Cocoa-flavanols enhance moderate-intensity pulmonary V̇O2 kinetics but not exercise tolerance in sedentary middle-aged adults: A randomised controlled trial

Daniel Sadler
Liverpool John Moores University https://orcid.org/0000-0001-6737-5630
Richard Draijer
Unilever Innovation Vlaardingen
Claire E. Stewart
Liverpool John Moores University
Helen Jones
Liverpool John Moores University
Simon Marwood
Liverpool Hope University
Dick H. J. Thijssen (d.thijssen@ljmu.ac.uk)
https://orcid.org/0000-0002-7707-5567

Research

Keywords: Cocoa flavanols, oxygen uptake kinetics, heart rate, exercise tolerance, middle-age

Posted Date: June 16th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-35412/v1

Cocoa-flavanols enhance moderate-intensity pulmonary $\dot{V}O_2$ kinetics but not exercise tolerance in sedentary middle-aged adults: A randomised controlled trial

Daniel G. Sadler[1]; Richard Draijer[3]; Claire E. Stewart[1]; Helen Jones[1]; Simon Marwood[2]; Dick H. J. Thijssen[1].

[1]School of Sport and Exercise Science, Liverpool John Moores University, Liverpool, United Kingdom

[2]School of Health Sciences, Liverpool Hope University, Liverpool, United kingdom

[3]Unilever Research & Development, Olivier van Noortlaan 120, 3133 AT, Vlaardingen, The Netherlands

Corresponding Author: Dick H. J. Thijssen

Address Correspondence: Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Byrom Street, L3 3AF, Liverpool, UK

Telephone number: +44 (0)151 904 6284

E-mail address: D.Thijssen@ljmu.ac.uk

Running head: Cocoa-flavanols enhance pulmonary $\dot{V}O_2$ kinetics

Abbreviations: *A*HR, amplitude of the fundamental heart rate response; *A*$\dot{V}O_2$, amplitude of the fundamental oxygen uptake response; CF, cocoa flavanol; GET, gas exchange threshold; HR, heart rate; HR_b, baseline heart rate; HRτ, time constant of the fundamental response; NO, nitric oxide; O_2, oxygen; PL, placebo; SC$\dot{V}O_2$, magnitude of the slow component; $\tau\dot{V}O_2$, time constant of the fundamental response; $TD_{SC\dot{V}O_2}$, time delay of the $\dot{V}O_2$ slow component;

23 $TD\dot{V}O_2$, time delay of the fundamental response; T_{lim}, limit of exercise tolerance; $\dot{V}O_2$, oxygen

24 uptake; $\dot{V}O_{2b}$, baseline oxygen uptake; $\tau\dot{V}O_2$, time constant of the fundamental response.

ABSTRACT

Background: Cocoa flavanols (CF) may exert health benefits through their potent vasodilatory effects which are perpetuated by elevations in nitric oxide (NO) bioavailability. These vasodilatory effects may contribute to improved delivery of blood and oxygen to exercising muscle. **Objective:** Therefore, the objective of this study was to examine how CF supplementation impacts pulmonary oxygen uptake ($\dot{V}O_2$) kinetics and exercise tolerance in sedentary middle-aged adults. **Methods:** We employed a double-blind cross-over, placebo-controlled design whereby 17 participants (11 male, 6 female; mean±SD, 45±6 years) randomly received either 7 days of daily CF (400 mg) or placebo (PL) supplementation. On day 7, participants completed a series of 'step' moderate- and severe-intensity exercise tests for the determination of oxygen uptake kinetics. **Results:** During moderate-intensity exercise, the time constant of the fundamental phase of $\dot{V}O_2$ kinetics ($\tau\dot{V}O_2$) was decreased by 15% in CF as compared to PL (mean±SD; PL: 40±12 vs. CF: 34±9 s, P=0.019), with no differences in the amplitude of $\dot{V}O_2$ ($A\dot{V}O_2$; PL: 0.77±0.32 vs. CF: 0.79±0.34 l min^{-1}, P=0.263). However, during severe-intensity exercise, $\tau\dot{V}O_2$,the amplitude of the slow component ($SC\dot{V}O_2$) and exercise tolerance (PL: 435±58 vs. CF: 424±47 s, P=0.480) were unchanged between conditions. **Conclusions:** Our data show that acute CF supplementation enhanced oxygen uptake kinetics during moderate-, but not severe-intensity exercise in middle-aged participants. These novel effects of CFs, in this demographic, may contribute to improved tolerance of moderate-activity physical activities, which appear commonly present in daily life.

Registered under ClinicalTrials.gov Identifier no. NCT04370353

Key words: Cocoa flavanols, oxygen uptake kinetics, heart rate, exercise tolerance, middle-age

INTRODUCTION

Skeletal muscle contraction and force production form the basis for the ability to perform physical activity, both for daily life activities as well as during sports-related events. Repeated muscle contractions require continuous regeneration of adenosine triphosphate (ATP). The production of ATP during (prolonged) physical activity is driven through oxidative phosphorylation, which depends on sufficient delivery of oxygen (O_2) (1). Impairment of pathways involved in the delivery of O_2 to working skeletal muscle, such as with older age or physical inactivity, leads to slower rates of pulmonary O_2 uptake ($\dot{V}O_2$) and therefore greater O_2 deficit (2–4). Impaired $\dot{V}O_2$ kinetics in response to physical activity are associated with reduced exercise tolerance (5–7) and may also affect capacity to perform daily life activities that require moderate-intensity physical activity.

The slower dynamic adjustment of $\dot{V}O_2$ observed in older adults across a metabolic transient is thought to be due to a mismatch of O_2 delivery to O_2 utilisation. Indeed, attenuations in microvascular blood flow supply and distribution (and thus O_2 delivery) within aged skeletal muscle are well documented (3,8–10). These reductions in O_2 transport to active skeletal muscle are likely caused by impaired vascular endothelial function and diminished nitric oxide (NO) bioavailability (9,11–13). Interestingly, lifestyle interventions, such as exercise training and dietary strategies (14–18), have demonstrated potent effects to enhance NO bioavailability and improve endothelial function. Consequently, a number of studies have shown improved $\dot{V}O_2$ kinetics in concert with increased O_2 availability (7,19–21).

Cocoa flavanols (CFs) represent a group of flavonoids present in cocoa derived from seeds of the fruit of the Theobroma cacao tree. Previous studies have found CFs act primarily through the monomer (-)-epicatechin, to stimulate NO production, resulting in improved vasodilation

and endothelial function in healthy adults (22–25). Given the direct impact of CFs on NO production and vascular endothelial function, and the negative impact of ageing on O_2 delivery and O_2 uptake kinetics at the onset of exercise, our objective was to test the hypothesis that, compared with placebo (PL), CF supplementation enhances $\dot{V}O_2$ kinetics during moderate-intensity physical activity and increases exercise tolerance in healthy middle-aged individuals.

METHODOLOGY

Participants

Seventeen healthy middle-aged adults (11 male: mean±SD, age 45±6 years; body mass 87.7±13.5 kg; height 1.75±0.07 m; and 6 female: aged 47±5 years; body mass 68.2±17.7 kg; height 1.62±0.09 m) volunteered and gave written informed consent to participate in the study (see **Figure 1**). All procedures conformed to the CONSORT statement (see **Additional File 1**) and the Declaration of Helsinki and were approved by Liverpool John Moores University Research Ethics Committee (approval reference number: 18/SPS/014). Participants engaged in less than two hours of structured exercise training per week. All participants were non-smokers and had no history of cardiovascular, respiratory or metabolic diseases. Participants were not taking any dietary supplements or medication.

Participants reported to the laboratory at least 3 hours postprandial in a rested state, having completed no strenuous exercise within the previous 24 hours and avoided alcohol and caffeine for 24 and 6 hours, preceding each exercise test, respectively. Participants were advised to avoid consumption of flavonoid-rich foodstuffs (e.g. green tea, dark chocolate and berries) in the 24 hours preceding each experimental trial.

Procedures

Participants visited the temperature-controlled laboratory (19-22 °C) on 4 occasions during a 4-5-week period, with each test scheduled at the same time of day (± 1 h) and at least 48 h between visits. Participants completed two preliminary trials and two experimental trials. Exercise bouts were performed on an electrically operated cycle ergometer (Lode Corival, Groningen, The Netherlands). Saddle and handlebar height/angle were recorded at the first visit and replicated during each subsequent visit for each individual participant. Throughout all exercise tests, participants were instructed to maintain a cadence of 65-80 rev min^{-1}, and exhaustion was defined as when the participants cadence dropped 10 rev min^{-1} below the target work rate. Time to exhaustion was measured to the nearest second (s) in all tests.

Preliminary trial(s)

Upon arrival to the laboratory, participants height and weight were recorded. Subsequently, each participant undertook an incremental step test until the limit of tolerance to establish $\dot{V}O_2$ peak, the gas exchange threshold (GET) and the power outputs for later tests. The incremental step test consisted of 3-min of baseline pedalling at 0 W, followed by a continuous, stepped increase in power output of 30 or 25 W min^{-1} (for males and females, respectively) until the limit of tolerance was established. Gas exchange and ventilatory variables were measured continuously at the mouth breath-by-breath throughout each test. $\dot{V}O_2$ peak was defined as the highest $\dot{V}O_2$ value obtained over 30 s. The GET was determined using a collection of previously established criteria (26) including (1) a disproportionate rise in CO_2 production ($\dot{V}CO_2$) relative to $\dot{V}O_2$; (2) an increase in minute ventilation ($\dot{V}E$) relative to $\dot{V}O_2$ ($\dot{V}E/\dot{V}O_2$) without an increase in $\dot{V}E$ relative to $\dot{V}CO_2$ ($\dot{V}E/\dot{V}CO_2$); and (3) an increase in end tidal O_2 tension without decreasing end tidal CO_2 tension.

During the familiarisation trial (visit 2), participants were requested to perform two bouts of severe-intensity exercise at a fixed power output to exhaustion (e.g. T_{lim}), each separated by 45 min of seated rest. The power outputs of these severe-intensity bouts were selected based upon performance during the incremental test and were calculated to be 60%Δ (i.e., a work rate calculated to require 60% of the difference between GET and $\dot{V}O_2$ peak). On occasion, adjustments were made to the power output of the subsequent exercise tests based upon performance in the familiarisation trials; the prescribed power output was lowered for participants who failed to exercise for up to 360 s during the severe-intensity bouts.

After completion of the familiarisation trial, participants were randomly assigned (computer generated), using a double-blind cross-over design (**Figure 2**), to receive 7 consecutive days of CF supplementation or a PL that was matched for caffeine and theobromine content. Nine participants began with the CF condition, and eight participants began with the PL condition. Participants were advised to consume 4 capsules daily, each providing 2.9 mg caffeine and 22.5 mg theobromine (Fagron, Netherlands), whilst CF contained 316 mg CocoActiv (Naturex, Netherlands; ~100 mg total flavanols of which 22 mg DP1 = catechin + epicatechin) and PL contained 0 mg flavanols. Remaining empty volumes of PL and CF capsules were filled with microcrystalline cellulose (Fagron, Netherlands). Two capsules were taken in the morning and two in the evening following ingestion of a mixed meal (27). A 7-day wash-out period separated the supplementation periods and the order between CF and PL supplementation was randomised. Throughout the study period participants were instructed to maintain their normal daily activities and diet. Participants kept a food dairy and were instructed to consume an identical diet in the two periods of exercise testing. Physical activity levels were measured by accelerometery in the 6 days preceding testing via a hip-mounted activity monitor (Actigraph GT3X).

149

150 *Experimental trials*

151 On the 7th day of supplementation, participants were advised to consume 4 capsules 45 min
152 prior to arrival at the laboratory. The supplementation protocol was chosen so that participants
153 commenced exercise testing ~90 min following supplement ingestion, which coincided with
154 reported peak plasma flavanol concentrations (27). The participants completed a series of
155 "step" exercise tests from an unloaded (0 W) baseline to moderate and severe-intensity work
156 rates for the determination of pulmonary $\dot{V}O_2$ kinetics. Tests began with 3 minutes of 0 W
157 baseline cycling, before a step change in power output to 80% GET or 60%Δ for 6 minutes and
158 until T_{lim}, respectively. Participants completed three bouts of moderate- and one bout of severe-
159 intensity exercise, each separated by 10 min of passive recovery. This protocol was employed
160 with the knowledge that prior moderate-intensity exercise does not impact the $\dot{V}O_2$ kinetics of
161 subsequent heavy intensity exercise (28,29).

162

163 *Measurements*

164 During all exercise tests, pulmonary gas exchange and ventilation were measured at the mouth
165 breath-by-breath using a metabolic cart (Jaeger Oxycon Pro, Hoechberg, Germany).
166 Participants wore a facemask and breathed through a low dead space (90 ml), low resistance
167 (0.75 mmHg l^{-1} s^{-1} at 15 l/s) impeller turbine assembly (Jaeger Triple V, Hoechberg,
168 Germany). The inspired and expired gas volumes and gas concentration signals were
169 continuously sampled at 100 Hz, the latter using paramagnetic (O_2) and infrared (CO_2)
170 analysers (Jaeger Oxycon Pro, Hoechberg, Germany) via a capillary line connected to the
171 mouthpiece. These analysers were calibrated before each test with gases of known
172 concentrations (16% O_2 and 4% CO_2), and the turbine volume transducer was calibrated using

a 3-liter syringe (Hans Rudolph, Kansas City, MO). The volume and concentration signals were time aligned by accounting for the delay in capillary gas transit and analyser rise time relative to the volume signal. Breath-by-breath fluctuations in lung gas stores were corrected for by computer algorithms (30). Heart rate was measured during all tests via short-range radiotelemetry (Polar H10, Polar Electro, Kempele, Finland). During one of the transitions to moderate- and severe-intensity exercise for both supplementation periods, a blood sample was collected from a fingertip over the last 30 s preceding the step transition in work rate and within the last 15 s of exercise. Blood samples were immediately analysed using a hand-held device (Lactate Pro, Nova Biomedical, USA) to determine blood lactate concentration. Blood lactate accumulation was calculated as the difference between blood lactate at end exercise and blood lactate at baseline.

After arrival to the laboratory, participants underwent an assessment of the previous 7 days physical activity levels and sedentary behaviour by the International Physical Activity Questionnaire (IPAQ) and by accelerometery (ActiGraph GTX3). Following 10 min of seated rest, participants blood pressure was measured in the brachial artery. Blood pressure was measured three times and the mean of the responses was recorded.

Data analysis

Breath-by-breath $\dot{V}O_2$ data were edited to remove data points lying more than 3 standard deviations (SD) outside the local 5-breath mean (31). The resultant data were then linearly interpolated to provide second-by-second values. For $\dot{V}O_2$ and heart rate data in response to moderate exercise transitions, second-by- second data for the three transitions were averaged together to produce a single dataset. The severe-intensity exercise bout for each condition was not repeated and was modelled separately. For each exercise transition, the first 20 s of data

after the onset of exercise (i.e., the cardiodynamic or phase I response) were deleted (32,33) and a mono-exponential model (Equation 1) with time delay was then fitted to the data (4), as follows:

$$\dot{V}O_2 = \dot{V}O_{2(b)} + A\dot{V}O_2 \left(1 - e^{-\left(t - TD_{\dot{V}O_2} / \tau\dot{V}O_2\right)}\right) \quad (1)$$

Where $\dot{V}O_2(t)$ is the $\dot{V}O_2$ at any time t, $\dot{V}O_{2b}$ is the baseline $\dot{V}O_2$, which was taken as the mean $\dot{V}O_2$ over the final 30 s of the baseline period preceding the transition, $A\dot{V}O_2$ is the amplitude of the fundamental response above baseline, $TD_{\dot{V}O_2}$ is the time delay of the fundamental relative to the onset of exercise, and $\tau\dot{V}O_2$ is the time constant of the fundamental response. For moderate intensity exercise, data were modelled to 360s. For severe intensity exercise, the onset of the $\dot{V}O_2$ slow component ($TD_{SC\dot{V}O_2}$) was determined using purpose-designed programming in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA), which iteratively fits a monoexponential function to the $\dot{V}O_2$ data, starting at 60 s until the window encompasses the entire response. The resulting fundamental phase time constants are plotted against time, and the $TD_{SC\dot{V}O_2}$ was identified as the point at which $\tau\dot{V}O_2$ consistently deviates from a previously 'flat' profile, and the demonstration of a local threshold in the $\chi 2$ value (34). This method allows the fitting of eqn (1) to the fundamental component of the response isolated from the slow component, thus avoiding the possibility of arbitrarily parameterizing the slow component. The amplitude of the $\dot{V}O_2$ slow component was determined by calculating the difference between the end-exercise $\dot{V}O_2$ (i.e. mean $\dot{V}O_2$ over final 30 s of exercise) and ($A\dot{V}O_2$ + $\dot{V}O_{2b}$). In instances where exercise duration was too short to allow the slow component to be discerned the $\dot{V}O_2$ response was modelled using Equation 1 to the end of exercise and the slow component was assigned a value of 0.

Heart rate kinetics were modelled for each exercise transition using a monoexponential function (Equation 2) with the response constrained to the start of exercise (at t = 0; i.e., with no time delay):

$$HR_{(t)} = HR_b + AHR * \left(1 - e^{(t/\tau HR)}\right) \quad (2)$$

where HR_b is the mean HR measured over the final 30 s of baseline cycling, and *A*HR and τHR are the amplitude and the time constant of the response, respectively.

Statistics

Based on previous knowledge of a meaningful change in $\tau\dot{V}O_2$ during intervention studies (5 s), and a common standard deviation of 4.3 s (32), the necessary calculated sample size was 12. Differences in the cardiorespiratory variables between conditions were determined with two-tailed, paired-samples *t*-tests (GraphPad, Prism, USA). Data are presented as means±SD. Statistical significance was accepted when $P<0.05$.

RESULTS

Peak $\dot{V}O_2$ was 2.45±0.61 l min^{-1} (28.1±5.7 ml kg^{-1} min^{-1}), with the mean GET occurring at 1.51±0.46 l min^{-1} (108±39 W). The peak work rate attained from the incremental test was 207±49 W and the work rates calculated to require 80% of the GET and 60%Δ were 87±29 W and 166±40 W, respectively.

244 **Table 1.** Heart rate and blood lactate responses during moderate- and severe-intensity exercise following CF and PL supplementation

Parameter	HR_b (b min^{-1})	*A*HR (b min^{-1})	HRτ (s)	End exercise HR (b min^{-1})	Baseline blood lactate (mM)	End exercise blood lactate (mM)	Δ blood lactate (mM)	Blood lactate at exhaustion (mM)
Moderate-intensity exercise								
PL	83±13	31±8	53±22	114±16	1.5±0.7	2.6±0.5	1.2±0.9	-
CF	83±14	32±8	47±13	115±18	1.3±0.4	2.5±0.7	1.3±0.8	-
Severe-intensity exercise								
PL	89±15	69±16	89±17	159±14	1.9±0.9	8.8±2.0	7.4±2.5	9.5±2.3#
CF	92±17	67±17	89±29	160±17	1.8±0.9	8.4±2.3	7.1±2.8	9.7±1.9#

245
246
247
248
249
250
251
252
253
254

255 HR_b, baseline heart rate; *A*HR, amplitude of the fundamental response; HRτ, time constant of the fundamental response; PL, placebo; CF, cocoa

256 flavanol. Values are mean±SD. #Significantly different from baseline blood lactate (P<0.05).

257 *Heart rate kinetics, blood lactate profiles and blood pressure*

258 There were no differences in the primary τHR between PL and CF for moderate- or severe-
259 intensity bouts (P=0.219 and 0.956, respectively, **Table 1**). Despite significant changes in
260 blood lactate concentrations at T_{lim} compared to baseline (P<0.05; Table 1), there were no
261 significant differences in blood [lactate] from pre- to post-exercise between conditions during
262 moderate- and severe-intensity exercise (see Table 1). Overall, there were no differences
263 between resting systolic (PL: 128±12 vs. CF: 127±12 mmHg, P=0.66) or diastolic (PL: 78±7
264 vs. 78±7 mmHg, P=0.75) blood pressure following either PL or CF administration.
265
266 *$\dot{V}O_2$ kinetics and exercise tolerance*

267 The $\dot{V}O_2$ kinetic parameters for moderate intensity exercise are presented in **Table 2**, and the
268 $\dot{V}O_2$ response of a representative participant to moderate-intensity exercise is shown in **Figure**
269 **3.** Compared with PL, $\tau\dot{V}O_2$ was smaller during moderate-intensity exercise following CF
270 supplementation (PL: 40±12 vs. CF: 34±9 s, P=0.019). However, there were no differences in
271 $\dot{V}O_{2b}$ (P=0.175), $A\dot{V}O_2$ (P=0.263), $TD\dot{V}O_2$ (P=0.961), Gain (P=0.478) or end exercise $\dot{V}O_2$
272 (P=0.565) between PL and CF.
273
274 The pulmonary $\dot{V}O_2$ response to severe-intensity exercise for a representative participant is
275 shown in **Figure 4A** and group mean responses are shown in **Figure 4B.** The associated
276 modelled parameters are presented in Table 2. No impact of CF supplementation on the $\tau\dot{V}O_2$
277 (P=0.799) for exercise initiated at 60% Δ over PL was evident. There were no differences in
278 $\dot{V}O_{2b}$ (P=0.246), $A\dot{V}O_2$ (P=0.427), $TD\dot{V}O_2$ (P=0.617), $SC\dot{V}O_2$ (P=0.887), Gain (P=0.640), or
279 end exercise $\dot{V}O_2$ (P=0.954) between conditions. $TD_{SC\dot{V}O_2}$was lower following CF vs. PL
280 supplementation (PL: 110±15 vs. CF: 95±13 s, P=0.002). Both end-exercise $\dot{V}O_2$ (P=0.959)

and T_{lim} (P=0.480) were not significantly different following PL and CF supplementation during severe-intensity exercise (see Table 2).

305 **Table 2.** Pulmonary O_2 uptake responses to moderate- and severe-intensity exercise following CF and PL supplementation

Parameter	$\dot{V}O_{2b}$ (l min^{-1})	$A\dot{V}O_2$ (l min^{-1})	$TD\dot{V}O_2$ (s)	$\tau\dot{V}O_2$ (s)	Gain (ml min^{-1} W^{-1})	End exercise $\dot{V}O_2$ (l min^{-1})	$TD_{SC\dot{V}O_2}$ (s)	$SC\dot{V}O_2$ (l min^{-1})	T_{lim} (s)
Moderate-intensity exercise									
PL	0.69±0.12	0.77±0.32	13±6	40±12	8.90±1.25	1.50±0.35	-	-	-
CF	0.66±0.13	0.79±0.34	13±7	34±9*	9.06±1.49	1.50±0.38	-	-	-
Severe-intensity exercise									
PL	0.78±0.14	1.40±0. 40	17±4	27±9	8.20±0.86	2.60±0.66	110±15	0.50±0.20	435±58
CF	0.74±0.13	1.50±0.52	16±4	28±6	8.36±1.21	2.60±0.65	95±13*	0.50±0.20	424±47

306

307 $\dot{V}O_{2b}$, baseline oxygen uptake; $A\dot{V}O_2$, amplitude of the fundamental response; $TD\dot{V}O_2$, time delay of the fundamental response; $\tau\dot{V}O_2$, time constant
308 of the fundamental response; Gain, increase in $\dot{V}O_2$ per unit increase in power output; $TD_{SC\dot{V}O_2}$, time delay of the $\dot{V}O_2$ slow component; $SC\dot{V}O_2$,
309 magnitude of the slow component; T_{lim}, limit of exercise tolerance; PL, placebo; CF, cocoa flavanol. Values are mean±SD. *Significantly different
310 from PL (P<0.05).

DISCUSSION

The purpose of this study was to examine the impact of CFs on pulmonary $\dot{V}O_2$ kinetics during two intensities of cycling exercise in healthy, normotensive middle-aged individuals. Congruent with our hypothesis, the major finding of this study was that 7-days CF supplementation enhanced pulmonary $\dot{V}O_2$ kinetics during moderate-intensity exercise as demonstrated by a significant reduction in $\tau\dot{V}O_2$. These effects of CFs, however, were not apparent during severe-intensity exercise when compared with a PL. Ultimately, the findings of the present study may have clinical potential in contributing to improved tolerance of daily life activity in middle-aged adults.

Effects of CFs on the Physiological Responses to Moderate-Intensity Exercise

This study is the first to investigate whether CFs modulate pulmonary $\dot{V}O_2$ kinetics. We show that 7 days CF supplementation significantly reduced the $\tau\dot{V}O_2$ (40 vs. 34 s) associated with the transition from unloaded to moderate-intensity cycling in middle-aged adults. Notably, the magnitude of change in $\tau\dot{V}O_2$ (~6 s) reported is important, as it exceeds the minimum physiologically relevant change of ~5 s (32). The reduction in $\tau\dot{V}O_2$ observed after CF supplementation in our middle-aged individuals reflects a shift towards oxygen kinetics typically observed in younger healthy individuals (35), whereby $\dot{V}O_2$ kinetics are not limited by O_2 delivery *per se* (36). Theoretically, a lowered $\tau\dot{V}O_2$ would reduce the O_2 deficit incurred during the exercise transition, thereby causing less perturbations to the intracellular milieu (i.e., Δ phosphocreatine, ADP, H_+, inorganic phosphate, glycogen) and enhancing exercise tolerance (5–7). Therefore, our data suggest CFs may lower the O_2 deficit incurred during moderate-intensity activity by negating age-associated impairments to O_2 delivery and pulmonary $\dot{V}O_2$ kinetics.

Since the purpose of the study was to examine the impact of CFs on $\dot{V}O_2$ kinetics, our data raise the question about the potential underlying mechanisms contributing to the lowered $\tau\dot{V}O_2$ with CF supplementation. It is acknowledged $\tau\dot{V}O_2$ is sensitive to manipulations in O_2 delivery (36,37). Further, the slowing of $\dot{V}O_2$ kinetics with advancing age occurs primarily as a consequence of lowered O_2 availability in oxidative skeletal muscle (2,8,10). Given that CFs exert potent NO-dependent vasodilatory effects (25,27,38,39), CF supplementation may have sped $\dot{V}O_2$ kinetics by augmenting muscle blood flow and O_2 availability. In addition, it is important to acknowledge CFs have powerful antioxidant properties (40,41), which may influence $\dot{V}O_2$ responses to exercise by quenching of reactive oxygen species and increasing NO bioavailability (42,43).

In spite of differences in the kinetics of $\dot{V}O_2$, no changes in the O_2 cost of moderate-intensity exercise were observed after CF supplementation. Similarly, Patel and colleagues (2015) demonstrated no significant reduction in $\dot{V}O_2$ during twenty minutes of moderate-intensity cycling after 14 days dark chocolate supplementation (44). Together these data contrast with those published employing alternate dietary means of augmenting NO bioavailability, such as dietary nitrate, which reduces the O_2 cost of moderate-intensity activity (17,45–48). Such discrepancies may be explained by recent evidence linking dietary nitrate to improved contractile function (49), an effect that has not been reported with CF supplementation. Possibly, the mechanisms by which CFs impact physiological responses to exercise relate to muscle O_2 delivery rather than contractile function.

Effects of CFs on the Physiological Responses to Severe-Intensity Exercise

In contrast to our observations during moderate-intensity exercise, acute CF supplementation had no measurable impact on pulmonary $\dot{V}O_2$ kinetics during severe-intensity cycling. For

361 instance, the $\tau\dot{V}O_2$ of the fundamental component was similar between PL and CF (62 vs. 60
362 s, respectively). The kinetics of $\dot{V}O_2$ are considered to be an important determinant of exercise
363 tolerance (5,50). In line with this principle, we observed no effect of CF supplementation on
364 T_{lim} during severe-intensity exercise. Whilst no previous studies have examined the impact of
365 CF supplementation on $\dot{V}O_2$ kinetics in the severe-intensity exercise domain, a number have
366 studied their effects on exercise performance. Our findings corroborate these data showing no
367 beneficial impact of acute or sub-chronic CF supplementation on time-trial or time-to-
368 exhaustion performance in healthy male adults (51–54).
369
370 Our data demonstrate divergent effects of CFs on $\dot{V}O_2$ kinetics between moderate- and severe-
371 intensity exercise domains. Given that the pattern of muscle-fibre activation within moderate-
372 and severe-intensity exercise domains differs (type I and type II predominant, respectively),
373 future studies should investigate a potential muscle fibre-type dependency of CF
374 supplementation on the physiological responses to exercise. Another potential explanation for
375 the differences between exercise intensity domains presented herein relates to the dose of CFs
376 administered. Recent published evidence suggests that the 400 mg CF prescribed is the
377 minimum dose necessary to exert beneficial effects during exercise (39). Therefore, the dose
378 used in the present study may not have been high enough to raise blood flow sufficiently during
379 severe-intensity exercise to detect a measurable effect upon $\dot{V}O_2$ kinetics. In addition, CFs had
380 no beneficial impact on resting systolic or diastolic blood pressure over PL, which may be
381 attributable to insufficient dosage (55). Besides, another limitation of the study is that only a
382 single bout of severe-intensity exercise was conducted. As we were unable to feasibly include
383 additional visits for testing, we could not carry out multiple severe-intensity bouts to enhance
384 the signal-to-noise ratio of these $\dot{V}O_2$ responses and potentially detect differences between
385 conditions.

CONCLUSION

In the present study, seven days supplementation with a flavanol-rich cocoa-extract resulted in a reduced $\tau\dot{V}O_2$ following moderate-, but not severe-intensity exercise in normotensive, middle-aged adults. Whilst the O_2 cost of exercise was similar between CF and PL, the faster $\dot{V}O_2$ kinetics response at the onset of moderate-intensity exercise suggests an improvement in O_2 delivery with acute CF intake. Such effects were not found with severe-intensity exercise. Overall, CF administration may reduce the metabolic perturbations associated with moderate-intensity exercise in middle-aged adults.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures conformed to the Declaration of Helsinki and were approved by Liverpool John Moores University Research Ethics Committee (approval reference number: 18/SPS/014).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

Daniel G. Sadler, Claire E. Stewart, Helen Jones, Simon Marwood and Dick H. J. Thijssen had no conflict of interest associated with this manuscript. Richard Draijer is employed by Unilever R & D Vlaardingen, The Netherlands.

FUNDING

The study was supported by funding received from the Biotechnology and Biological Sciences Research Council (BBSRC) and Unilever R & D Vlaardingen, The Netherlands.

AUTHORS' CONTRIBUTIONS

DGS, CS, HJ and DHJT conceived and designed the project, DGS collected and analysed the data, DGS, HJ, CS, RD, SM and DHJT interpreted the data. DGS drafted the manuscript and all authors revised it critically. All authors provided final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All people designated as authors qualify for authorship, and all those who qualify for authorship are listed. DHJT is the guarantor for the work and/or conduct of the study, had full access to all the data in the study and takes responsibility for the integrity of data and the accuracy of the data analysis, and controlled the decision to publish.

ACKNOWLEDGEMENTS

The authors would like to thank all participants for their contributions to this study.

REFERENCES

1. Poole DC, Barstow TJ, Mcdonough P, Jones AM. Control of oxygen uptake during exercise. Medicine and Science in Sports and Exercise. 2008.

2. DeLorey DS, Kowalchuk JM, Paterson DH. Effect of age on O2 uptake kinetics and the adaptation of muscle deoxygenation at the onset of moderate-intensity cycling exercise. J Appl Physiol. 2004;

3. Dumanoir GR, Delorey DS, Kowalchuk JM, Paterson DH. Differences in exercise limb blood flow and muscle deoxygenation with age: Contributions to O2 uptake kinetics. Eur J Appl Physiol. 2010;

4. Whipp BJ, Rossiter HB. The kinetics of oxygen uptake: Physiological inferences from the parameters. In: Oxygen Uptake Kinetics in Sport, Exercise and Medicine. 2013.

5. Grassi B, Porcelli S, Salvadego D, Zoladz JA. Slow V̇O2 kinetics during moderate-intensity exercise as markers of lower metabolic stability and lower exercise tolerance. Eur J Appl Physiol. 2011;

6. Goulding RP, Roche DM, Marwood S. “Work-to-Work” exercise slows pulmonary oxygen uptake kinetics, decreases critical power, and increases W’ during supine cycling. Physiol Rep. 2018;

7. Goulding RP, Roche DM, Marwood S. Prior exercise speeds pulmonary oxygen uptake kinetics and increases critical power during supine but not upright cycling. Exp Physiol. 2017;

8. Behnke BJ, Delp MD. Aging blunts the dynamics of vasodilation in isolated skeletal muscle resistance vessels. J Appl Physiol. 2010;

9. Muller-Delp JM, Spier SA, Ramsey MW, Delp MD. Aging impairs endothelium-

454 dependent vasodilation in rat skeletal muscle arterioles. Am J Physiol - Hear Circ
455 Physiol. 2002;

456 10. Musch TI, Eklund KE, Hageman KS, Poole DC. Altered regional blood flow
457 responses to submaximal exercise in older rats. J Appl Physiol. 2004;

458 11. Woodman CR, Price EM, Laughlin MH. Aging induces muscle-specific impairment of
459 endothelium-dependent dilation in skeletal muscle feed arteries. J Appl Physiol. 2002;

460 12. Sindler AL, Delp MD, Reyes R, Wu G, Muller-Delp JM. Effects of ageing and
461 exercise training on eNOS uncoupling in skeletal muscle resistance arterioles. J
462 Physiol. 2009;

463 13. Spier SA, Delp MD, Meininger CJ, Donato AJ, Ramsey MW, Muller-Delp JM. Effects
464 of ageing and exercise training on endothelium-dependent vasodilation and structure
465 of rat skeletal muscle arterioles. J Physiol. 2004;

466 14. Belman MJ, Gaesser GA. Exercise training below and above the lactate threshold in
467 the elderly. Med Sci Sports Exerc. 1991;

468 15. Green DJ, Maiorana A, O'Driscoll G, Taylor R. Effect of exercise training on
469 endothelium-derived nitric oxide function in humans. Journal of Physiology. 2004.

470 16. Maiorana A, O'Driscoll G, Cheetham C, Dembo L, Stanton K, Goodman C, et al. The
471 effect of combined aerobic and resistance exercise training on vascular function in
472 type 2 diabetes. J Am Coll Cardiol. 2001;

473 17. Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, et al.
474 Acute and chronic effects of dietary nitrate supplementation on blood pressure and the
475 physiological responses to moderate-intensity and incremental exercise. Am J Physiol
476 - Regul Integr Comp Physiol. 2010;

18. Bahra M, Kapil V, Pearl V, Ghosh S, Ahluwalia A. Inorganic nitrate ingestion improves vascular compliance but does not alter flow-mediated dilatation in healthy volunteers. In: Nitric Oxide - Biology and Chemistry. 2012.

19. Breese BC, Mcnarry MA, Marwood S, Blackwell JR, Bailey SJ, Jones AM. Beetroot juice supplementation speeds O2 uptake kinetics and improves exercise tolerance during severe-intensity exercise initiated from an elevated metabolic rate. Am J Physiol - Regul Integr Comp Physiol. 2013;

20. Bailey SJ, Varnham RL, DiMenna FJ, Breese BC, Wylie LJ, Jones AM. Inorganic nitrate supplementation improves muscle oxygenation, O2 uptake kinetics, and exercise tolerance at high but not low pedal rates. J Appl Physiol. 2015;

21. Murias JM, Kowalchuk JM, Peterson DH. Speeding of Vo2 kinetics with endurance training in old and young men is associated with improved matching of local O2 delivery to muscle O2 utilization. J Appl Physiol. 2010;

22. Berry NM, Davison K, Coates AM, Buckley JD, Howe PRC. Impact of cocoa flavanol consumption on blood pressure responsiveness to exercise. Br J Nutr. 2010;

23. Davison K, Coates AM, Buckley JD, Howe PRC. Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects. Int J Obes. 2008;

24. Ramirez-Sanchez I, Maya L, Ceballos G, Villarreal F. (-)-Epicatechin activation of endothelial cell endothelial nitric oxide synthase, nitric oxide, and related signaling pathways. Hypertension. 2010;

25. Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, et al. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc Natl Acad Sci U S A. 2006;

501 26. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic
502 threshold by gas exchange. J Appl Physiol. 2016;

503 27. Cifuentes-Gomez T, Rodriguez-Mateos A, Gonzalez-Salvador I, Alañon ME, Spencer
504 JPE. Factors Affecting the Absorption, Metabolism, and Excretion of Cocoa Flavanols
505 in Humans. In: Journal of Agricultural and Food Chemistry. 2015.

506 28. Gerbino A, Ward SA, Whipp BJ. Effects of prior exercise on pulmonary gas-exchange
507 kinetics during high-intensity exercise in humans. J Appl Physiol. 1996;

508 29. Burnley M, Jones AM, Carter H, Doust JH. Effects of prior heavy exercise on phase II
509 pulmonary oxygen uptake kinetics during heavy exercise. J Appl Physiol. 2000;

510 30. Beaver WL, Lamarra N, Wasserman K. Breath-by-breath measurement of true alveolar
511 gas exchange. J Appl Physiol Respir Environ Exerc Physiol. 1981;

512 31. Lamarra N, Whipp BJ, Ward SA, Wasserman K. Effect of interbreath fluctuations on
513 characterizing exercise gas exchange kinetics. J Appl Physiol. 1987;

514 32. Benson AP, Bowen TS, Ferguson C, Murgatroyd SR, Rossiter HB. Data collection,
515 handling, and fitting strategies to optimize accuracy and precision of oxygen uptake
516 kinetics estimation from breath-by-breath measurements. J Appl Physiol. 2017;

517 33. McNarry MA, Kingsley MIC, Lewis MJ. Influence of exercise intensity on pulmonary
518 oxygen uptake kinetics in young and late middle-aged adults. Am J Physiol - Regul
519 Integr Comp Physiol. 2012;

520 34. Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR, Whipp BJ. Effects of
521 prior exercise on oxygen uptake and phosphocreatine kinetics during high-intensity
522 knee-extension exercise in humans. J Physiol. 2001;

523 35. Grassi B, Porcelli S, Marzorati M, Lanfranconi F, Vago P, Marconi C, et al. Metabolic

myopathies: Functional evaluation by analysis of oxygen uptake kinetics. Med Sci Sports Exerc. 2009;

36. Poole DC, Jones AM. Oxygen uptake kinetics. Compr Physiol. 2012;

37. Poole DC, Musch TI. Mechanistic insights into how advanced age moves the site of V̇O 2 kinetics limitation upstream. Journal of Applied Physiology. 2010;

38. Quiñones M, Sánchez D, Muguerza B, Moulay L, Laghi S, Miguel M, et al. Long-term intake of CocoanOX attenuates the development of hypertension in spontaneously hypertensive rats. Food Chem. 2010;

39. Decroix L, Soares DD, Meeusen R, Heyman E, Tonoli C. Cocoa Flavanol Supplementation and Exercise: A Systematic Review. Sports Medicine. 2018.

40. Lee KW, Kim YJ, Lee HJ, Lee CY. Cocoa Has More Phenolic Phytochemicals and a Higher Antioxidant Capacity than Teas and Red Wine. J Agric Food Chem. 2003;

41. Arteel GE, Sies H. Protection against peroxynitrite by cocoa polyphenol oligomers. FEBS Lett. 1999;

42. Donato AJ, Uberoi A, Bailey DM, Wray DW, Richardson RS. Exercise-induced brachial artery vasodilation: Effects of antioxidants and exercise training in elderly men. Am J Physiol - Hear Circ Physiol. 2010;

43. Wray DW, Uberoi A, Lawrenson L, Bailey DM, Richardson RS. Oral antioxidants and cardiovascular health in the exercise-trained and untrained elderly: A radically different outcome. Clin Sci. 2009;

44. Patel RK, Brouner J, Spendiff O. Dark chocolate supplementation reduces the oxygen cost of moderate intensity cycling. J Int Soc Sports Nutr. 2015;

45. Lansley KE, Winyard PG, Fulford J, Vanhatalo A, Bailey SJ, Blackwell JR, et al.

547 Dietary nitrate supplementation reduces the O2 cost of walking and running: A
548 placebo-controlled study. J Appl Physiol. 2011;

549 46. Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, et al.
550 Dietary nitrate supplementation enhances muscle contractile efficiency during knee-
551 extensor exercise in humans. J Appl Physiol. 2010;

552 47. Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, et al.
553 Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and
554 enhances tolerance to high-intensity exercise in humans. J Appl Physiol. 2009;

555 48. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Effects of dietary nitrate on oxygen
556 cost during exercise. Acta Physiol. 2007;

557 49. Bailey SJ, Gandra PG, Jones AM, Hogan MC, Nogueira L. Incubation with sodium
558 nitrite attenuates fatigue development in intact single mouse fibres at physiological
559 PO2. J Physiol. 2019;

560 50. Whipp BJ, Ward SA. Pulmonary gas exchange dynamics and the tolerance to muscular
561 exercise: effects of fitness and training. The Annals of physiological anthropology =
562 Seiri Jinruigaku Kenkyūkai kaishi. 1992.

563 51. Peschek K, Pritchett R, Bergman E, Pritchett K. The effects of acute post exercise
564 consumption of two cocoa-based beverages with varying flavanol content on indices of
565 muscle recovery following downhill treadmill running. Nutrients. 2013;

566 52. Stellingwerff T, Godin JP, Chou CJ, Grathwohl D, Ross AB, Cooper KA, et al. The
567 effect of acute dark chocolate consumption on carbohydrate metabolism and
568 performance during rest and exercise. Appl Physiol Nutr Metab. 2014;

569 53. Davison G, Callister R, Williamson G, Cooper KA, Gleeson M. The effect of acute
570 pre-exercise dark chocolate consumption on plasma antioxidant status, oxidative stress

and immunoendocrine responses to prolonged exercise. Eur J Nutr. 2012;

54. Allgrove J, Farrell E, Gleeson M, Williamson G, Cooper K. Regular dark chocolate consumption's reduction of oxidative stress and increase of free-fatty-acid mobilization in response to prolonged cycling. Int J Sport Nutr Exerc Metab. 2011;

55. Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, et al. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: A systematic review and meta-analysis of randomized trials. Am J Clin Nutr. 2012;

TABLE LEGENDS

Table 1. Heart rate and blood lactate responses during moderate- and severe-intensity exercise following CF and PL supplementation

Table 2. Pulmonary O_2 uptake responses to moderate- and severe-intensity exercise following CF and PL supplementation

FIGURE LEGENDS

Figure 1. CONSORT diagram showing the flow of participants through each stage of the randomised trial.

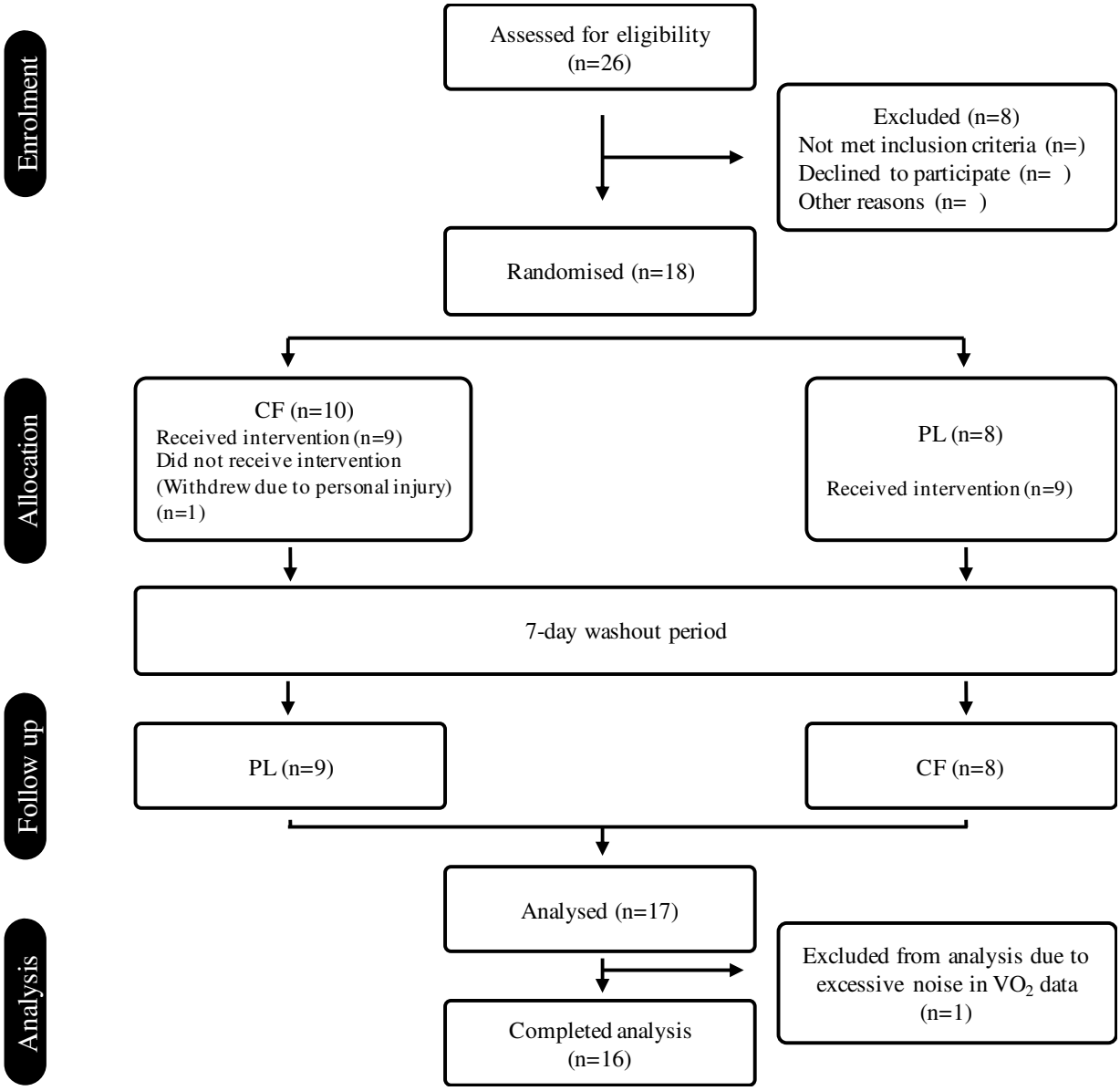
Figure 2. Schematic of experimental design.

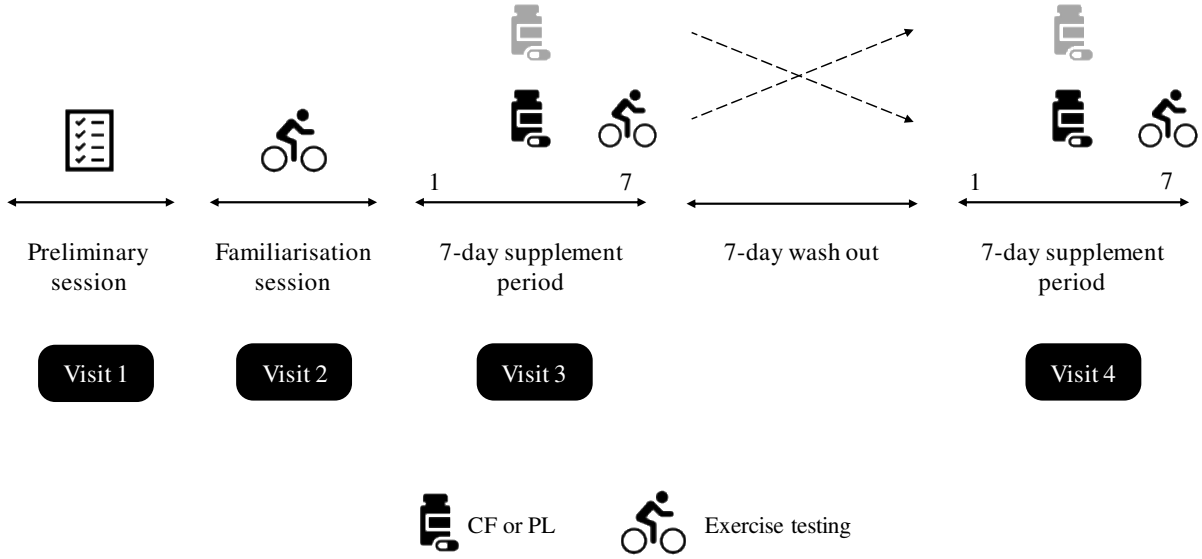
593 **Figure 3.** Pulmonary $\dot{V}O_2$ and best-fit modelled responses of a representative participant to
594 moderate-intensity exercise following PL (solid black circles) and CF (clear circles)
595 supplementation. $\tau\dot{V}O_2$ values are displayed for each transition, with the solid grey lines
596 representing the modelled fits.

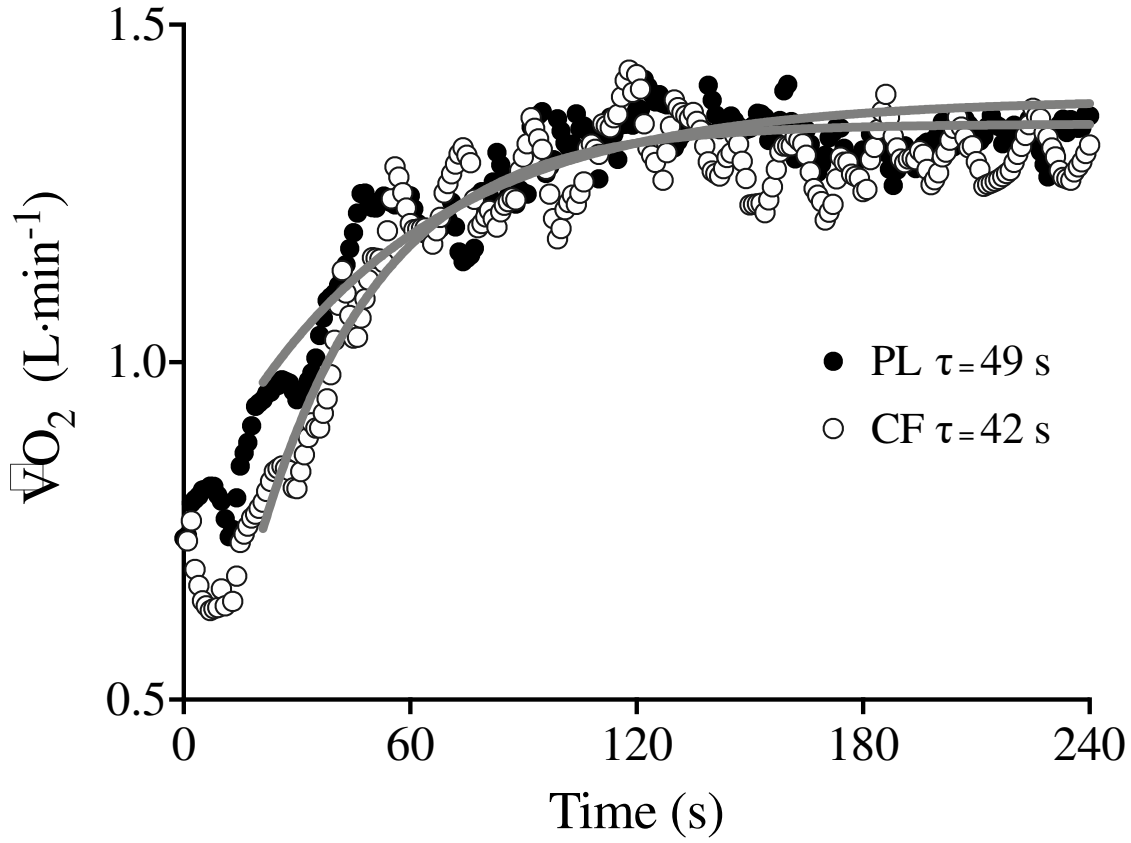
597

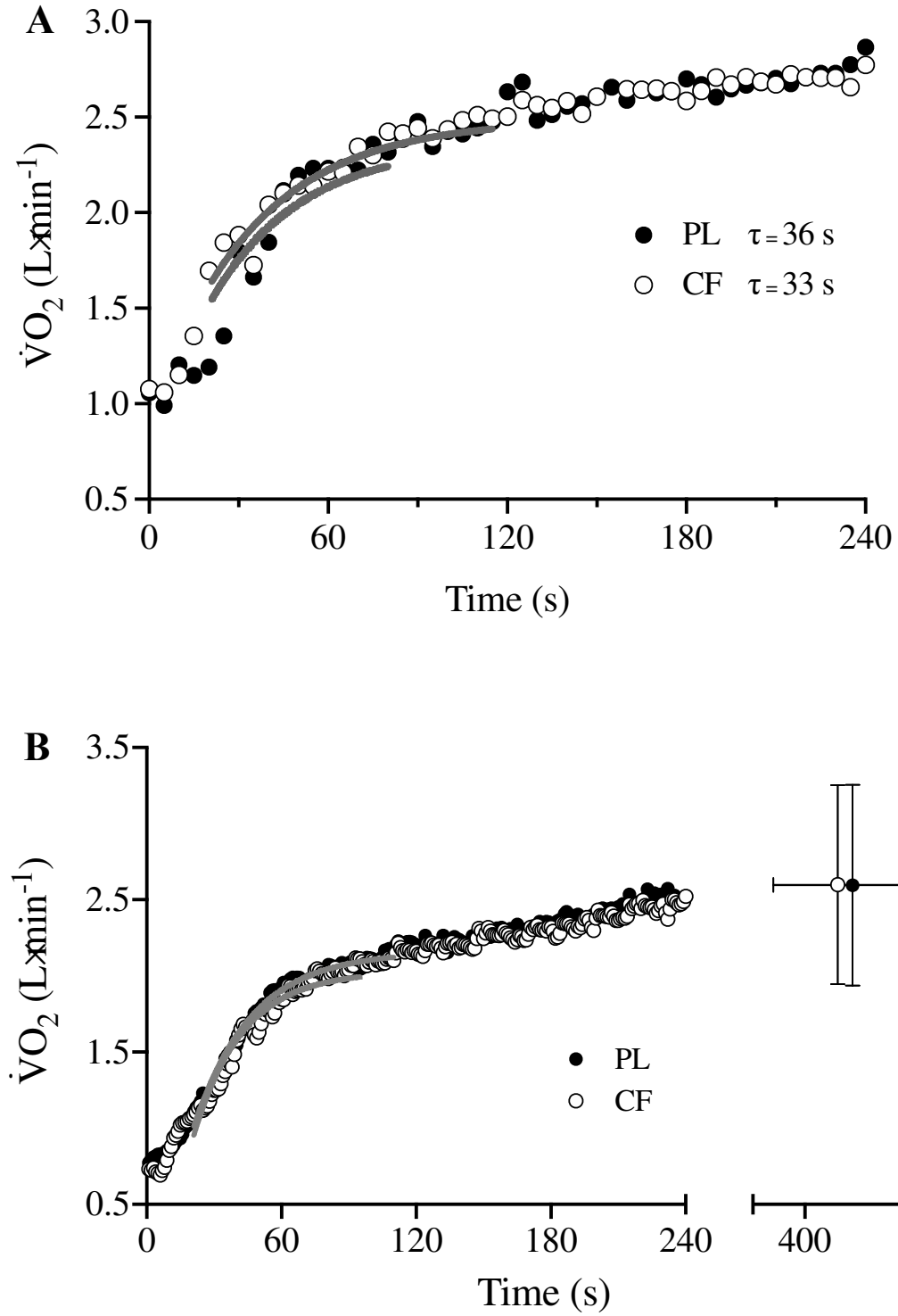
598 **Figure 4.** Pulmonary $\dot{V}O_2$ and best-fit modelled responses to severe-intensity exercise
599 following PL (solid black squares) and CF (clear black squares) supplementation. Panel A)
600 Pulmonary $\dot{V}O_2$ responses of a representative participant displayed with associated $\tau\dot{V}O_2$.
601 Panel B) Group mean $\dot{V}O_2$ responses during the rest-to-exercise transition following PL and
602 CF supplementation. Group mean ± SD $\dot{V}O_2$ at limit of exercise tolerance also shown. Solid
603 grey lines represent the modelled fits.

604
605


Enrolment
Assessed for eligibility (n=26)
Excluded (n=8)
Not met inclusion criteria (n=)
Declined to participate (n=)
Other reasons (n=)
Randomised (n=18)
Allocation
CF (n=10)
Received intervention (n=9)
Did not receive intervention (Withdrew due to personal injury) (n=1)
PL (n=8)
Received intervention (n=9)
7-day washout period
Follow up
PL (n=9)
CF (n=8)
Analysis
Analysed (n=17)
Excluded from analysis due to excessive noise in VO_2 data (n=1)
Completed analysis (n=16)


1
7
1
7
Preliminary session
Familiarisation session
7-day supplement period
7-day wash out
7-day supplement period
Visit 1
Visit 2
Visit 3
Visit 4
CF or PL
Exercise testing


1.5
1.0
0.5
$\dot{V}O_2$ (L·min^{-1})
0
60
120
180
240
Time (s)
PL τ = 49 s
CF τ = 42 s


A
B
PL τ = 36 s
CF τ = 33 s
PL
CF
Time (s)
Time (s)

Figures

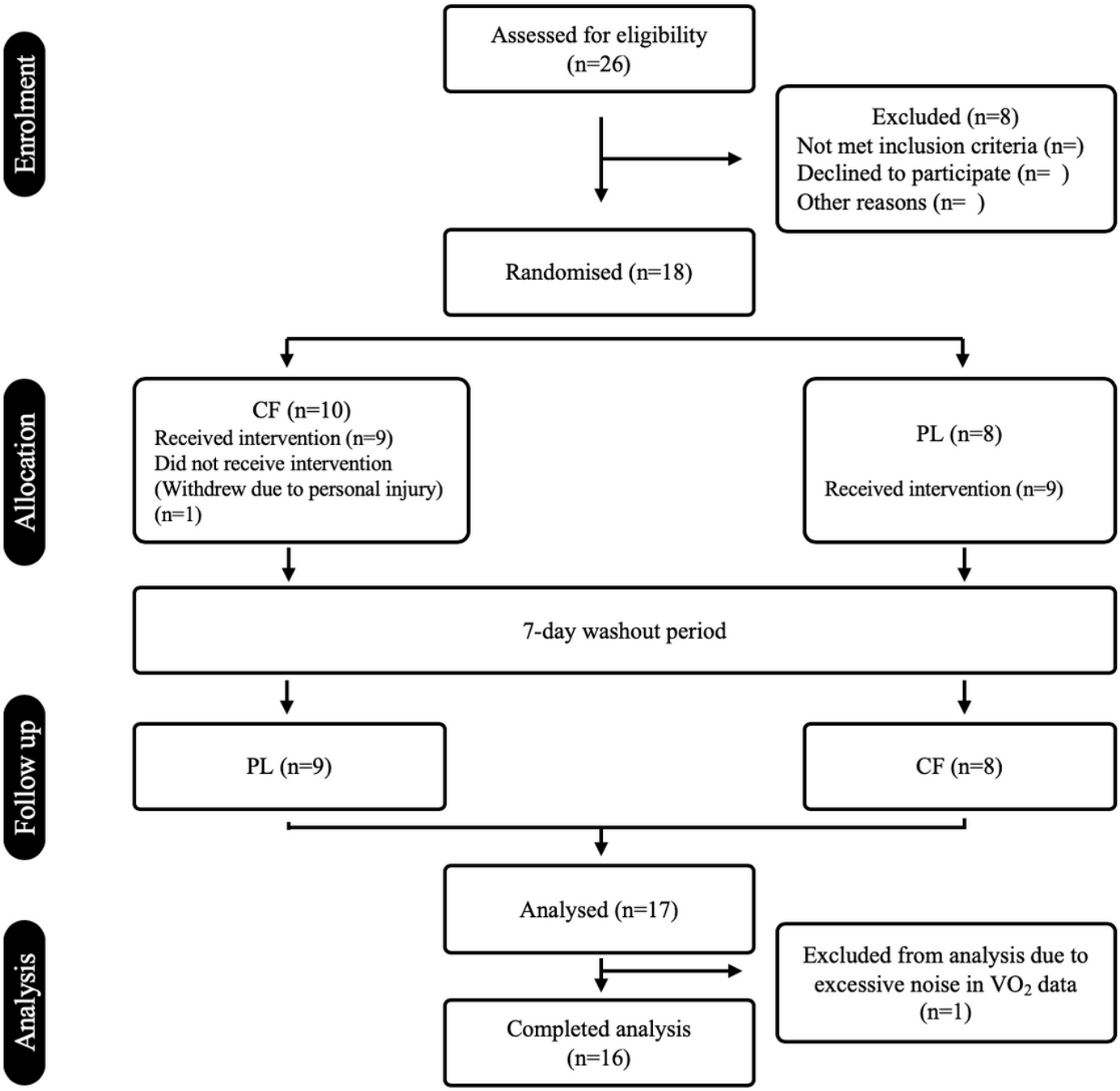


Figure 1

CONSORT diagram showing the flow of participants through each stage of the randomised trial.

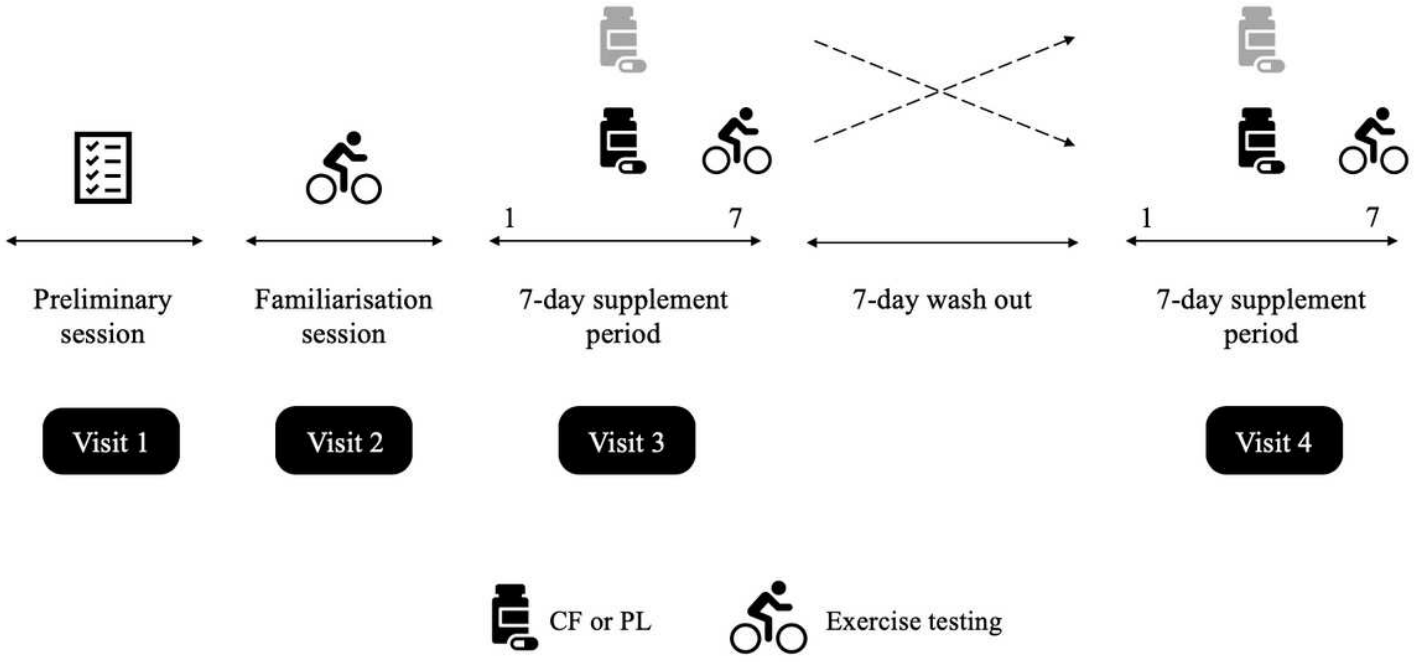


Figure 2

Schematic of experimental design.

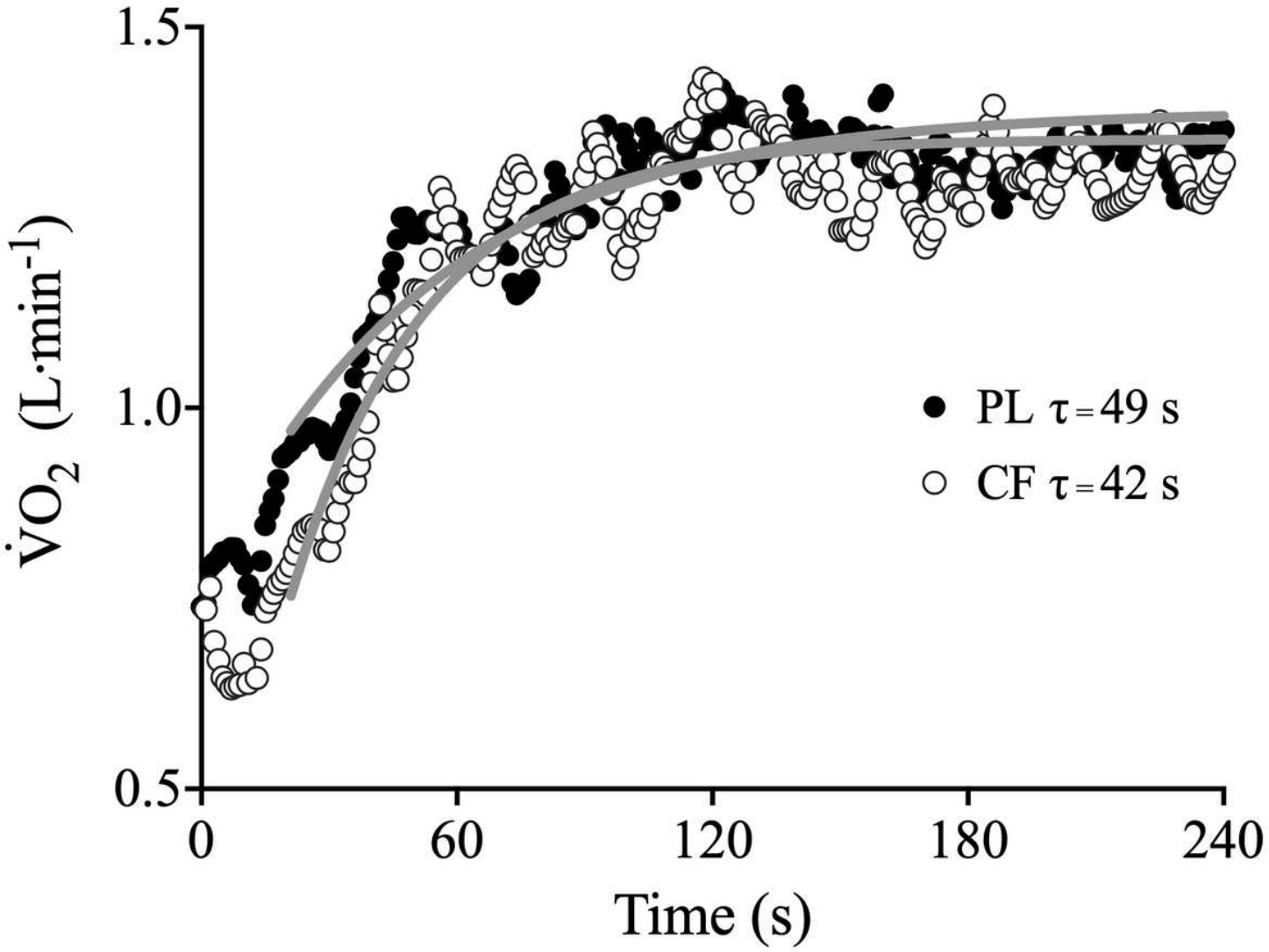


Figure 3

Pulmonary V̇O2 and best-fit modelled responses of a representative participant to moderate-intensity exercise following PL (solid black circles) and CF (clear circles) supplementation. τV̇O2 values are displayed for each transition, with the solid grey lines representing the modelled fits.

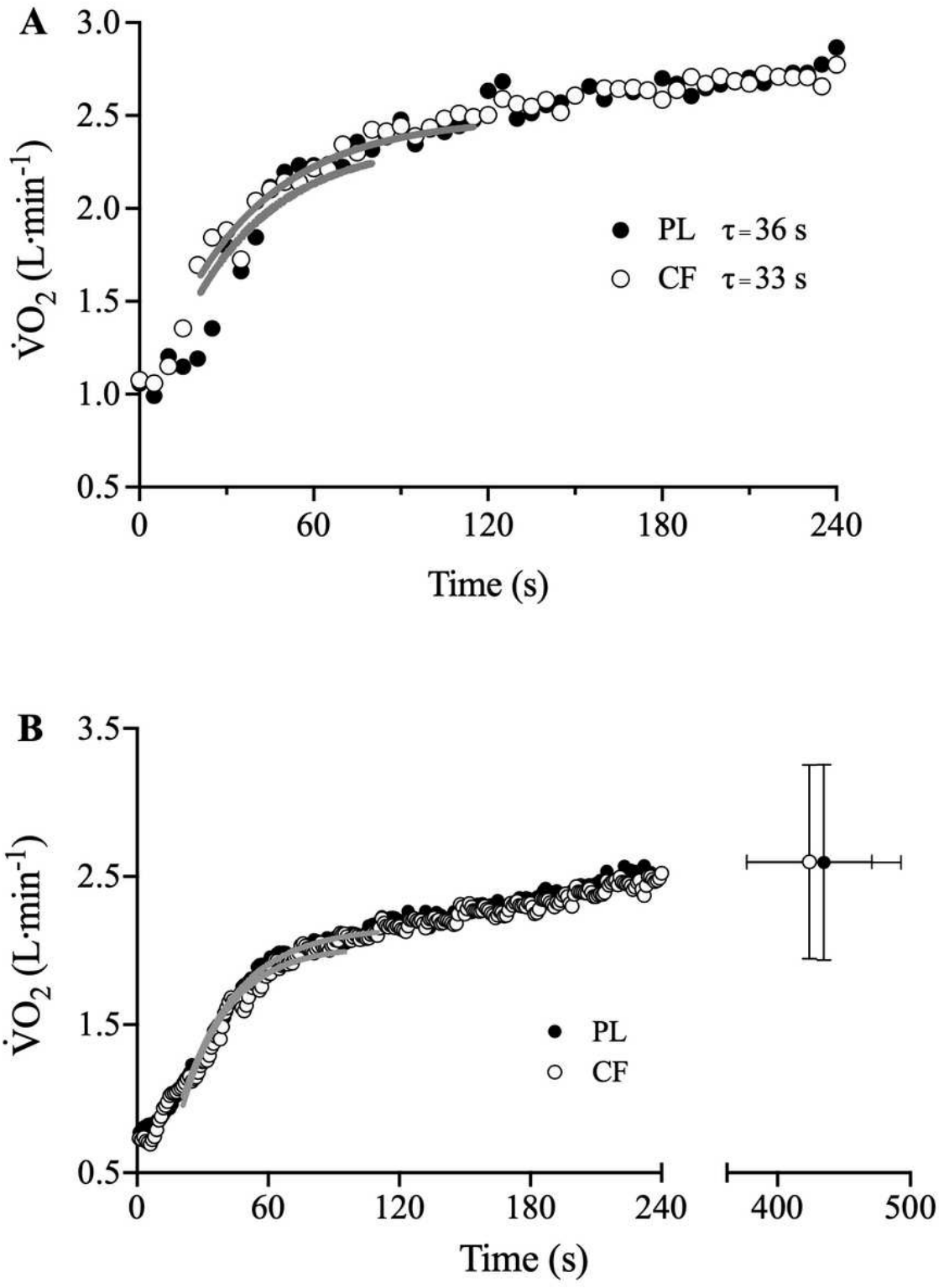


Figure 4

Pulmonary V̇O2 and best-fit modelled responses to severe-intensity exercise following PL (solid black squares) and CF (clear black squares) supplementation. Panel A) Pulmonary V̇O2 responses of a representative participant displayed with associated τV̇O2. Panel B) Group mean V̇O2 responses during the rest-to-exercise transition following PL and CF supplementation. Group mean ± SD V̇O2 at limit of exercise tolerance also shown. Solid grey lines represent the modelled fits.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- OnlineSupplementaryinformationfile.docx