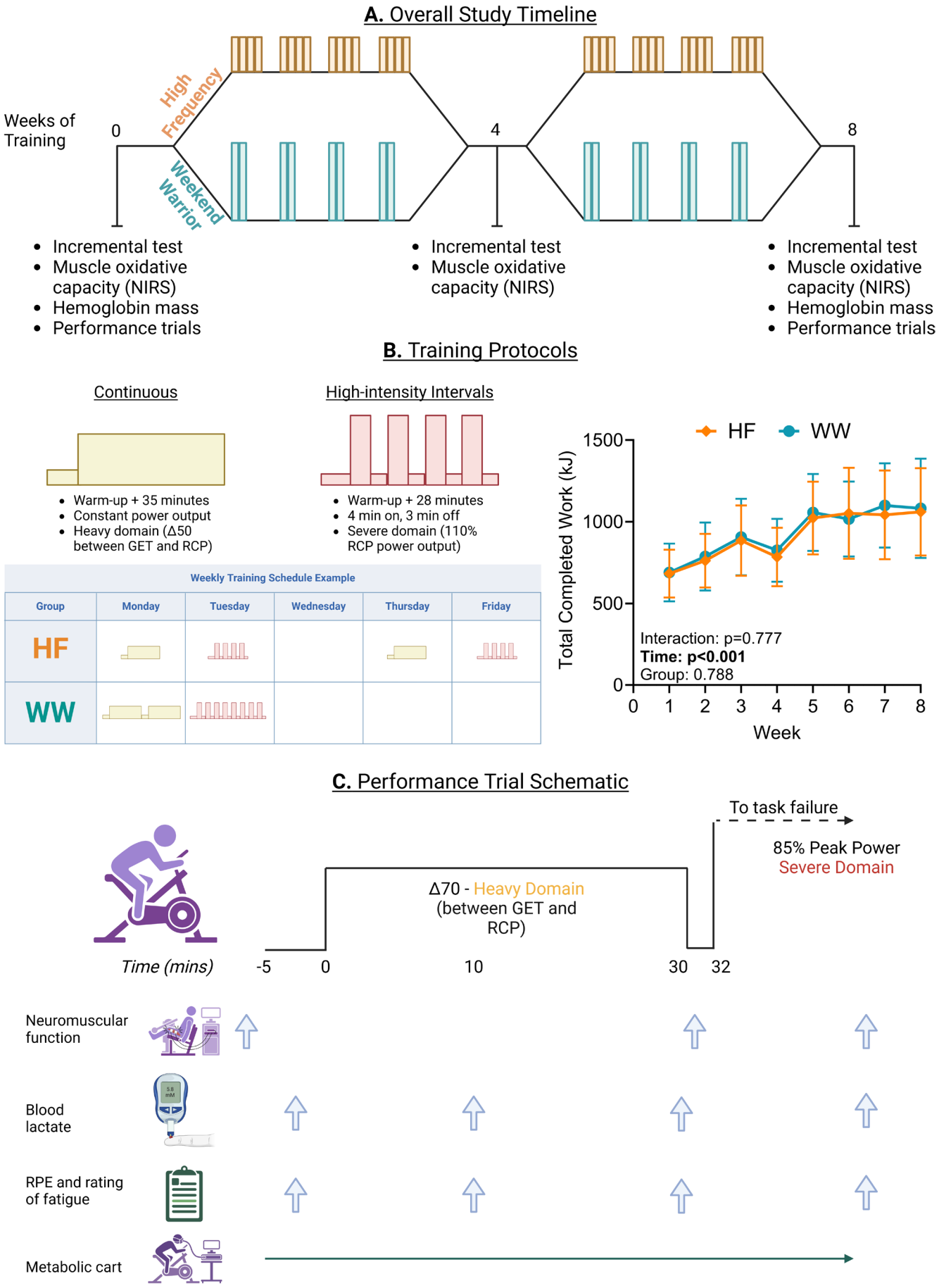

FIGURE 1 | Legend on next page.

FIGURE 1 | A schematic of the experimental overview. The overall study consisted of baseline testing, followed by random allocation to either the high-frequency (HF) or low-frequency (Weekend Warrior, WW) training protocols. Following week 4, participants completed partial testing, and following week 8, participants repeated the full testing protocol (A). Both training protocols consisted of continuous and high-intensity interval training sessions performed on a cycle ergometer. The HF group completed four workouts spread throughout each training week. The WW group completed two workouts on back-to-back days, performing each set of two training units as one prolonged bout on each training day. Total completed work was very similar between groups throughout the protocol (B). During the performance trial (C) participants cycled in the heavy domain for 30 min, at a power output 70% of the way between the gas exchange threshold (GET) and respiratory compensation point (RCP) power outputs. After a 2 min break for neuromuscular function assessment, participants completed a time-to-task failure (TTF) bout in the severe domain (85% of peak power output). Neuromuscular function was measured using the interpolated twitch technique and isometric maximal voluntary contractions of the knee extensors (pre-exercise, post-heavy exercise, post-TTF). Blood lactate, rating of perceived exertion (RPE) and rating of fatigue were measured (during 50 W warm-up, at 10 and 30 min of the heavy trial, and post-TTF). Gas exchange and heart rate were measured throughout the trial.

2.2.3 | Post-Training Testing (8 Week)

All baseline testing visits and measurements were repeated, beginning 5–7 days after the final training session to ensure complete recovery. The only difference relative to baseline testing was that two performance trials were performed: one at the same absolute intensity (i.e., same power output) and one at the same relative intensity (i.e., 70% of the difference between gas exchange threshold [GET] and respiratory compensation point [RCP] power outputs) as at 0 week.

2.3 | Training Interventions

Participants completed training on stationary cycle ergometers (Tacx NEO Smart Trainer 2; Garmin Ltd., Olathe, KS, USA) in a temperature-controlled room (~22°C–24°C) with researcher supervision. All training was controlled using the Tacx Training app (v4.60.0; Garmin Ltd.) on tablets connected to the training ergometers. Two participants trained on a Velotron ergometer (Racermate, Seattle, WA, USA) due to sizing issues with the Tacx trainers. During each training session, participants wore a chest heart rate strap (Polar H10; Polar, Kempele, Finland) connected to a smart watch (Vantage V; Polar). At the end of each session, participants provided a session rating of perceived exertion (sRPE) on the 0–10 scale based on the entire workout, from beginning to end [25].

Training interventions were matched for relative intensity and total weekly exercise duration, resulting in equal weekly training volumes (Figure 1B). The WW group trained twice per week on back-to-back days while the HF group trained four times per week without specific requirements for the number of consecutive days exercising. As a result of the volume matching, training sessions for the WW group were twice the duration of the HF group throughout the study.

As shown in Figure 1B, training consisted of continuous sessions (2× per week for HF, 1× per week for WW) and high-intensity interval training (HIIT) sessions (2× per week for HF, 1× per week for WW). Continuous sessions were performed at 50% of the difference between the power outputs associated with the GET and RCP. HIIT sessions consisted of 4 min at 110% of RCP power output separated by 3 min of recovery at 50 W. Over the first 3 weeks, the session duration gradually increased (continuous: 25, 30, 35 min; HIIT: 3 intervals, 4 intervals; double each duration for WW); thereafter, the session intensity was periodically increased (week 4, week 5, week 6–7) to maintain the training stimulus. Training intervention progression specifics are shown in Table S1.

Four participants ($n = 2$ HF, $n = 2$ WW) completed a portion of the training remotely (no more than 8/32 training units). Remote sessions were performed on a stationary bike with instructions for session timing and intensity (i.e., power outputs, approximate heart rate targets, and/or target sRPE) and occurred after a minimum of 2 weeks of supervised training to ensure participant familiarization with the training protocol.

2.4 | Measurements

2.4.1 | NIRS-Derived Muscle Oxidative Capacity

Vastus lateralis muscle oxidative capacity was measured with a NIRS probe (Portamon; Artinis Medical Systems, Elst, The Netherlands) as previously described [26], using the protocol described by [27]. Following a standardized isometric contraction warm-up, participants performed two maximal voluntary contractions (MVC). Each trial consisted of two 10 s isometric contractions at a standardized intensity of 40% MVC, separated by 10 s rest, followed by 4.5 min of NIRS monitoring during transient arterial occlusions (5 s on, 10 s off). Trials were performed in duplicate.

NIRS signals were analyzed as previously described [26]. Briefly, oxy- and deoxyhemoglobin signals were corrected for changes in total hemoglobin [28] using previously published equations [29]. The rate of change in corrected HHb during a 3 s window of each arterial occlusion was calculated and the resulting values were plotted against time post-contraction and fit with a mono-exponential decay function. The rate constant (k) was calculated for each trial, and the average k was taken as the skeletal muscle oxidative capacity.

2.4.2 | Step-Ramp-Step Incremental Test

A step-ramp-step ramp incremental test protocol [30] was used to determine maximal oxygen uptake ($\dot{V}O_2max$) and peak power output (PPO), as well as the $\dot{V}O_2$ and power output at the GET and RCP. The protocol was completed on an electromagnetically braked cycle ergometer (Velotron; Racermate, Seattle, WA, USA), with power output controlled by an external

computer, independent of cycling cadence. Participants first completed 2 min of cycling at 25 W before a step transition to 75 W (50 W for participants < 55 kg) for 5 min. The power output returned to 50 W (25 W for participants < 55 kg) for 4 min and then the ramp incremental portion of the test began. The external power output smoothly increased by 20 W/min (1 W every 3 s) until volitional exhaustion or failure to maintain a cadence above 60 rpm despite verbal encouragement. During the ramp portion of the protocol, participants were blinded to elapsed time and power output. After 15–20 min of rest, participants completed a 12-min bout at an intensity in the heavy domain.

Participants wore a facemask over their mouth and nose connected to a mixing chamber via a two-way, non-rebreathing valve (Hans Rudolph, Incorporated; Shawnee, KS, USA) during exercise tests. Expired gases were analyzed in 10 s bins using a metabolic cart (CPET Quark; COSMED, Rome, Italy). The turbine flowmeter and gas analyzers were calibrated prior to each test. The highest 30 s average $\dot{V}O_2$ during the ramp incremental test was taken as the $\dot{V}O_2$max, provided a plateau in $\dot{V}O_2$ was observed or end-test heart rate was within 10 bpm of age-predicted maximal heart rate and end-test RER was > 1.10 [31]. Endurance exercise thresholds (GET and RCP) were determined using the exphyslab.com website following published guidelines [32]. The initial moderate step was used to correct the $\dot{V}O_2$-power relationship for the mean response time, and the post-ramp heavy step was used to correct the $\dot{V}O_2$-power relationship for the $\dot{V}O_2$ slow component in the heavy domain. This approach allowed us to estimate the power outputs eliciting the GET and RCP [30, 33]. Previously, we and others have shown that this method provides a reasonable estimate of the maximum metabolic steady state [34, 35].

2.4.3 | Dual-Energy X-Ray Absorptiometry

A whole-body DXA scan was performed with a Lunar iDXA device (General Electric Healthcare, Chicago, IL, USA) to determine fat-free mass and body fat percentage. Participants arrived for the DXA scan following an overnight fast (~10 h).

2.4.4 | Carbon Monoxide Rebreathe: Hemoglobin Mass and Vascular Volumes

Total Hb_{mass} and vascular volumes were measured using the modified carbon monoxide (CO) rebreathe technique as previously described [36, 37]. Briefly, we collected a resting venous blood sample from an antecubital vein and a resting measure of exhaled CO concentration. Participants then rebreathed a small dose of CO (~1 mL/kg) in a closed spirometry system (Blood tec GmbH, Bayreuth, Germany) with ~3 L of O_2 for 2 min. After disconnecting the participant from the spirometer, we collected a breath sample (2 min post) and a blood sample (5 min post).

Hb_{mass} was calculated from the change in carboxyhemoglobin, which was measured in duplicate for each sample using a blood gas analyzer (ABL 80-FLEX; Radiometer, Copenhagen, Denmark). Blood volume, red blood cell volume (RBCV), and plasma volume were calculated from Hb_{mass} using standard equations [38] and quadruplicate measures of hemoglobin concentration and hematocrit (twice from each blood sample). To limit variability, we performed the CO rebreathe twice at each measurement point on separate days and repeated the test a third time if Hb_{mass} was > 5% different between the first two measures. In those instances ($n = 10$ at 0 week, $n = 5$ at 8 week), the final value was the average of the two closest values. The coefficients of variation for the repeated measures that were averaged to calculate Hb_{mass} at 0 week and 8 week were 3.1% and 1.7%, respectively.

2.4.5 | Performance Trial

The performance trial consisted of 5 min baseline cycling at 50 W, 30 min of cycling in the heavy domain (Δ70 GET-RCP; 143 ± 33 W), 2 min rest for an NMF assessment, and a severe domain time-to-task-failure (TTF) bout (85% PPO; 224 ± 43 W), with cycling performed on an electromagnetically braked cycle ergometer (Velotron; Racermate). At baseline, participants performed a familiarization trial to become accustomed to the NMF assessments and the prescribed cycling intensities. If participants were unable to complete the 30 min portion ($n = 3$), the power output was reduced by ~5% for the actual baseline trial. At the post-training timepoint, participants completed performance trials at the same absolute (i.e., same power output) and relative (i.e., Δ70 GET-RCP post-training [174 ± 37 W] and 85% post-training PPO [246 ± 49 W]) intensities as at baseline, on separate days in a random order.

2.4.5.1 | Cardiorespiratory and Metabolic Measurements. As described above, cardiopulmonary data were measured continuously during the performance trial to determine HR, $\dot{V}O_2$, $\dot{V}CO_2$, respiratory exchange rate (RER), minute ventilation ($\dot{V}_E$), respiratory frequency (f_R), and tidal volume (V_T). Absolute rates of carbohydrate and fat oxidation were calculated using previously published equations [39]. Total energy expenditure was calculated by multiplying the absolute rates of substrate oxidation by the energy density of each substrate (4.07 kcal/g carbohydrate, 9.75 kcal/g lipid) [39]. For analysis, values were averaged from 10 to 30 min of the heavy bout.

Blood lactate concentration ([BLa]) was measured from a finger capillary sample using a portable blood lactate analyzer (Lactate Plus, Nova Biomedical, Cheshire, UK) during baseline cycling, and at 10 and 30 min of heavy cycling.

2.4.5.2 | Neuromuscular Function (NMF) Assessment. As previously described, participants moved from the cycle ergometer to a custom-built isometric dynamometer positioned directly above the fly wheel for each NMF assessment [36]. There were two NMF assessments performed prior to the baseline cycling, one immediately after the 30 min (~30 s delay) and one immediately after the TTF.

Each NMF assessment consisted of a brief isometric MVC (~5 s) of the knee extensors and four electrical stimuli applied to the femoral nerve. A 100 Hz doublet (Db100) was applied during the force plateau of the MVC to elicit a superimposed twitch (SIT). A second Db100 was delivered ~2 s following the MVC, followed by a 10 Hz doublet (Db10) and a single resting potentiated twitch

(Pt) separated by 3 s each [40]. The stimulation intensity was determined in the resting state (prior to the isometric warm-up) as 130% of the optimal current, defined as a plateau in force and compound muscle action potential (M-wave) while current was gradually increased.

The change in MVC force was used to measure overall NMF. Voluntary activation (VA) was calculated using the SIT force, MVC force, and Db100 force [41], and changes in VA were taken as the central component of NMF. Changes in the Db10:Db100 force ratio (indicative of low-frequency fatigue) and the Pt force were taken as peripheral components of NMF.

2.4.5.3 | Perceptual Responses. During baseline cycling, at 10 and 30 min of heavy cycling, and immediately following the TTF, participants were asked to rate their perceived effort ("How hard are you working?") and their overall sense of fatigue ("How fatigued do you feel?") using a Borg 6–20 scale [42] and a 0–10 rating-of-general-fatigue scale [43], respectively. The scales were explained to participants prior to the familiarization trial.

2.5 | Experimental Controls

Participants were instructed to record their diet for 24 h prior to the experimental performance trial at baseline. Prior to the post-training performance trials, participants were provided with their diet records and instructed to replicate the 24-h intake as best as possible. Participants were instructed to avoid exhaustive exercise, alcohol, and caffeine intake for 24, 12, and 12 h, respectively, prior to the ramp incremental test and performance trials, and to arrive at the lab in a fed state (last meal > 2 h prior to measurement). To avoid potential diurnal effects, time of day was standardized within ±1 h for all testing measurements.

Throughout the training protocol, participants in both groups were reminded to maintain their habitual physical activity, not take up any additional exercise training programs, and maintain their typical diet. Participants were provided with a recording sheet to document their non-protocol physical activity as well as their weekly step count. According to participant records, some individuals engaged in other physical activities on 1–3 days per week, consistent with their habitual activity patterns (e.g., climbing, coaching sports practices, weightlifting) throughout the study in each group.

2.6 | Statistical Analysis

All data were assessed for normality and equality of variance (two groups) or sphericity (> 2 groups) prior to analysis. Where these assumptions were violated (i.e., RPE and fatigue ratings), appropriate non-parametric statistical tests (Mann–Whitney *U*) were used.

The primary statistical analysis for this trial was the non-inferiority test for the improvement in relative $\dot{V}O_2$max. The non-inferiority test was conducted by constructing a 95% confidence interval for the mean difference in relative $\dot{V}O_2$max improvement between HF and WW and examining whether the upper bound crossed the non-inferiority margin. Improvements in absolute and relative (body mass and fat-free mass) $\dot{V}O_2$max were also analyzed using two-way, repeated measures ANOVAs, as described below.

The secondary analyzes for this trial (exercise thresholds, vascular volumes, muscle oxidative capacity, performance trial outcomes aside from perceptual scales) were conducted mainly using two-way, repeated measures ANOVAs with training (i.e., 0 vs. 4 vs. 8 weeks) as the within-subjects factor and group allocation (i.e., HF vs. WW) as the between-subjects factor. When the ANOVA detected a significant interaction effect, pairwise Sidak's multiple comparison post hoc tests were applied to identify the means that were significantly different. When the ANOVA detected a significant main effect, Sidak's multiple comparison post hoc tests were applied as appropriate (e.g., comparing 0 vs. 8 weeks). For all variables reported in-text, marginal means–WW and HF values collapsed into one group–are reported in Supporting Information Table 2 when only a significant effect of training was detected. In instances with uneven data (e.g., a participant missing a value at one time point), ANOVAs were replaced with linear mixed models, using the same analysis flow (interaction effects, main effects, post hoc tests [Sidak's post hoc tests], etc.), to avoid entire removal of participants from the analysis.

We performed a tertiary analysis to examine potential sex differences in training responses. For this analysis, participants were collapsed across training interventions and grouped by sex. These analyzes were performed with two-way, repeated measures ANOVAs with training (i.e., 0 vs. 4 vs. 8 weeks) as the within-subjects factor and sex (i.e., male vs. female) as the between-subjects factor. The same analysis flow as described above was used for adjusting for uneven data, examining interaction or main effects, and applying post hoc testing. Statistical analyzes were performed in GraphPad Prism (v10.3; Dotmatics, Boston, MA, USA). The level of significance was set at $\alpha = 0.05$.

3 | Results

3.1 | Training Intervention

All participants completed a minimum of 30 of the 32 prescribed training units (30 for $n = 1$ HF and $n = 2$ WW; 31 for $n = 1$ HF; 32 for remaining participants), with average adherence of 99.2% session completion. Training load (completed external work per week) is shown in Figure 1B, and the internal load per session (sRPE · session duration [min]) and the median sRPE for each week of training are presented in Figure S1.

3.2 | Maximal Oxygen Uptake

The $\dot{V}O_2$max per kg body mass increased throughout the training intervention in both groups with no significant interaction (Table 1, Figure 2A). The difference in the improvement after 8 weeks was not significantly different between training frequencies (Figure 2C; $p = 0.43$), and the bounds of the 95% confidence interval (−1.5 to 2.9 mL/kg/min) did not include the a

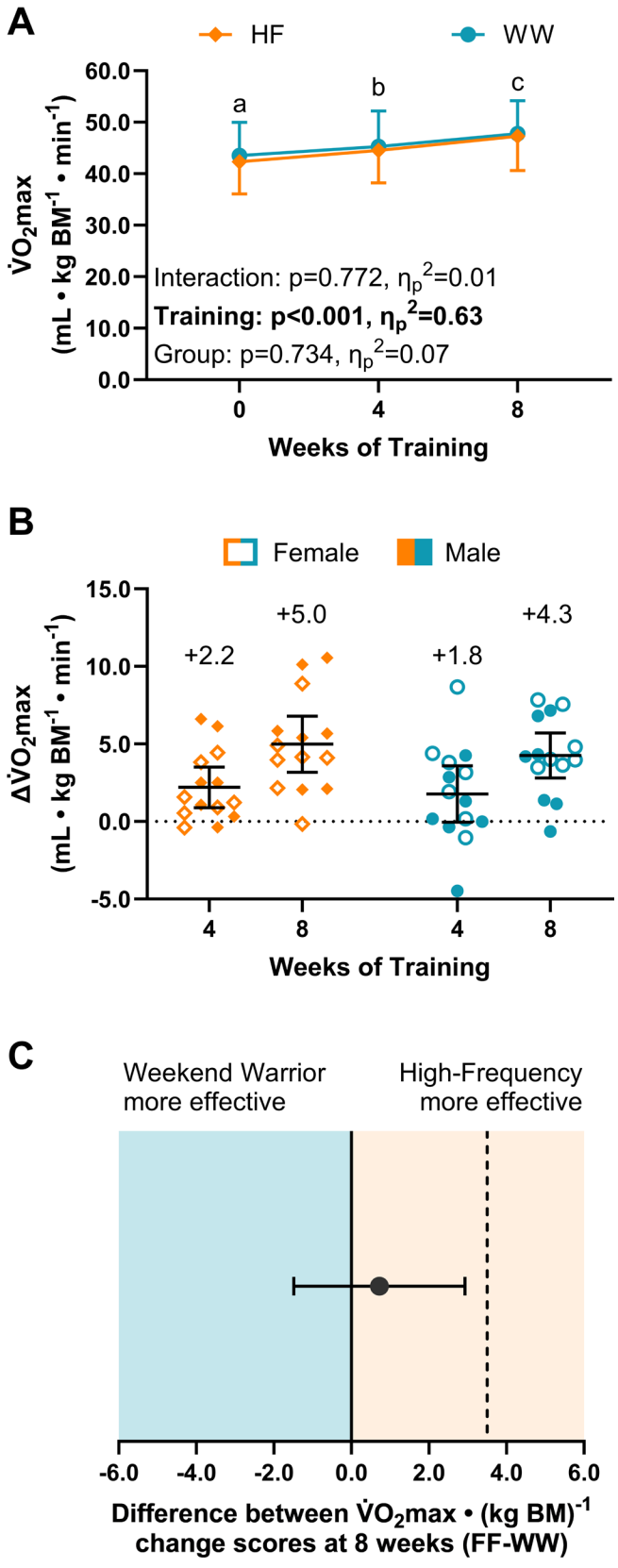

FIGURE 2 | Legend on next page.

priori determined non-inferiority margin of +3.5 mL/kg/min (Figure 2C). When expressed relative to FFM, $\dot{V}O_2$max also increased after 8 weeks without significant differences between groups (Table 1).

FIGURE 2 | Relative $\dot{V}O_2$max (per total body mass) in response to high-frequency (HF) and Weekend Warrior (WW) exercise training. The mean (± standard deviation) $\dot{V}O_2$max (A) across the training period and the individual change scores for $\dot{V}O_2$max (mean and 95% confidence interval) from baseline to the respective time point (B) are shown for each group, with the mean change at week 8 presented numerically. An estimation plot, showing the point estimate and 95% confidence interval, is presented for the difference in training-induced $\dot{V}O_2$max change scores between the two groups, with the dashed line representing the a priori specified non-inferiority margin of 3.5 mL/kg/min (C). Data in Panel A were analyzed with a two-way ANOVA, with the *p*-value for each effect shown. Significant effects (bolded) were followed up with post hoc tests as appropriate. Time points with different letters indicate statistically significant differences for the main effect of training ($p < 0.05$). $n = 14$ (HF) and $n = 14$ (WW) for all panels.

3.3 | Anthropometrics

Body mass was lower following the WW training but was unchanged following the HF training (Table 1), though the post hoc testing did not identify any specific points that were significantly different from one another. There was also a significant decrease in body fat percentage irrespective of group (Table 1). Aside from $\dot{V}O_2$max (due to the non-inferiority test being based on the values relative to body mass), relevant variables are reported expressed relative to fat-free mass to isolate effects independent of body composition changes throughout the intervention.

3.4 | Incremental Exercise Test: Exercise Thresholds and Power Outputs

The $\dot{V}O_2$ at the RCP (Figure 3A,B) and the $\dot{V}O_2$ at the GET (Figure 3C,D) both increased with training, with no significant interaction between training and frequency. The power outputs associated with the RCP (Figure S2A,B) and GET (Figure S2C,D) also both increased with training, with no significant interaction between training and frequency. Similarly, there was a significant main effect of training and no significant interaction for any expression of PPO (Table 1).

3.5 | Hemoglobin Mass and Vascular Volumes

Absolute and relative hematological parameters are presented in Table 2 and Figure 3E,F (relative Hb_{mass}). Expressed in absolute units or relative to FFM, Hb_{mass}, blood volume, RBCV, and plasma volume all increased with training without a significant interaction between training and frequency. Hemoglobin concentration and hematocrit were not different between groups and were unchanged following training (Table 2).

3.6 | Muscle Oxidative Capacity

NIRS-derived skeletal muscle oxidative capacity significantly increased following 4 weeks of training (Figure 3G,H) but did

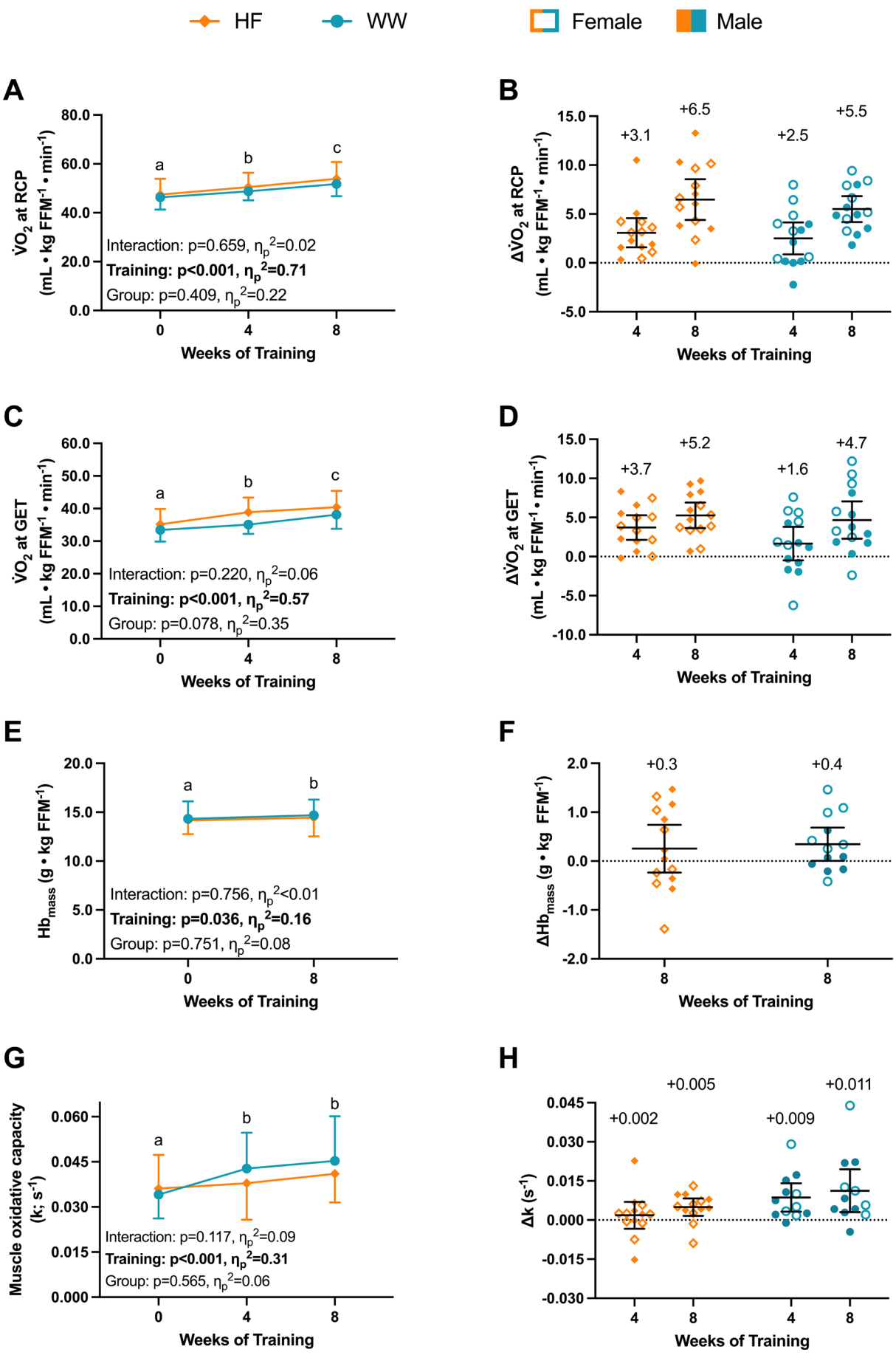

FIGURE 3 | Legend on next page.

FIGURE 3 | Respiratory compensation point (RCP), gas exchange threshold (GET), hemoglobin mass (Hb_{mass}) and near-infrared spectroscopy (NIRS)-derived muscle oxidative capacity (*k*) in response to high-frequency (HF) and Weekend Warrior (WW) exercise training. The group mean (± standard deviation) across the training period (A, C, E, G) and the individual change scores (mean and 95% confidence interval) from baseline to the respective time point (B, D, F, H) are shown for RCP (A, B), GET (C, D), Hb_{mass} (E, F), and muscle oxidative capacity (G, H). Data were analyzed with two-way ANOVAs, with the *p*-value for each effect shown. Significant effects (bolded) were followed up with post hoc tests as appropriate. Time points with different letters indicate statistically significant differences for the main effect of training ($p < 0.05$). FFM, fat-free mass. $n = 14$ (HF) and $n = 14$ (WW) for RCP and GET. $n = 14$ (HF) and $n = 13$ (WW) for Hb_{mass}. $n = 13$ (HF) and $n = 12$ (WW) for *k*.

TABLE 2 | Effects of Training and Frequency on Vascular Volumes and Hematological Outcomes.

	HF			WW			Linear model (effect p [η_p^2])
Group	**0 week**	**8 weeks**	**Δ**	**0 week**	**8 weeks**	**Δ**	**G × T; G; T**
Hb_{mass} (g)	754 ± 185[a]	765 ± 202[b]	11 ± 42	771 ± 203[a]	790 ± 189[b]	18 ± 27	0.596 [0.01]; 0.780 [0.27]; **0.043 [0.15]**
Blood volume (L)	5.36 ± 1.02[a]	5.52 ± 1.13[b]	0.16 ± 0.29	5.55 ± 1.07[a]	5.74 ± 1.04[b]	0.19 ± 0.25	0.759 [< 0.01]; 0.622 [0.38]; **0.002 [0.31]** **0.31**
RBCV (L)	2.31 ± 0.57[a]	2.34 ± 0.62[b]	0.03 ± 0.13	2.37 ± 0.62[a]	2.42 ± 0.58[b]	0.05 ± 0.08	0.622 [0.01]; 0.778 [0.28]; **0.048 [0.15]**
Plasma volume (L)	3.05 ± 0.48[a]	3.18 ± 0.52[b]	0.13 ± 0.19	3.19 ± 0.47[a]	3.32 ± 0.51[b]	0.14 ± 0.22	0.905 [< 0.01]; 0.465 [0.33]; **0.003 [0.31]**
Relative blood volume (mL · kg FFM^{-1})	102.0 ± 9.2[a]	105.2 ± 10.4[b]	3.2 ± 5.1	104.2 ± 7.9[a]	107.5 ± 7.5[b]	3.4 ± 4.4	0.926 [< 0.01]; 0.499 [0.19]; **0.002 [0.33]**
Relative RBCV (mL · kg FFM^{-1})	43.5 ± 4.3[a]	44.2 ± 5.8[b]	0.8 ± 2.6	44.0 ± 5.4[a]	45.0 ± 4.9[b]	1.0 ± 1.7	0.744 [< 0.01]; 0.750 [0.08]; **0.045 [0.15]**
Relative plasma volume (mL · kg FFM^{-1})	58.5 ± 7.1[a]	61.0 ± 6.2[b]	2.4 ± 3.2	60.2 ± 4.8[a]	62.6 ± 5.4[b]	2.3 ± 4.0	0.938 [< 0.01]; 0.455 [0.18]; **< 0.001 [0.31]**
[Hb] (g · dL^{-1})	15.3 ± 1.2	15.0 ± 0.9	−0.3 ± 0.7	15.1 ± 1.2	15.0 ± 1.2	−0.1 ± 0.6	0.344 [0.04]; 0.812 [0.02]; 0.102 [0.10]
Hct (%)	47.0 ± 3.5	45.9 ± 2.8	−1.0 ± 2.0	46.3 ± 3.7	46.0 ± 3.5	−0.4 ± 1.9	0.389 [0.03]; 0.824 [0.02]; 0.076 [0.12]

Note: Data were analyzed with two-way, repeated measures ANOVAs. Results show the interaction (group [G] × training [T]) and main effects of group (G) and training (T). Significant effects ($p < 0.05$) are bolded. Data points with different letters are significantly different from one another (main effect of time). $n = 14$ (HF) and $n = 13$ (WW) for all variables.
Abbreviations: η_p^2, partial eta squared; FFM, fat-free mass; [Hb], hemoglobin concentration; Hct, hematocrit; HF, high-frequency; RBCV, red blood cell volume; WW, Weekend Warrior.

not show further significant increases after 8 weeks of training. There was no significant interaction between training and frequency for the increase in skeletal muscle oxidative capacity.

3.7 | Performance Trial

3.7.1 | Severe Domain Time to Task Failure

The TTF at the same absolute intensity in the severe domain (following 30 min of the same absolute heavy domain exercise) increased similarly following both training frequencies (Figure 4A,B). At the same relative intensity in the severe domain (following 30 min of the same relative heavy domain exercise), the TTF was not different from pre-training in either group (Figure 4A,B; 0 vs. 8 weeks. Rel, $p = 0.927$). For simplicity, the remainder of included analyzes compare only 0 and 8 week performance trials at the same absolute intensity. Additional comparisons between performance trials at the same relative intensity can be found in the supplemental materials (Tables S3 and S4).

3.7.2 | Cardiorespiratory and Metabolic Outcomes During Heavy Exercise

Mean values for each cardiorespiratory and metabolic outcome throughout the heavy exercise portion of the performance trial are shown in Supporting Information Figure 3. Values from

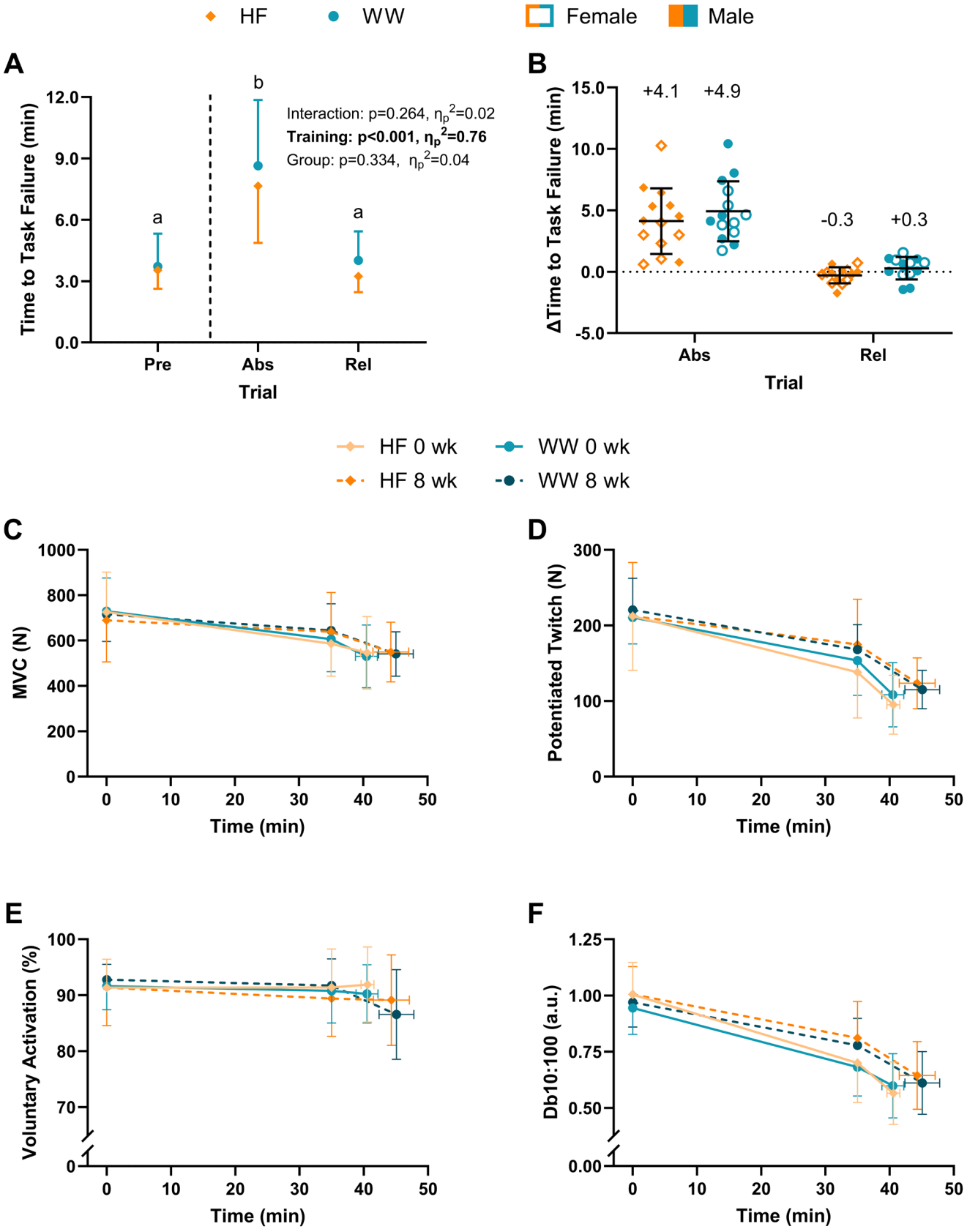

FIGURE 4 | Legend on next page.

the steady state portion of the heavy exercise (10–30 min) were averaged within each participant and are presented in Table 3. Training decreased steady state $\dot{V}O_2$, $\dot{V}CO_2$, RER, and HR without interactions between training and frequency. Steady state $\dot{V}_E$ decreased following training, due to reductions in f_R and V_T, with similar decreases in each group.

FIGURE 4 | Severe intensity time to task failure (TTF) and neuromuscular function in response to high-frequency (HF) and Weekend Warrior (WW) exercise training. The mean (± standard deviation) TTF at baseline and after 8 weeks of training at the same absolute (Abs) and relative (Rel) intensities (A) and the individual change scores (with mean and 95% confidence interval, B) are shown. Data were analyzed with a two-way repeated measures ANOVA. Statistically significant effects (bolded) were followed up with post hoc tests. Trials with different letters were significantly different for one another for the main effect of training. Muscle contractile responses and voluntary contractile properties at baseline (0 min) and in response to 30 min of heavy exercise (35 min) and the TTF trial (final data points, time value varies) are shown at 0 weeks (HF 0 wk and WW 0 wk) and following 8 weeks of training (HF 8 wk and WW 8 wk) for the same absolute intensity trial. Overall voluntary muscle function was assessed using maximal voluntary contraction force (MVC; C). Decrements in peripheral components of muscle contractile function were assessed using potentiated twitch force (D) and the ratio of 10–100 Hz stimulation forces (Db10:100; F) and central adjustments assessed using voluntary activation (E) are plotted separately, with statistical analysis presented in Table 4. $n = 14$ (HF) and $n = 14$ (WW) for TTF. $n = 13$ (HF) and $n = 14$ (WW) for MVC, potentiated twitch, Db10:100, and voluntary activation.

Steady state energy expenditure was decreased following training in both groups, due to decreases in the rate of carbohydrate oxidation and no significant change in fat oxidation rate. The percentage of energy from each substrate changed, as indicated by a significant increase in the percentage of energy from fat in both groups following training. Blood lactate concentration at the end of the 30-min heavy session was decreased similarly following both training frequencies.

3.7.3 | Perceptual Responses to Heavy Exercise

End-trial perceptual responses to heavy exercise are presented in Table 3. Pooling the two groups, RPE at 30 min was significantly lower, representing a change from a rating of "hard (heavy)" to a rating between "light" and "somewhat hard". The rating of general fatigue at 30 min was also significantly lower, corresponding to a change from a rating between "moderately fatigued" and "very fatigued" to a rating between "a little fatigued" and "moderately fatigued."

3.7.4 | Neuromuscular Function Following Heavy Exercise and Task Failure

Absolute values for NMF outcomes across the performance trial before and after training (MVC force, Pt force, Db10:100 ratio, and VA) are displayed in Figure 4C–F. Relative values (changes within a trial from pre-exercise values) are displayed in Table 4 and were statistically analyzed to assess training and frequency effects.

For NMF in response to the 30-min heavy exercise, training attenuated the decline in MVC and Db10:100 ratio. The decline in Pt force was lessened following the HF training only (Table 4). Changes in VA in response to 30-min heavy exercise were not affected by training or different between groups (Table 4).

For NMF at task failure (i.e., changes relative to Pre-exercise in response to 30-min heavy exercise and severe exercise to task failure), training attenuated the decline in MVC regardless of training frequency. The HF group showed an attenuated decline in Pt force at task failure while the WW group showed similar declines at 0 and 8 weeks (Table 4). There were no statistically significant effects of training or frequency on Db10:100 ratio at task failure (Table 4). In both groups, a decline in VA at task failure was noted post-training that was not present pre-training.

3.7.5 | Sex Differences in Training Responses

Data were pooled across training groups and analyzed to explore any sex × training interactions as a tertiary analysis. There was a statistically significant interaction between sex and training for the decline in [Hb] (Male vs. Female: -0.4 ± 0.6 vs. -0.1 ± 0.7 g/dL, $p = 0.029$) and the decline in Hct (Male vs. Female: $-1.3\% \pm 1.8\%$ vs. $-0.2\% \pm 2.0\%$, $p = 0.025$). There was also a statistically significant interaction between sex and training for attenuation of the pre-exercise to post-TTF decline in Pt force (Male vs. Female: $+4.2\% \pm 11.1\%$ vs. $+14.4\% \pm 12.1\%$, $p = 0.031$). There were no statistically significant interactions for the change in any of $\dot{V}O_2$max, exercise thresholds ($\dot{V}O_2$ or power outputs), skeletal muscle oxidative capacity, vascular volumes, TTF, cardiorespiratory or metabolic responses to heavy exercise, or other NMF outcomes ($p > 0.05$ for all, data not shown).

4 | Discussion

The present study compared 8 weeks of training prescribed either two or four times per week while matching for total weekly volume and exercise intensity. As the increase in $\dot{V}O_2$max for WW was not inferior to that of HF, our results suggest that exercise frequency is not an important determinant of improvements in cardiorespiratory fitness for a given volume of weekly exercise during short-term training. We also demonstrated that both HF and WW training decreased the physiological and perceived stress at a given exercise intensity and increased the capacity for high-intensity exercise. Underlying these physiological changes were improvements in factors that contribute to oxygen delivery (Hb_{mass}) and utilization (muscle oxidative capacity) as well as reductions in overall (MVC) and peripheral (Pt and Db10:100 ratio) indices of NMF.

Despite large differences in the duration of a single session—and the internal training load per session (Figure S1C)—and the distribution of sessions throughout the week, improvements in $\dot{V}O_2$max following WW training were not inferior to HF training. The margin of non-inferiority was set at 3.5 mL/kg/min, equivalent to 1 MET. Our data suggest the protocols are much closer than this limit (0.7 mL/kg/min), but powering the study

TABLE 3 | Effects of training and frequency on steady state cardiorespiratory and metabolic responses during 30 min of heavy exercise at the same absolute intensity.

	HF			WW			
Group	**0 weeks**	**8 weeks**	**Δ**	**0 weeks**	**8 weeks**	**Δ**	**ANOVA (effect p [η_p^2]) G × T; G; T**
$\dot{V}O_2$ (L/min)	2.30 ± 0.48[a]	2.14 ± 0.48[b]	−0.16 ± 0.16	2.29 ± 0.43[a]	2.18 ± 0.46[b]	−0.11 ± 0.11	0.310 [0.04]; 0.954 [0.01]; **< 0.001 [0.50]**
$\dot{V}CO_2$ (L/min)	2.08 ± 0.45[a]	1.90 ± 0.46[b]	−0.18 ± 0.17	2.08 ± 0.44[a]	1.94 ± 0.41[b]	−0.14 ± 0.12	0.425 [0.02]; 0.928 [0.01]; **< 0.001 [0.58]**
RER ($\dot{V}O_2/\dot{V}CO_2$)	0.90 ± 0.03[a]	0.88 ± 0.03[b]	−0.02 ± 0.05	0.90 ± 0.04[a]	0.89 ± 0.04[b]	−0.02 ± 0.04	0.900 [< 0.01]; 0.771 [< 0.01]; **0.048 [0.14]**
Heart rate (bpm)	170 ± 14[a]	157 ± 15[b]	−13 ± 14	166 ± 13[a]	153 ± 13[b]	−12 ± 9	0.839 [< 0.01]; 0.382 [0.12]; **< 0.001 [0.57]**
$\dot{V}_E$ (L/min)	75.0 ± 15.1[a]	63.4 ± 15.6[b]	−11.6 ± 5.2	77.3 ± 14.7[a]	65.4 ± 11.6[b]	−12.0 ± 8.3	0.899 [< 0.01]; 0.688 [0.09]; **< 0.001 [0.76]**
f_R (breaths/min)	34.5 ± 5.7[a]	32.1 ± 6.1[b]	−2.4 ± 3.3	36.1 ± 6.9[a]	31.3 ± 3.6[b]	−4.8 ± 6.4	0.239 [0.05]; 0.844 [0.01]; **< 0.001 [0.35]**
V_T (L)	2.22 ± 0.51[a]	2.02 ± 0.51[b]	−0.20 ± 0.20	2.20 ± 0.44[a]	2.13 ± 0.42[b]	−0.08 ± 0.25	0.163 [0.07]; 0.279 [0.05]; **0.004 [0.28]**
Energy expenditure (kJ/min)	47.9 ± 10.0[a]	44.5 ± 10.0[b]	−3.4 ± 3.4	47.6 ± 9.1[a]	45.3 ± 9.5[b]	−2.3 ± 2.3	0.306 [0.04]; 0.952 [0.01]; **< 0.001 [0.52]**
Carbohydrate oxidation (g/min)	1.95 ± 0.52[a]	1.64 ± 0.52[b]	−0.30 ± 0.44	1.97 ± 0.62[a]	1.67 ± 0.54[b]	−0.28 ± 0.45	0.890 [< 0.01]; 0.864 [0.01]; **0.002 [0.32]**
Fat oxidation (g/min)	0.36 ± 0.12	0.40 ± 0.13	0.04 ± 0.19	0.34 ± 0.11	0.41 ± 0.20	0.06 ± 0.20	0.811 [< 0.01]; 0.830 [< 0.01]; 0.171 [0.07]
Energy from fat (%)	31.5 ± 9.9[a]	37.8 ± 9.6[b]	6.3 ± 15.4	30.8 ± 12.4[a]	36.4 ± 14.9[b]	5.6 ± 14.9	0.913 [< 0.01]; 0.772 [< 0.01]; **0.048 [0.15]**
Blood lactate (mM)	6.0 ± 1.5[a]	3.6 ± 1.5[b]	−2.4 ± 1.9	5.7 ± 2.2[a]	3.3 ± 1.3[b]	−2.4 ± 1.3	0.972 [< 0.01]; 0.611 [0.03]; **< 0.001 [0.72]**
RPE (6–20 scale)	15 (13–17)	13 (9–16)	−2 (−8–0)	15 (11–17)	13 (9–15)	−3 (−4–0)	HF: $W = 55$, **$p = 0.002$** WW: $W = 88$, **$p = 0.001$**
Fatigue (0–10 scale)	5.5 (3–7)	4 (3–7)	−1 (−3–1)	6 (4–8)	4.5 (2–7)	−2 (−3–0)	HF: $W = 70$, **$p = 0.004$** WW: $W = 66$, **$p = 0.002$**

Note: All data were analyzed with two-way, repeated measures ANOVAs, except for RPE and Fatigue (Wilcoxon matched-pairs signed rank tests). Results show the interaction (group [G] × training [T]) and main effects of group (G) and training (T). Significant effects ($p < 0.05$) are bolded. RPE and Fatigue were analyzed using non-parametric Wilcoxon matched-pairs signed rank tests to compare pre- to post-training ratings within each training group. $n = 14$ (HF) and $n = 14$ (WW) for all variables.

Abbreviations: η_p^2, partial eta squared; f_R, respiratory frequency; HF, high-frequency; RPE, rating of perceived exertion; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen consumption; V_T, tidal volume; WW, Weekend Warrior.

TABLE 4 | Effects of training and frequency on neuromuscular function following 30 min of heavy exercise and subsequent time-to-task-failure at the same absolute intensity.

Trial timepoints	Group	HF			WW			Linear model (effect p [η_p^2]) G × T; G; T
		0 weeks	8 weeks	Δ	0 weeks	8 weeks	Δ	
Pre-exercise to post-heavy	MVC (%Δ)	−18.6 ± 8.9[a]	−7.4 ± 5.0[b]	11.5 ± 8.0	−16.9 ± 9.7[a]	−9.9 ± 9.6[b]	7.0 ± 9.5	0.202 [0.06]; 0.879 [< 0.01]; **< 0.001 [0.54]**
	Pt (%Δ)	−34.4 ± 15.5	−17.2 ± 7.1	19.3 ± 12.1*	−27.9 ± 13.1	−23.3 ± 11.3	4.7 ± 12.3	**0.010;** 0.934 [< 0.01]; **< 0.001 [0.47]**
	Db10:100 (%Δ)	−30.6 ± 12.1[a]	−19.4 ± 12.1[b]	11.6 ± 12.0	−27.4 ± 13.3[a]	−19.4 ± 11.0[b]	7.9 ± 10.5	0.426 [0.03]; 0.712 [0.01]; **< 0.001 [0.44]**
	VA (%Δ)	0.0 ± 4.6	−2.2 ± 5.0	−1.9 ± 5.2	−0.9 ± 4.8	−1.1 ± 3.7	−0.2 ± 6.0	0.385 [0.03]; 0.986 [< 0.01]; 0.289 [0.04]
Post-heavy to post-TTF	MVC (%Δ)	−7.3 ± 12.3	−13.0 ± 7.6	−6.2 ± 15.5	−12.6 ± 9.4	−14.9 ± 12.8	−2.3 ± 12.1	0.514 [0.02]; 0.262 [0.05]; 0.140 [0.08]
	Pt (%Δ)	−29.6 ± 17.7	−27.6 ± 11.3	1.1 ± 19.9	−29.6 ± 14.3	−30.0 ± 14.4	−0.7 ± 10.6	0.742 [< 0.01]; 0.831 [< 0.01]; 0.850 [< 0.01]
	Db10:100 (%Δ)	−19.1 ± 13.4[a]	−20.3 ± 9.6[b]	−3.6 ± 17.6	−10.7 ± 13.3[a]	−21.5 ± 12.6[b]	−9.2 ± 8.4	0.088 [0.11]; 0.345 [0.03]; **0.029 [0.18]**
	VA (%Δ)	0.8 ± 5.7	−0.3 ± 6.3	−1.3 ± 8.5	−0.6 ± 3.3	−5.5 ± 9.2	−5.0 ± 10.3	0.278 [0.02]; 0.067 [0.06]; 0.091 [0.05]
Pre-exercise to post-TTF	MVC (%Δ)	−25.1 ± 8.5[a]	−19.5 ± 5.9[b]	5.4 ± 11.9	−27.4 ± 12.8[a]	−23.6 ± 12.5[b]	3.6 ± 8.6	0.654 [0.01]; 0.364 [0.03]; **0.027 [0.18]**
	Pt (%Δ)	−55.6 ± 8.0	−40.2 ± 9.0	16.1 ± 10.9*	−48.9 ± 14.9	−46.4 ± 14.1	2.6 ± 10.4	**0.003 [0.31];** 0.999 [< 0.01]; **0.001 [0.46]**
	Db10:100 (%Δ)	−43.6 ± 10.6	−35.8 ± 12.7	7.6 ± 13.3	−35.5 ± 16.5	−36.4 ± 15.2	−0.7 ± 11.3	0.081 [0.12]; 0.421 [0.03]; 0.159 [0.08]
	VA (%Δ)	0.5 ± 5.0[a]	−2.3 ± 8.7[b]	−3.0 ± 9.5	−1.4 ± 4.8[a]	−6.6 ± 8.3[b]	−5.2 ± 8.1	0.482 [0.02]; 0.139 [0.08]; **0.032 [0.19]**

Note: Data were analyzed with linear mixed-effects models. Results show the interaction (group [G] × training [T]) and main effects of group (G) and training (T). Significant effects ($p < 0.05$) are bolded and in instances with a significant interaction effect, an asterisk denotes statistical differences between HF and WW for the Δ value. Data points with different letters are significantly different from each other (main effect of time). $n = 14$ (HF 0 weeks, WW 0 weeks, WW 8 weeks) and $n = 13$ (HF 8 weeks).

Abbreviations: η_p^2, partial eta squared; Db10:100, 10:100 Hz doublet force ratio; HF, high-frequency; MVC, maximal voluntary contraction; Pt, potentiated twitch; TTF, time-to-task-failure; VA, voluntary activation; WW, Weekend Warrior.

with a non-inferiority approach allows for specific estimation of the range of possible differences, a strength compared to traditional superiority designs. Although even smaller differences in $\dot{V}O_2$max are associated with health benefits [44], this margin was selected to balance a functionally important effect size [23] with feasibility to assess this primary outcome alongside other important physiological outcomes. Both training protocols improved $\dot{V}O_2$max by ~10%, in agreement with other protocols of approximately this length and training volume [45] and with two previous studies that interrogated the question of exercise frequency (8 weeks of volume-matched training either 2 or 5 times per week) [14, 15]. The magnitudes of training improvement we report fall between these two previous studies, potentially due to differences in study populations: in a cohort of obese females, predicted $\dot{V}O_2$max increased by 30% (HF) to 40% (WW) [14] and in a cohort of middle-aged males and females $\dot{V}O_2$max increased by 4% (HF, not significantly different to control) to 9% (WW, significantly greater than control) [15]. One study did not see improvements in $\dot{V}O_2$max following low-frequency training, but that may have been due to the training frequency of once per week or the other types of training participants were performing [13]. Our finding that improvements in cardiorespiratory fitness are not inferior for low-frequency training seems to support the general consensus of these studies.

The oxygen uptake and the external power output at each of the measured exercise thresholds were increased by training, with no advantage to either HF or WW training. These findings agree with data suggesting that improvements in lactate threshold power output [14] or the $\dot{V}O_2$ at anaerobic threshold [15] do not depend on training frequency. Improvements in the oxygen uptake at the GET and RCP indicate that greater metabolic rates can be sustained without additional physiological stress, while improvements in the external power output are a combination of increased metabolic power and improved exercise efficiency. Exercise threshold improvements imply that greater aerobic ATP resynthesis rates can be sustained before an increase in blood lactate (in the case of GET) [32, 46] and before oxidative metabolic rates become unsustainable (in the case of RCP) [47, 48]. These shifts in the exercise intensity domain boundaries increase the range of intensities in the moderate domain and extend the upper boundary of sustainable exercise. Decreasing the physiological stress associated with a given power output would be expected to impact exercise performance, which was evident in the performance trial.

One of the major adaptations to endurance training is an increased capacity for high-intensity exercise as measured by the ability to sustain a high work rate. Both the HF and WW groups improved severe domain TTF at the same absolute intensity (Figure 4A,B) and were able to maintain TTF at the same relative intensity (and therefore a higher absolute intensity, Figure 4A,B). As our performance trials consisted of a set-duration, heavy-intensity component prior to the severe-intensity portion, improvements in the TTF reflect training adaptations affecting exercise tolerance in both domains. After training, participants began the severe-intensity bout feeling less fatigued (Table 3) with a lower degree of overall (MVC) NMF impairment (Figure 4, Table 4), adaptations that agree with previous interventional studies [20]. One notable difference between frequencies was HF, but not WW, training attenuating the decline in peripheral components of NMF following 30 min of heavy exercise and severe TTF, including a statistically significant interaction in Pt and a similar numerical pattern in Db10:100 (without a statistically significant interaction). We recently showed that the large majority (~85%) of the total decline in Pt and Db10:100 forces caused by an 8×4 HIIT protocol are present after 4 intervals [7], so the HF training group more frequently reaching this level of muscle function decline could have increased the contractile resilience to future disruptions. Despite this group difference in peripheral NMF, overall force decline (MVC) and TTF improvements were not different between groups after training. Lower physiological stress from the heavy bout in the trained state, indicated by the lower total energy expenditure, HR, and $\dot{V}_E$ (Table 3), could explain attenuated decrements in neuromuscular function and increased TTF. We further observed decreased rates of carbohydrate oxidation and lower accumulation of blood lactate during the heavy exercise, both of which suggest participants likely spared glycogen through the 30 min. Although muscle glycogen was not directly measured in this study, previous research has shown similar glycogen-sparing metabolic adaptations with training [18]. This combination of numerous physiological adaptations appears to have contributed to lessened exercise stress and the increased tolerance for severe domain work evident in both WW and HF groups after training.

The duration of the heavy exercise bout represents a potential limitation for detecting frequency/duration-mediated effects on exercise performance improvements in the present study. The 30-min session resembled exercise that both groups undertook throughout the training period, although at a slightly higher intensity (i.e., Δ70 GET-RCP vs. Δ50 GET-RCP for training). We can speculate that the WW group, in which participants would've grown accustomed to greater durations of cycling, could have demonstrated greater durability [49] and a greater relative improvement in TTF for a longer exercise task (e.g., 60 min) compared to the HF group, who completed a maximum continuous exercise duration of ~35 min during training. Familiarity with a task can increase self-efficacy [50] and thus may have impacted the perceived effort or fatigue rating at the end of a longer heavy bout (e.g., 60 min) [51].

Underlying the exercise performance and integrative physiological improvements with both training frequencies are physiological adaptations affecting the cardiorespiratory and muscle metabolic systems. In the present study, we measured vascular volumes and skeletal muscle oxidative capacity, which each showed training-induced augmentations that did not differ between training frequencies. While there is no previous data on the effect of frequency (independent of volume) to compare against our results, the improvements we observed are aligned with those reported in studies using traditional exercise training protocols. Plasma volume expansion is considered an "early" hematological adaptation to exercise training [52] and was increased by ~5% across the pooled study cohort. Typically, increases in RBCV lag behind increased plasma volume [53], and, as expected, the changes in RBCV and Hb_{mass} were proportionally lower (~2%). The magnitude of these improvements aligns with studies employing similar training durations [16, 54]. Skeletal muscle oxidative capacity measured using

NIRS increased during the first half of the training protocols (Figure 3G). The rapid responsiveness of skeletal muscle oxidative capacity to periods of training [17] and de-training [55] is well-established. Notably, many previous studies used invasive measurements of mitochondrial respiration to quantify these training-induced changes [56], though factors such as capillarization also contribute to the overall functional muscle oxidative capacity [57, 58]. The NIRS-derived method has been used to quantify improvements following training interventions in multiple patient populations. The magnitude of those improvements was much greater (~70%–120%) than we report here (~23% increase after 8 weeks), potentially due to longer interventions or the relatively low state of initial fitness of the patient participants [59, 60]. Previous research suggests underlying variables like mitochondrial volume density continue to increase as exercise volume accumulates during training [56], so the lack of a significant improvement during the final 4 weeks of training may represent a limitation of the NIRS method or a type II statistical error. Regardless, training-induced improvements in factors affecting both oxygen delivery and oxygen consumption were observed following the entirety of the training protocol in both HF and WW groups.

The similarity in adaptations across a range of outcomes from $\dot{V}O_2$max to mitigated NMF impairment suggests that the downstream 'adaptive impulse' was, on average, comparable between the two training protocols with similar interindividual variability. The main differences in the training protocols were the magnitude of exercise stress from a single session (the WW group completed workouts that were twice the duration) and the frequency of signaling pathway stimulation (the HF group performed twice as many workouts). One potential signaling mechanism that might be duration-sensitive is glycogen degradation, which we would expect to be greater at the end of a given WW session compared to a given HF session [61]; however, muscle glycogen was not measured in the present study. Rather, the magnitude of the exercise stress appears to be mostly dictated by exercise intensity [62]. We and others have shown that the size of muscle contractile decrements [7], the concentration of key metabolites [8], and signaling kinase activity [63] display non-linear relationships to exercise duration; however, the exposure time of the physiological system to the peak signal would be increased by increasing session duration. Indeed, in the WW group, signaling pathways were stimulated by training twice per week. Conversely, the HF group had a maximum of three consecutive days without a training stress and nearly all participants performed their training sessions across 5 days, meaning a maximum of two consecutive resting days.

4.1 | Experimental Considerations

That these different exercise protocols can elicit comparable training adaptations raises important questions about the magnitude/frequency of gene expression, protein expression, and regulatory pathway activation in numerous physiological systems, but it was not within the scope of the present study to investigate underlying signaling mechanisms throughout training. We recruited both male and female participants to increase the generalizability of the study findings, but the study was not specifically powered to detect sex specific responses to training or sex × frequency interaction effects. Additionally, we did not collect information pertaining to menstrual cycle history or contraceptive use for participants in the study. We included participants across a range of baseline fitness levels from sedentary to recreationally active to increase the generalizability of our findings. This inclusion criterion means that participants could have been performing physical activities outside of the prescribed training; thus, our results do not reflect conditions where the only physical activity was performed on two (WW) or four (HF) days per week, but rather conditions where the only structured endurance training program was administered on two (WW) or four (HF) days per week.

Although we consider it a strength of the study that training was performed under supervision (except for four participants that remotely completed ≤¼ of the sessions), the ecological validity of the study is lower as a result. Allowing for potential differences in participant adherence as a result of the training interventions would increase the ecological validity of our results, but whether adherence would change the overall interpretation of the effectiveness of WW compared to HF training protocols cannot be determined here. From a practical perspective, the higher average baseline fitness of our study sample means that individuals seeking to begin training with fewer, longer exercise sessions may not be able to tolerate exercise sessions of ~75 min on back-to-back days. As with all exercise prescription, ensuring adequate progression plans (e.g., starting with shorter sessions and gradually increasing session duration) would be most appropriate.

We chose to use an active comparator (i.e., high-frequency training that has been well-established to improve cardiorespiratory fitness) rather than a true control group. This design served to answer the question of whether Weekend Warrior training was inferior to another training protocol, but also means that we cannot fully conclude whether all observed changes were due to the two exercise training protocols rather than other factors. Furthermore, because the study was powered for the non-inferiority test of improvements in $\dot{V}O_2$max, other comparisons may have been underpowered to detect a statistically significant interaction that would suggest an effect of frequency. We have included effect size estimates for ANOVA effects throughout to provide information on the size of interactions that were potentially undetectable in the present study.

5 | Perspective

In a cohort of healthy males and females, low-frequency training was not inferior to high-frequency training for improving $\dot{V}O_2$max during 8 weeks of volume- and intensity-matched endurance exercise. The integrative physiological effects of endurance training induced by each protocol extended to exercise performance in the heavy and severe-intensity domains and ranged from decreased reliance on carbohydrate oxidation to improved NMF and lower perceived effort. These results provide evidence that can inform decisions about endurance exercise prescription, while generating numerous questions about the signaling pathways that link acute exercise stresses to long-term training adaptations for future research.

Acknowledgments

The authors would like to thank Paige Boklaschuk and Jim Griffiths for their assistance with training supervision during the study.

Ethics Statement

Ethical approval for this trial was received from the University of Calgary Conjoint Health Research Ethics Board (23-0467). All participants provided written informed consent prior to beginning the trial.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. T. Reilly, T. Morris, and G. Whyte, "The Specificity of Training Prescription and Physiological Assessment: A Review," *Journal of Sports Sciences* 27, no. 6 (2009): 575–589, https://doi.org/10.1080/02640410902729741.

2. B. Egan and A. P. Sharples, "Signal Transduction and Exercise," in *Molecular Exercise Physiology* (Routledge, 2022), 152–189, https://doi.org/10.4324/9781315110752-7.

3. C. Granata, N. A. Jamnick, and D. J. Bishop, "Principles of Exercise Prescription, and How They Influence Exercise-Induced Changes of Transcription Factors and Other Regulators of Mitochondrial Biogenesis," *Sports Medicine* 48, no. 7 (2018): 1541–1559, https://doi.org/10.1007/s40279-018-0894-4.

4. M. J. MacInnis and M. J. Gibala, "Physiological Adaptations to Interval Training and the Role of Exercise Intensity," *Journal of Physiology* 595, no. 9 (2017): 2915–2930, https://doi.org/10.1113/JP273196.

5. D. Montero and C. Lundby, "Refuting the Myth of Non-Response to Exercise Training: 'Non-Responders' Do Respond to Higher Dose of Training," *Journal of Physiology* 595, no. 11 (2017): 3377–3387, https://doi.org/10.1113/JP273480.

6. R. J. Shepherd, "Intensity, Duration and Frequency of Exercise as Determinants of the Response to a Training Regime," *International Journal of Applied Physiology Including Work Physiology* 26 (1968): 272–278.

7. T. R. Tripp, A. M. Caswell, S. J. Aboodarda, and M. J. MacInnis, "The Effect of Duration on Performance and Perceived Fatigability During Acute High-Intensity Interval Exercise in Young, Healthy Males and Females," *Scandinavian Journal of Medicine & Science in Sports* 34, no. 7 (2024): 1–14, https://doi.org/10.1111/sms.14692.

8. M. J. Davies, A. P. Benson, D. T. Cannon, et al., "Dissociating External Power From Intramuscular Exercise Intensity During Intermittent Bilateral Knee-Extension in Humans," *Journal of Physiology* 595, no. 21 (2017): 6673–6686, https://doi.org/10.1113/JP274589.

9. G. O'Donovan, O. L. Sarmiento, and M. Hamer, "The Rise of the "Weekend Warrior"," *Journal of Orthopaedic and Sports Physical Therapy* 48, no. 8 (2018): 604–606, https://doi.org/10.2519/jospt.2018.0611.

10. G. O'Donovan, I. M. Lee, M. Hamer, and E. Stamatakis, "Association of 'Weekend Warrior' and Other Leisure Time Physical Activity Patterns With Risks for All-Cause, Cardiovascular Disease, and Cancer Mortality," *JAMA Internal Medicine* 177, no. 3 (2017): 335–342, https://doi.org/10.1001/JAMAINTERNMED.2016.8014.

11. I. M. Lee, H. D. Sesso, Y. Oguma, and R. S. Paffenbarger, "The 'Weekend Warrior' and Risk of Mortality," *American Journal of Epidemiology Hopkins Bloomberg School of Public Health all Rights Reserved* 160, no. 7 (2004): 636–641, https://doi.org/10.1093/aje/kwh274.

12. S. Khurshid, M. A. Al-Alusi, T. W. Churchill, J. S. Guseh, and P. T. Ellinor, "Accelerometer-Derived "Weekend Warrior" Physical Activity and Incident Cardiovascular Disease," *Journal of the American Medical Association* 330, no. 3 (2023): 247–252, https://doi.org/10.1001/jama.2023.10875.

13. A. Kilen, L. B. Hjelvang, N. Dall, N. L. Kruse, and N. B. Nordsborg, "Adaptations to Short, Frequent Sessions of Endurance and Strength Training Are Similar to Longer, Less Frequent Exercise Sessions When the Total Volume Is the Same," *Journal of Strength and Conditioning Research* 29, no. 11 (2015): S46–S51, www.nsca.com.

14. E. Manthou, J. M. R. Gill, and D. Maikova, "Effect of Exercise Programs With Aerobic Exercise Sessions of Similar Intensity but Different Frequency and Duration on Health-Related Measures in Overweight Women," *Journal of Physical Activity & Health* 12 (2015): 80–86, https://doi.org/10.1123/lpah.2013-0047.

15. T. Meyer, M. Auracher, K. Heeg, A. Urhausen, and W. Kindermann, "Does Cumulating Endurance Training at the Weekends Impair Training Effectiveness?," *European Journal of Cardiovascular Prevention and Rehabilitation* 13 (2006): 578–584.

16. M. N. Sawka, V. A. Convertino, E. R. Eichner, S. M. Schneider, and A. J. Young, "Blood Volume: Importance and Adaptations to Exercise Training, Environmental Stresses, and Trauma/Sickness," *Medicine and Science in Sports and Exercise* 32, no. 2 (2000): 332–348, https://doi.org/10.1097/00005768-200002000-00012.

17. J. O. Holloszy and E. F. Coyle, "Adaptations of Skeletal Muscle to Endurance Exercise and Their Metabolic Consequences," *Journal of Applied Physiology* 121, no. 4 (1984): 831–838, https://doi.org/10.1152/jappl.1984.56.4.831.

18. H. J. Green, S. Jones, M. Ball-Burnett, B. Farrance, and D. Ranney, "Adaptations in Muscle Metabolism to Prolonged Voluntary Exercise and Training," *Journal of Applied Physiology* 78, no. 1 (1995): 138–145.

19. S. J. Aboodarda, J. Mira, M. Floreani, et al., "Effects of Endurance Cycling Training on Neuromuscular Fatigue in Healthy Active Men. Part II: Corticospinal Excitability and Voluntary Activation," *European Journal of Applied Physiology* 118, no. 11 (2018): 2295–2305, https://doi.org/10.1007/s00421-018-3951-7.

20. J. Mira, S. J. Aboodarda, M. Floreani, et al., "Effects of Endurance Training on Neuromuscular Fatigue in Healthy Active Men. Part I: Strength Loss and Muscle Fatigue," *European Journal of Applied Physiology* 118, no. 11 (2018): 2281–2293, https://doi.org/10.1007/s00421-018-3950-8.

21. R. B. D'Agostino, J. M. Massaro, and L. M. Sullivan, "Non-Inferiority Trials: Design Concepts and Issues—The Encounters of Academic Consultants in Statistics," *Statistics in Medicine* 22, no. 2 (2003): 169–186, https://doi.org/10.1002/sim.1425.

22. E. C. Inglis, D. Iannetta, L. Rasica, et al., "Heavy-, Severe-, and Extreme-, But Not Moderate-Intensity Exercise Increase VO_2max and Thresholds After 6 Week of Training," *Medicine and Science in Sports and Exercise* 56, no. 7 (2024): 1307–1316, https://doi.org/10.1249/MSS.0000000000003406.

23. R. Ross, S. N. Blair, R. Arena, et al., "Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association," *Circulation* 134, no. 24 (2016): e653–e699, https://doi.org/10.1161/CIR.0000000000000461.

24. R. Mazzolari, S. Porcelli, D. J. Bishop, and D. Lakens, "Myths and Methodologies: The Use of Equivalence and Non-Inferiority Tests for Interventional Studies in Exercise Physiology and Sport Science,"

Experimental Physiology 107, no. 3 (2022): 201–212, https://doi.org/10.1113/EP090171.

25. C. Foster, J. A. Florhaug, J. Franklin, et al., "A New Approach to Monitoring Exercise Training," *Journal of Strength and Conditioning Research* 15, no. 1 (2001): 109–115.

26. T. R. Tripp, R. M. McDougall, B. P. Frankish, J. Preston Wiley, and M. J. MacInnis, "Contraction Intensity Affects NIRS-Derived Muscle Oxidative Capacity but Not Its Relationships to Mitochondrial Protein Content or Aerobic Fitness," *Journal of Applied Physiology* 136, no. 2 (2024): 298–312, https://doi.org/10.1152/japplphysiol.00342.2023.

27. M. Motobe, N. Murase, T. Osada, et al., "Noninvasive Monitoring of Deterioration in Skeletal Muscle Function With Forearm Cast Immobilization and the Prevention of Deterioration," *Dynamic Medicine* 11 (2004): 1–11.

28. T. E. Ryan, M. L. Erickson, J. T. Brizendine, H. J. Young, and K. K. McCully, "Noninvasive Evaluation of Skeletal Muscle Mitochondrial Capacity With Near-Infrared Spectroscopy: Correcting for Blood Volume Changes," *Journal of Applied Physiology* 113, no. 2 (2012): 175–183, https://doi.org/10.1152/japplphysiol.00319.2012.

29. A. T. Beever, T. R. Tripp, J. Zhang, and M. J. MacInnis, "NIRS-Derived Skeletal Muscle Oxidative Capacity Is Correlated With Aerobic Fitness and Independent of Sex," *Journal of Applied Physiology* 129, no. 3 (2020): 558–568, https://doi.org/10.1152/japplphysiol.00017.2020.

30. D. Iannetta, E. C. Inglis, S. Pogliaghi, J. M. Murias, and D. A. Keir, "A "Step-Ramp-Step" Protocol to Identify the Maximal Metabolic Steady State," *Medicine and Science in Sports and Exercise* 52, no. 9 (2020): 2011–2019, https://doi.org/10.1249/MSS.0000000000002343.

31. N. M. Beltz, A. L. Gibson, J. M. Janot, L. Kravitz, C. M. Mermier, and L. C. Dalleck, "Graded Exercise Testing Protocols for the Determination of VO2max: Historical Perspectives, Progress, and Future Considerations," *Journal of Sports Medicine* 2016 (2016): 1–12, https://doi.org/10.1155/2016/3968393.

32. D. A. Keir, D. Iannetta, F. Mattioni Maturana, J. M. Kowalchuk, and J. M. Murias, "Identification of Non-Invasive Exercise Thresholds: Methods, Strategies, and an Online App," *Sports Medicine* 52, no. 2 (2022): 237–255, https://doi.org/10.1007/s40279-021-01581-z.

33. D. A. Keir, D. H. Paterson, J. M. Kowalchuk, and J. M. Murias, "Using Ramp-Incremental $\dot{V}O_2$ Responses for Constant-Intensity Exercise Selection," *Applied Physiology, Nutrition, and Metabolism* 43, no. 9 (2018): 882–892, https://doi.org/10.1139/apnm-2017-0826.

34. K. Caen, J. G. Bourgois, L. Stuer, V. Mermans, and J. Boone, "Can We Accurately Predict Critical Power and W′ From a Single Ramp Incremental Exercise Test?," *Medicine and Science in Sports and Exercise* 55, no. 8 (2023): 1401–1408, https://doi.org/10.1249/MSS.0000000000003171.

35. C. R. Van Rassel, O. O. Ajayi, K. M. Sales, R. A. Azevedo, J. M. Murias, and M. J. Macinnis, "A "Step-Ramp-Step" Protocol to Identify Running Speed and Power Associated With the Maximal Metabolic Steady State," *Medicine and Science in Sports and Exercise* 55, no. 3 (2023): 534–547, https://doi.org/10.1249/MSS.0000000000003066.

36. H. Kontro, C. Bertagnolli, J. M. Murias, and M. J. MacInnis, "Impairment in Maximal Lactate Steady State After Carbon Monoxide Inhalation Is Related to Training Status," *Experimental Physiology* 107, no. 11 (2022): 1–18, https://doi.org/10.1113/EP090642.

37. W. Schmidt and N. Prommer, "The Optimised CO-Rebreathing Method: A New Tool to Determine Total Haemoglobin Mass Routinely," *European Journal of Applied Physiology* 95, no. 5–6 (2005): 486–495, https://doi.org/10.1007/s00421-005-0050-3.

38. S. Keiser, A. K. Meinild-Lundby, T. Steiner, et al., "Detection of Blood Volumes and Haemoglobin Mass by Means of CO Re-Breathing and Indocyanine Green and Sodium Fluorescein Injections," *Scandinavian Journal of Clinical and Laboratory Investigation* 77, no. 3 (2017): 164–174, https://doi.org/10.1080/00365513.2016.1271908.

39. A. E. Jeukendrup and G. A. Wallis, "Measurement of Substrate Oxidation During Exercise by Means of Gas Exchange Measurements," *International Journal of Sports Medicine* 26, no. 1 (2005): 528–537.

40. R. L. Krüger, S. J. Aboodarda, L. M. Jaimes, P. Samozino, and G. Y. Millet, "Cycling Performed on an Innovative Ergometer at Different Intensities–Durations in Men: Neuromuscular Fatigue and Recovery Kinetics," *Applied Physiology, Nutrition and Metabolism* 44, no. 12 (2019): 1320–1328, https://doi.org/10.1139/apnm-2018-0858.

41. V. Strojnik and P. V. Komi, "Neuromuscular Fatigue After Maximal Stretch-Shortening Cycle Exercise," *Journal of Applied Physiology* 84, no. 1 (1998): 344–350.

42. G. A. V. Borg, "Psychophysical Bases of Perceived Exertion," *Medicine and Science in Sports and Exercise* 14, no. 5 (1982): 377–381.

43. D. Micklewright, A. St Clair Gibson, V. Gladwell, and A. Al Salman, "Development and Validity of the Rating-of-Fatigue Scale," *Sports Medicine* 47, no. 11 (2017): 2375–2393, https://doi.org/10.1007/s40279-017-0711-5.

44. J. A. Laukkanen, F. Zaccardi, H. Khan, S. Kurl, S. Y. Jae, and R. Rauramaa, "Long-Term Change in Cardiorespiratory Fitness and All-Cause Mortality: A Population-Based Follow-Up Study," *Mayo Clinic Proceedings* 91, no. 9 (2016): 1183–1188, https://doi.org/10.1016/j.mayocp.2016.05.014.

45. J. Helgerud, K. Høydal, E. Wang, et al., "Aerobic High-Intensity Intervals Improve $\dot{V}O_2$max More Than Moderate Training," *Medicine and Science in Sports and Exercise* 39, no. 4 (2007): 665–671, https://doi.org/10.1249/mss.0b013e3180304570.

46. D. C. Poole, H. B. Rossiter, G. A. Brooks, and L. B. Gladden, "The Anaerobic Threshold: 50+ Years of Controversy," *Journal of Physiology* 599, no. 3 (2021): 737–767, https://doi.org/10.1113/JP279963.

47. A. M. Jones, M. Burnley, M. I. Black, D. C. Poole, and A. Vanhatalo, "The Maximal Metabolic Steady State: Redefining the 'Gold Standard'," *Physiological Reports* 7, no. 10 (2019): 1–16.

48. D. A. Keir, S. Pogliaghi, E. C. Inglis, J. M. Murias, and D. Iannetta, "The Respiratory Compensation Point: Mechanisms and Relation to the Maximal Metabolic Steady State," *Sports Medicine* 54, no. 12 (2024): 2993–3003, https://doi.org/10.1007/s40279-024-02084-3.

49. A. M. Jones, "The Journal of Physiology the Fourth Dimension: Physiological Resilience as an Independent Determinant of Endurance Exercise Performance," *Journal of Physiology* 602, no. 17 (2024): 4113–4128, https://doi.org/10.1113/JP284205#support-information-section.

50. P. A. Anstiss, C. Meijen, and S. M. Marcora, "The Sources of Self-Efficacy in Experienced and Competitive Endurance Athletes," *International Journal of Sport and Exercise Psychology* 18, no. 5 (2020): 622–638, https://doi.org/10.1080/1612197X.2018.1549584.

51. L. Hu, E. Mcauley, R. W. Motl, and J. F. Konopack, "Influence of Self-Efficacy on the Functional Relationship Between Ratings of Perceived Exertion and Exercise Intensity," *Journal of Cardiopulmonary Rehabilitation and Prevention* 27 (2007): 303–308.

52. V. A. Convertino, "Blood Volume: Its Adaptation to Endurance Training," *Medicine and Science in Sports and Exercise* 23, no. 12 (1991): 1338–1348.

53. D. Montero and C. Lundby, "Regulation of Red Blood Cell Volume With Exercise Training," *Comprehensive Physiology* 9, no. 1 (2019): 149–164, https://doi.org/10.1002/cphy.c180004.

54. Ø. Skattebo, A. Wold, M. Auensen, et al., "Blood Volume Expansion Does Not Explain the Increase in Peak Oxygen Uptake Induced by 10 Weeks of Endurance Training," *European Journal of Applied Physiology* 120, no. 5 (2020): 0123456789, https://doi.org/10.1007/s00421-020-04336-2.

55. E. F. Coyle, W. H. Martin, III, S. A. Bloomfield, O. H. Lowry, and J. O. Holloszy, "Effects of Detraining on Responses to Submaximal Exercise," *Journal of Applied Physiology* 59, no. 3 (1985): 853–859.

56. C. Granata, N. A. Jamnick, and D. J. Bishop, "Training-Induced Changes in Mitochondrial Content and Respiratory Function in Human Skeletal Muscle," *Sports Medicine* 48, no. 8 (2018): 1809–1828, https://doi.org/10.1007/s40279-018-0936-y.

57. H. Hoppeler and S. R. Kayar, "Capillarity and Oxidative Capacity of Muscles," *Physiology* 3 (1988): 113–116.

58. K. Schwerzmann, H. Hoppeler, S. R. Kayar, and E. R. Weibel, "Oxidative Capacity of Muscle and Mitochondria: Correlation of Physiological, Biochemical, and Morphometric Characteristics," *Proceedings of the National Academy of Sciences of the United States of America* 86 (1989): 1583–1587.

59. M. L. Erickson, T. E. Ryan, D. Backus, and K. K. McCully, "Endurance Neuromuscular Electrical Stimulation Training Improves Skeletal Muscle Oxidative Capacity in Individuals With Motor-Complete Spinal Cord Injury," *Muscle & Nerve* 55, no. 5 (2017): 669–675, https://doi.org/10.1002/MUS.25393.

60. T. B. Willingham, J. Melbourn, M. Moldavskiy, K. K. McCully, and D. Backus, "Effects of Treadmill Training on Muscle Oxidative Capacity and Endurance in People With Multiple Sclerosis With Significant Walking Limitations," *International Journal of MS Care* 21, no. 4 (2019): 166–172, https://doi.org/10.7224/1537-2073.2018-021.

61. L. Hermansen, E. Hultman, and B. Saltin, "Muscle Glycogen During Prolonged Severe Exercise," *Acta Physiologica Scandinavia* 71 (1967): 29–139.

62. C. Granata, R. S. F. Oliveira, J. P. Little, K. Renner, and D. J. Bishop, "Training Intensity Modulates Changes in PGC-1α and p53 Protein Content and Mitochondrial Respiration, but Not Markers of Mitochondrial Content in Human Skeletal Muscle," *FASEB Journal* 30, no. 2 (2016): 959–970, https://doi.org/10.1096/fj.15-276907.

63. A. J. Rose and M. Hargreaves, "Exercise Increases Ca^{2+}-Calmodulin-Dependent Protein Kinase II Activity in Human Skeletal Muscle," *Journal of Physiology* 553, no. 1 (2003): 303–309, https://doi.org/10.1113/jphysiol.2003.054171.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.