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TITLE

Influence of priming exercise on oxygen uptake and muscle deoxygenation kinetics during moderate-intensity cycling in type 2 diabetes.

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RUNNING HEAD: Fractional O_2 extraction & $\dot{V}O_2$ dynamics following PE in T2D

NEW AND NOTEWORTHY (75 words):

Heavy-intensity 'priming' exercise (PE) elicited faster pulmonary oxygen uptake ($\dot{V}O_2$) kinetics during moderate-intensity cycling exercise in middle-aged individuals with type 2 diabetes (T2D). This was accompanied by greater near–infrared spectroscopy-derived muscle deoxygenation (i.e. deoxygenated haemoglobin and myoglobin concentration, [HHb+Mb]) responses and a reduced Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio. This suggests that the PE-induced acceleration in oxidative metabolism in T2D is as a result of greater O_2 extraction and better matching between O_2 delivery and utilisation.

Abstract

The pulmonary oxygen uptake ($\dot{V}O_2$) kinetics during the transition to moderate-intensity exercise is slowed in individuals with type 2 diabetes (T2D), at least in part due to limitations in O_2 delivery. The present study tested the hypothesis that a prior heavy-intensity warm-up or 'priming exercise' (PE) bout would accelerate $\dot{V}O_2$ kinetics in T2D, due to a better matching of O_2 delivery to utilisation. Twelve middle-aged individuals with T2D and 12 healthy controls (ND) completed moderate-intensity constant-load cycling bouts either without (ModA) or with (ModB) prior PE. The rate of muscle deoxygenation (i.e. deoxygenated haemoglobin and myoglobin concentration, [HHb+Mb]) and oxygenation (i.e. total oxygenation index, TOI) were continuously measured by near-infrared spectroscopy at the vastus lateralis muscle. The local matching of O_2 delivery to O_2 utilization was assessed by the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio. Both groups demonstrated an accelerated $\dot{V}O_2$ kinetics response during ModB compared with ModA (T2D: 32±9 *vs.* 42±12 s; ND: 28±9 *vs.* 34±8 s), and an elevated muscle oxygenation throughout ModB, while the [HHb+Mb] amplitude was greater during ModB only in individuals with T2D. The [HHb+Mb] kinetics remained unchanged in both groups. In T2D ModB was associated with a decrease in the 'overshoot' relative to steady-state in the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio (1.17±0.17 *vs.* 1.05±0.15), while no overshoot was observed in the control group prior to (1.04±0.12) or after (1.01±0.12) PE. Our findings support a favourable priming-induced acceleration of the $\dot{V}O_2$ kinetics response in middle-aged individuals with uncomplicated T2D attributed to an enhanced matching of microvascular O_2 delivery to utilisation.

Keywords: near-infrared spectroscopy, oxygen extraction, cycling, exercise tolerance, priming exercise

Introduction

Young and middle-aged individuals with uncomplicated type 2 diabetes (T2D) demonstrate a slowed adjustment of oxidative metabolism at the onset of moderate-intensity exercise represented by a prolonged time constant of the primary phase of oxygen uptake ($\dot{V}O_2$) kinetics ($\tau\dot{V}O_{2p}$) (3, 30, 39, 48, 49, 59). This means that individuals with T2D exhibit an increased oxygen deficit placing greater reliance on non-oxidative energy sources to sustain any given activity (26). Clinically these findings are important because it is likely that they contribute to premature muscular fatigue (65), and reduced exercise tolerance in individuals with T2D (19), which is associated with an increased risk of cardiovascular outcomes and all-cause mortality (33, 66).

The aetiology of impaired $\dot{V}O_2$ responses in T2D is not well understood but it must relate to O_2 delivery to, and/or O_2 dependent metabolism within contracting muscle. In healthy active populations that present with an initial fast $\dot{V}O_2$ kinetics response (<~20s) during moderate-intensity cycling exercise, the $\tau\dot{V}O_{2p}$ appears to be limited by intracellular mechanisms (i.e. oxidative capacity of skeletal muscle) rather than O_2 delivery (54). However, $\dot{V}O_2$ kinetics in young and middle-aged individuals with T2D appear to be impaired, at least in part, due to limitations in O_2 delivery/supply to contracting muscle (3, 29, 32, 37, 41, 52), although defects in O_2 extraction have also been observed (28, 60). For instance, Bauer et al. (3) observed reduced microvascular blood flow responses (calculated using $\tau\dot{V}O_{2p}$ divided by near-infrared spectroscopy (NIRS)-derived quadriceps muscle deoxygenated haemoglobin and myoglobin, [HHb+Mb], responses) in individuals with T2D at the onset of moderate-intensity cycling exercise, which has been attributed to a relative mismatch in muscle O_2 delivery to $\dot{V}O_2$. In addition, leg vascular conductance kinetics during calf plantar-flexion exercise are slowed (29, 41), steady-state femoral artery blood flow responses during cycling

and knee extension exercise are reduced (32, 37) and endothelium-dependent vasodilation of the resting femoral and brachial arteries is blunted (32, 43), in uncomplicated individuals with T2D, suggesting a maldistribution of active muscle blood flow in this clinical population. However, findings of reduced O_2 delivery as the source of the impairment in $\dot{V}O_2$ control in T2D are not unanimous (11, 53, 70), likely owing to varied methodology in exercise modalities, participant characteristics or animal models used.

Heavy-intensity priming exercise (PE, or heavy warm-up) is an intervention that reduces $\tau\dot{V}O_{2p}$ during subsequent moderate-intensity step transitions in older healthy individuals (12, 13, 64), a population consistently demonstrating slowed $\tau\dot{V}O_{2p}$ responses (45, 56), and in young healthy individuals that present with an initially slow $\dot{V}O_2$ kinetics response (9, 17, 21, 22, 46). However, this is not observed in young individuals presenting initially with fast $\tau\dot{V}O_{2p}$ responses (13, 64). These PE-induced reductions in $\tau\dot{V}O_{2p}$ have been reported to be associated with improved local muscle oxygenation (13, 22) and/or elevated activity of the mitochondrial pyruvate dehydrogenase (PDH) complex (20, 21). However, activation of PDH prior to the onset of exercise via administration of dichloroacetate in the absence of augmented O_2 delivery failed to demonstrate a faster $\tau\dot{V}O_{2p}$ (36, 61). On the contrary, PE-induced speeding of $\tau\dot{V}O_{2p}$ was associated with a smaller NIRS-derived muscle Δ[HHb+Mb] to $\dot{V}O_2$ ratio (i.e. reduced Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio) throughout the exercise on-transient in young (46) and older (12) individuals, suggesting that the matching of microvascular O_2 delivery to utilisation plays a key role in limiting $\tau\dot{V}O_{2p}$ during moderate-intensity exercise when the initial $\dot{V}O_2$ kinetics is slow.

The present study aimed to investigate the influence of heavy-intensity PE on oxygen uptake and muscle oxygenation & deoxygenation kinetics on a subsequent moderate-intensity

submaximal exercise bout in T2D. We hypothesized that in middle-aged adults with T2D, PE would speed the $\dot{V}O_2$ kinetics response and reduce the $\Delta[HHb+Mb]/\Delta\dot{V}O_2$ ratio (i.e. reflecting a better matching of O_2 delivery to O_2 utilization) in a subsequent bout of moderate-intensity cycling exercise. To avoid the potential confounding effects of age on the T2D-related impairments on exercise tolerance, previously established in men (48, 69), we limited the age of participants to < 60 yr.

Methods

Participants

Twelve individuals with uncomplicated T2D (7 males/5 females) and 12 healthy controls (7 males/5 females) volunteered to participate in this study (Table 1). Participants in the control group (ND) were recruited from the general population, whilst participants with T2D were recruited from the Diabetes Outpatient Clinics of St. Columcille's Hospital (Louglinstown, Co. Dublin) and St. Vincent's University Hospital (SVUH, Dublin 4) following chart review. Four female participants were premenopausal (2 T2D and 2 ND) and six were postmenopausal (3 T2D and 3 ND). Participants were classified as untrained by self-report ($\leq$1.5 h.week^{-1} of moderate-intensity exercise in the preceding 6 months), which was confirmed by the use of 5-day RT3 triaxial accelerometry (Stayhealthy Inc, CA) in a subset of participants (Table 1) (62). All participants with T2D had a clinical history of diabetes between 2 and 10 years (mean ± SD = 5.9 ± 4.2 yrs.), were treated by oral hypoglycaemic agents and had adequately controlled HbA_{1c} levels (<10%). None of the participants with T2D was taking insulin or beta-blockers and all participants were non-smokers (had not smoked during the 12-month period preceding the study). Two of the healthy controls were on prescriptive medications (statins, n = 2), and individuals with T2D were taking oral (n = 10) and/or subcutaneous (n =2) hypoglycaemic prescription medications (metformin

monotherapy, n = 9; metformin & sulphonylurea, n = 1; glucagon-like peptide 1, n = 2). In addition, a subgroup of individuals with T2D was taking antihypertensive prescription drugs (angiotensin converting enzyme inhibitor, n = 3; angiotensin II receptor blocker, n = 2; calcium channel blocker, n = 3) and statins (n =5). All patients displayed no clinical evidence of cardiovascular disease (12-lead electrocardiogram treadmill stress test following the Bruce protocol), peripheral arterial disease (0.9 < Ankle-Brachial Index, ABI, < 1.3), kidney dysfunction (consistent urinary protein > 200 $mg \cdot dl^{-1}$) or liver dysfunction (urinary creatinine levels > 2.2 $mg \cdot dl^{-1}$). All participants provided written informed consent prior to participation. The study was approved by the Faculty of Health Sciences' Research Ethics Committee, Trinity College Dublin, and St Vincent's Healthcare Ethics and Medical Research Committee, and conducted in accordance with the principles outlined by the Declaration of Helsinki.

Study Protocol

Overview. Following the satisfactory completion of the 12-lead ECG stress test, all participants completed two visits to the laboratory. The controls undertook these tests in the cardiovascular performance laboratory in the Department of Physiology, Trinity College Dublin; whilst individuals with T2D did so in the exercise testing facility in St. Columcille's Hospital. All exercise tests were carried out in an upright position on an electrically braked cycle ergometer (Excalibur Sport; Lode B.V., Groningen, Netherlands). All participants were asked to refrain from consuming alcohol, caffeine and non-prescribed nutritional supplements as well as avoiding any strenuous exercise in the 24 hours prior to testing. All premenopausal participants were tested during the mid-follicular phase (days 5-12) of the menstrual cycle.

161 *Visit 1: Ramp incremental cycling test to exhaustion*. In the first visit all participants
162 performed a ramp incremental (RI) cycling test to exhaustion to determine $\dot{V}O_{2peak}$. The test
163 started with an initial workload of 10 W for 2 min (i.e. 'unloaded' cycling). This was
164 followed by 10/15 $W.min^{-1}$ increments in power output in women (*n*=2/8) or 15/20/25
165 $W.min^{-1}$ increments in men (*n*=5/8/1) based on participants' activity levels. Pedalling rate
166 was held constant at an individually selected cadence between 60-75 revolutions per minute
167 (rpm) and was maintained throughout all further testing. Failure in a test was determined as a
168 drop in cadence exceeding 10 rpm for >5 s. Peak workload was determined according to the
169 point of termination of the test. $\dot{V}O_{2peak}$ was determined by identifying the highest 15-s mean
170 $\dot{V}O_2$ value recorded before the participant's volitional termination of the test. The first
171 ventilatory threshold (VT) was determined as the $\dot{V}O_2$ at which $\dot{V}_E/\dot{V}O_2$ exhibited a
172 systematic exponential increase without a concomitant increase in $\dot{V}_E/\dot{V}CO_2$ (67) and the
173 deflection point of $\dot{V}CO_2$ vs. $\dot{V}O_2$ (V-slope method) during the RI test (1, 4). The first visit
174 lasted ~45-60 min.
175
176 *Visit 2: Priming effect on moderate-intensity cycling exercise*. In the second visit all
177 participants performed four bouts of constant-load moderate-intensity cycling at 80% of each
178 participant's VT obtained during the ramp incremental test. Two of these constant-load bouts
179 were completed without prior PE (Mod A) and two bouts were undertaken preceded by a
180 heavy- intensity PE bout (Mod B) at an intensity of 50% delta (Δ50%; the sum of the power
181 output at VT and 50% of the difference between the power output at VT and $\dot{V}O_{2peak}$). The
182 order of these bouts was fixed for all participants (Fig 1). The duration of each step transition
183 was 6 min and each transition was preceded by a 3 min 'baseline' cycling period at 10W.
184 There was a 12 min rest period between each of the cycling bouts, except following the first
185 primed moderate-intensity bout (Mod B) where participants remained seated in a chair for 45

186 min. This resting period has been shown to be sufficient for physiological parameters to
187 return to baseline levels, and therefore, not to influence $\dot{V}O_2$ kinetics responses during
188 subsequent exercise (8). Heart rate (HR), gas exchange/ventilatory variables and muscle
189 oxygenation & deoxygenation were continuously measured during each cycling bout. The
190 second visit lasted ~3 hours.
191
192 *Measurements*
193 During exercise, participants wore a facemask to continuously collect expired air using an
194 online metabolic system (Innocor, Innovision A/S, Odense, Denmark) that measured airflow
195 using a pneumotachometer. Carbon dioxide analysis was performed by using a photoacoustic
196 gas analyzer and oxygen was analyzed using an oxygen sensor (Oxigraf Inc., USA) based on
197 the principle of laser diode absorption spectroscopy. The volume was calibrated with a 3-litre
198 syringe, and the oxygen sensor was calibrated (against room air) prior to each test by the
199 researcher. Both the oxygen sensor and photoacoustic gas analyser require multi-point
200 calibration performed by the manufacturer periodically (6-12 months). Analysis of expired air
201 allowed determination of pulmonary O_2 uptake ($\dot{V}O_2$), CO_2 output ($\dot{V}CO_2$), minute
202 ventilation ($\dot{V}_E$) and the respiratory exchange ratio (RER) breath-by-breath. HR was recorded
203 every 5 s (Polar S610i, Polar Ltd, Finland), with peak HR defined as the highest HR attained
204 within the last 15 s of the point of termination of the test.
205
206 A continuous wave NIRS system (Hamamatsu Niro 200Nx; Hamamatsu Photonics,
207 Hamamatsu, Japan), was used to determine a muscle's oxygenation status non-invasively
208 through the spatially resolved spectroscopy (SRS) technique and modified Beer-Lambert
209 (MBL) principle with three wavelengths of emitting light (λ = 735, 810, and 850 nm). The
210 theoretical basis of NIRS and its use in exercise measurements have been described in detail

elsewhere (14) but briefly, this technique estimates the optical density changes of oxygenated (O_2Hb+Mb) and deoxygenated haemoglobin and myoglobin (HHb+Mb) based on the oxygen dependency of absorption changes for near-infrared light in these proteins. As the vastus lateralis (VL) muscle is a dominant locomotor muscle during cycling (38, 50), the present study examined the deoxy (Δ[HHb+Mb]) and tissue oxygenation index (TOI) profiles of the right quadriceps's vastus lateralis (VL) muscle. After shaving, cleaning and drying the skin, the probes were placed on the belly of the muscle, (5-8 cm above the lateral femoral condyle), parallel to the major axis of the thigh with a 3 cm spacing between the emitter and receiver. The probes were housed in a black rubber holder and secured on the skin surface with bi-adhesive tape and then covered with a dark elastic bandage, which minimised extraneous movement and the intrusion of stray light throughout the exercise protocol. Since the depth of the measured area was estimated to be approximately one-half the distance between the emitter and the receiver (~1.5 cm), the present study determined the thickness of the skin and adipose tissue at the site of the probe placement via 2D ultrasound operating in B-mode (Zonare Ultra Smart Cart, Software version 4.7, USA), to ensure that data largely represented absorption of near-infrared light in muscle tissue and not in subcutaneous fat. An exclusion criterion applied to individuals presenting with adiposity >1.5 cm over the site of interrogation on the vastus lateralis.

Data analysis

$\dot{V}O_2$ Kinetics: The breath-by-breath $\dot{V}O_2$ data for each transition were linearly interpolated to provide second-by-second values and time aligned such that time 0 represented the onset of exercise. Data from each transition were ensemble-averaged to yield a single, average response for each individual and further time-averaged into 5 s bins. Nine responses (from 6 participants) revealed a small slow component (Mod A: 3 T2D, 3 ND; Mod B: 2 T2D, 1 ND)

suggesting that the power outputs in these participants (3 participants showed a SC in both conditions, and 3 participans only during Mod A) were above their VT. This was likely due to the fact that in the present study the mean response times of $\dot{V}O_2$ during the ramp cycle exercise were not accounted for when calculating the target power outputs (27). Thus, the averaged and smoothed response for each participant was fitted to a monoexponential function (equation 1) or biexponential function (equation 2) as follows:

Equation 1 $$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + A_p[1\text{-}e^{-(t\text{-}TD_p)/\tau_p)}]\cdot F1$$

Equation 2 $$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + A_p[1\text{-}e^{-(t\text{-}TD_p)/\tau_p)}]\cdot F1 + A_s[1 - e^{-(t - TD_s)/\tau_s)}]\cdot F2$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t; $\dot{V}O_2$ baseline is the mean $\dot{V}O_2$ in the final 30 s of unloaded cycling; $A_p - A_s$, are the amplitudes of the increase in $\dot{V}O_2$ of the primary and slow component phases respectively; $TD_p - TD_s$ are the phase delays, and $\tau_p - \tau_s$ are the time constants, defined as the duration of time for which $\dot{V}O_2$ increases to a value equivalent to 63% of the amplitude. The conditional expressions F1-F2 limit the fitting of the phase to the period at and beyond the time delay associated with that phase. The first 20 s of data after the onset of exercise (i.e., the phase I $\dot{V}O_2$ response) were deleted, and while still allowing TD to vary freely (to optimize accuracy of parameter estimates), $\dot{V}O_2$ data were modelled from 20 s to 360 s of the step transition to ensure that each subject had attained a $\dot{V}O_2$ steady state (47). So, in this approach the TD is not used as a proxy for, nor is it synonymous with, phase I duration (47). The $\dot{V}O_2$ data were fitted to equation 1 or 2 using a weighted least-squares non-linear regression procedure (TableCurve 2D, Systat, USA). Data points lying outside the 95% prediction interval during the initial fit of a model were excluded. Parameter estimates of the best-fit function were used and only estimates representing the primary phase are presented. Whilst the presence of a slow component was detected in 9 responses, the presence of this phase does not appear to significantly affect the

parameter estimates of the earlier phases (68). The end-exercise $\dot{V}O_2$ response, referred to as End A, was calculated as the averaged $\dot{V}O_2$ over the last 30 s of the primary $\dot{V}O_2$ response. The functional "gain" of the primary $\dot{V}O_2$ response was calculated as the difference between End A and $\dot{V}O_2$ baseline normalized to the difference in power outputs between the moderate-intensity exercise and unloaded cycling.

Deoxygenated haemoglobin/myoglobin [HHb+Mb] and tissue oxygenation index (TOI) kinetics. To provide information on muscle deoxygenation throughout the protocol, we modelled the [HHb+Mb] and TOI response to exercise. As per the $\dot{V}O_2$ data, the NIRS-derived Δ[HHb+Mb] and TOI data for each transition were linearly interpolated to provide second-by-second values and time aligned. Data from each transition were ensemble-averaged to yield a single average response for each individual, and further time-averaged into 5 s bins. A time delay (TD) at the onset of exercise occurs in the [HHb+Mb] and TOI profiles before they increase and decrease, respectively. [HHb+Mb] data were fitted from the end of the TD to 180 s using *equation 1* as per $\dot{V}O_2$. The TOI data were fitted from the end of the TD to the lowest steady state within the first 180 s of exercise also using *equation 1*. The shorter fitting window of 180 s was selected to counteract the reported variations in the NIRS signal, which typically present between 180-240 s from exercise onset, from impacting the fitting of the on-transient response whilst permitting the reaching of a steady-state (15, 16, 46). The time course for the increase in Δ[HHb+Mb] and decrease in TOI can be described by the τΔ[HHb+Mb] and τTOI, however, the time course for the overall change of the Δ[HHb+Mb] and TOI responses, referred to as the effective response time (τ'Δ[HHb+Mb] and τ'TOI), was determined from the sum of the time delay and τ from the onset of exercise for each variable.

286 *Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio*. To calculate the Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio (44, 46) individual
287 second-by-second Δ[HHb+Mb] and $\dot{V}O_2$ data were firstly normalised (from 0%,
288 corresponding to the pre-transition 10W baseline value to 100% reflecting the post-transition
289 steady-state response). Then, Δ[HHb+Mb] and $\dot{V}O_2$ were time aligned by left-shifting the
290 normalised $\dot{V}O_2$ data by 20 s, accounting for the approximate duration of the cardiodynamic
291 phase, to ensure that the onset of exercise coincided with the beginning of the primary phase
292 of $\dot{V}O_2$. The normalised and time aligned data was then further averaged into 5 s bins for
293 statistical comparisons. The overall Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio for the adjustment during the
294 exercise on-transient was derived for each individual as the mean value from 20-120 s into
295 the transition. The commencement point of 20 s was selected as it is representative of the
296 region where the Δ[HHb+Mb] and $\dot{V}O_2$ signals meet, with the 120 s end point indicative of
297 the time point at which a steady-state value of 1.0 had been achieved by the
298 Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio (46). Values > 1.0 represent a time period whereby during the
299 exercise transition there was a greater reliance on fractional O_2 extraction compared with the
300 exercise steady-state (values = 1.0), thus reflecting a poorer local O_2 delivery relative to
301 muscle O_2 utilisation in the area of NIRS interrogation.
302
303 *Statistical analysis*
304 Statistical analysis was performed using SPSS for Windows (version 22.0, Chicago, IL).
305 Prior to analysis, normal distribution was assessed using the Shapiro-Wilk's test. Physical
306 characteristics between groups were compared using the unpaired Student's t-test for
307 parametric analyses, or the Mann-Whitney U test for non-parametric analyses. Based on *a*
308 *priori* evidence on the pre-determined reduced functional exercise capacity in individuals
309 with an uncomplicated T2D, the peak physiological responses between groups were
310 compared using unpaired 1-tailed Student's t-test for parametric analyses, or the Mann-

Whitney U test for non-parametric analyses. The Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio and kinetics parameter estimates for $\dot{V}O_2$, [HHb+Mb] and TOI during moderate-intensity exercise were analysed by using a two-way [Condition (Mod A, Mod B) x diabetes status (T2D, ND)] mixed model ANOVA. To assess whether the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio was different from 1 (i.e. to identify if there was a mismatch between local O_2 delivery relative to muscle O_2 utilisation) a Student's t-test was used. Finally, correlations between changes from Mod A to Mod B in $\tau\dot{V}O_{2p}$ and changes in Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratios were established using the Pearson product-moment correlation coefficient (Pearson r). Statistical significance was accepted as $P \leq 0.05$. All values are expressed as means ± standard deviation (SD) or as median and interquartile ranges for data that were deemed not normally distributed.

Results

Physical characteristics and activity levels.

Participants' physical characteristics are presented in Table 1. Both groups were well matched according to sex, age, body mass and BMI. Individuals with T2D recorded lower inactivity levels and higher light-intensity activity levels than controls. As expected, participants with T2D displayed higher HbA_{1c} and fasting plasma glucose levels. They also had higher total cholesterol and triglycerides than controls.

Performance data from ramp incremental cycling test

Absolute $\dot{V}O_{2peak}$ (T2D: 1.97 ± 0.60 L.min^{-1}; ND: 2.35 ± 0.50 L.min^{-1}; $P = 0.048$), $\dot{V}O_{2peak}$ normalised to body mass (T2D: 21.4 ± 4.1 mL.kg^{-1}.min^{-1}; ND: 27.6 ± 6.0 mL.kg^{-1}.min^{-1}; $P < 0.01$) and peak PO (T2D: 151± 46 W; ND: 188 ± 46 W; $P = 0.029$) were significantly reduced in individuals with T2D compared with healthy controls.

$\dot{V}O_2$ kinetics

The parameter estimates of the $\dot{V}O_2$ response to Mod A and Mod B are presented in Table 2, and responses for representative individuals are shown in Fig 2. Priming exercise (PE) resulted in an elevated $\dot{V}O_2$ baseline (P = 0.004) and a faster $\tau\dot{V}O_{2p}$ (P = <0.001) in both groups. There was an interactive effect of diabetes and priming on $\dot{V}O_2$ A (P = 0.022), $\dot{V}O_2$ end A (P = 0.043) and $\dot{V}O_2$ gain (P = 0.041), so that $\dot{V}O_2$ A and $\dot{V}O_2$ gain were lower during Mod B in T2D, but $\dot{V}O_2$ A and $\dot{V}O_2$ gain were higher in Mod B in the control group.

Muscle deoxygenation kinetics, total oxygenation index and Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio index

Kinetics parameters for Δ[HHb+Mb] and TOI responses, as well as Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratios are displayed in Table 3, while the normalised adaptation of Δ[HHb+Mb] and $\dot{V}O_2$ and the corresponding Δ[HHb+Mb]/$\Delta\dot{V}O_2$ index responses for representative individuals at the onset of exercise are shown in Fig 3. Due to a technical error with the NIRS responses data from 1 participant with T2D were excluded from the analyses. There was a diabetes status x condition interaction for Δ[HHb+Mb] A (P = 0.037) so that Mod B was higher than Mod A (P = 0.041) in participants with T2D but not in controls. PE did not influence any other Δ[HHb+Mb] kinetics parameters. The TOI baseline was higher during Mod B in both groups (main effect, condition, P = 0.001) and it was higher in the control than T2D group (main effect, diabetes status, P = 0.005). PE also resulted in an elevated TOI A (P = 0.006) in both groups. No other TOI kinetics parameters were influenced by PE. The overall Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio displayed tendencies for main effects on condition (P = 0.067) and diabetes status (P = 0.060) without a condition x diabetes status interaction (P = 0.221) (Table 3). Individuals with T2D exhibited a mismatch between local O_2 delivery relative to muscle O_2 utilisation (i.e. Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio $\neq$ 1) during Mod A (P = 0.007) but not

Mod B (P = 0.225). In contrast, no differences were present in controls during Mod A (P = 0.291) and Mod B (P = 0.697).

Correlations

Changes in $\tau\dot{V}O_{2p}$ from Mod A to Mod B and changes in $\Delta[HHb+Mb]/\Delta\dot{V}O_2$ ratios were significantly correlated in T2D (r = 0.75, P = 0.012) but not in controls (r = 0.46, P = 0.128). When all study participants were included in the analysis, these variables were significantly correlated (r = 0.72, P = <0.001).

Discussion

To our knowledge this is the first study investigating the effect of PE on the temporal relationship between the adaptation of muscle O_2 consumption and delivery during the on-transient of a subsequent bout of moderate-intensity cycling exercise in T2D. Consistent with our hypothesis, PE reduced the time of adjustment of the primary phase of the $\dot{V}O_2$ kinetics and this reduction was accompanied by the elimination of the "overshoot" in the $\Delta[HHb+Mb]/\Delta\dot{V}O_2$ ratio, suggesting a better matching of microvascular O_2 delivery to utilisation in participants with T2D.

Even if in the present study maximal oxygen uptake ($\dot{V}O_{2max}$) responses (55) were not assessed, the lower $\dot{V}O_{2peak}$ responses (18) in individuals with T2D compared with healthy controls observed herein are consistent with previously reported impairments in $\dot{V}O_{2peak}$ or $\dot{V}O_{2max}$ in adults with T2D (2, 39, 48, 49, 59). Likewise, in the present study, $\tau\dot{V}O_{2p}$ estimates during transition to Mod A in the group with T2D (~42 s) were similar to these reported previously in individuals with this clinical condition (~39-45 s) (19), and despite not reaching significance, the magnitude of the difference in the $\tau\dot{V}O_{2p}$ between the T2D and healthy

385 control groups (i.e. ~8 s or ~20% slower) is consistent with previous research supporting
386 impaired $\dot{V}O_2$ kinetics in young and middle-aged adults with T2D compared with age and
387 BMI-matched controls (3, 30, 39, 48, 49, 58). Similarly, the PE-induced faster adaptation of
388 $\tau\dot{V}O_{2p}$ during the on-transition to moderate-intensity exercise in healthy controls (initial
389 $\tau\dot{V}O_{2p}$: 34 s) is consistent with the literature surrounding PE in young healthy adults
390 presenting with slow $\tau\dot{V}O_{2p}$ and older adults (12, 20, 64). Moreover, the present study
391 demonstrates for the first time that such PE-induced effect is also present in individuals with
392 uncomplicated T2D.
393
394 In the present study the NIRS-derived TOI signal was accepted as surrogate for oxygen
395 availability within the examined vastus lateralis muscle. Despite the demonstration of a PE-
396 induced reduction in the $\tau\dot{V}O_{2p}$, the dynamic response of TOI was similar in both exercise
397 conditions for both groups. However, PE increased the resting (i.e. TOI baseline) as well as
398 exercising (i.e. TOI amplitude) microvascular O_2 availability throughout Mod B in both
399 groups. These findings are consistent with Gurd et al. (20) who showed elevated NIRS-
400 derived changes in total [Hb+Mb] during moderate-intensity exercise subsequent to PE in
401 older participants and suggest a greater muscle perfusion prior to and during Mod B.
402
403 The changes in the NIRS-derived [HHb+Mb] are indicative of the balance between O_2
404 availability and utilisation in the microvasculature within the region of NIRS interrogation,
405 and thus, were accepted as a surrogate for fractional O_2 extraction. In agreement with
406 previous studies on older adults (12, 20), the overall dynamic response of muscle
407 deoxygenation ($\tau'\Delta$[HHb+Mb]) herein was not affected by PE in both groups. In combination
408 with an accelerated $\tau\dot{V}O_{2p}$, this means a greater muscle blood flow and O_2 delivery to muscle
409 O_2 demand during the transition to ModB, although the role of these changes in the

enhancement of $\dot{V}O_2$ kinetics is not that clear. Thus, to better elucidate this, we examined the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio, an index indicative of the degree of O_2 extraction required for a given increment in $\dot{V}O_2$ (12, 44, 46). We observed that only individuals with T2D displayed an overshoot (relative to the steady-state ratio of 1.0) in the unprimed condition. This overshoot was abolished by the prior bout of heavy-intensity PE in the subsequent bout of submaximal exercise, owing to a significant reduction in the $\tau\dot{V}O_{2p}$ (which was indeed correlated with changes in the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio) and unchanged $\tau'\Delta$[HHb+Mb]. This thereby strengthened the notion that following an acute priming intervention, a reduction in the time constant of the $\dot{V}O_2$ kinetics response in middle-aged individuals with uncomplicated T2D is attributed to a better matching of microvascular O_2 delivery to utilisation. It should be noted that even if the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio for the control participants was not significantly different from the steady-state ratio of 1.0, it was numerically larger (1.04) and it was reduced following PE (1.01). As such, these results partially support previous findings by Murias et al. (46) who reported PE-induced significant reductions in the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio during moderate-intensity exercise bouts (unprimed bout: 1.08 ± 0.09; primed bout: 1.01 ± 0.06) in untrained healthy participants.

The present finding of a reduced O_2 availability imposing a limitation to $\dot{V}O_2$ kinetics in middle-aged individuals with T2D (enhanced with PE) is consistent with previous observations by Bauer et al. (3) whereby blunted microvascular blood flow responses upon initiation of a moderate-intensity cycle exercise were reported accompanied with slowed $\tau\dot{V}O_{2p}$ responses (43 s) in individuals with uncomplicated T2D. Similarly, blunted (i.e. slower) dynamic blood flow responses during intermittent calf plantar-flexion contractions in men and women with T2D (29, 41) or a transient lowering of capillary PO_2 responses at the onset of exercise, thereby limiting O_2 transport from the capillary to the myocyte in rodent

models with T2D (5) have also been reported. Thus, the present study extends the findings of reduced O_2 delivery as a contributing factor for the impairment in the control of oxidative metabolism in T2D observed in isolated muscle groups to that of whole body exercise modalities. The mechanisms underlying the PE-induced enhancement of the dynamic blood flow response in active muscles in T2D remain unclear. It is likely, as proposed by Gerbino et al. (17) that this enhanced O_2 delivery is, at least in part, mediated by a PE-induced increased lactic acidosis, via a greater perfusion and increased O_2 availability through a rightward shift of the oxyhaemoglobin dissociation curve. This may help explain the greater muscle deoxygenation (Δ[HHb+Mb] amplitude) during ModB in participants with T2D, suggestive of an increase in local O_2 utilization (extraction) following prior heavy-intensity PE. In addition, improvements in endothelium- and flow-mediated vasodilation responses have also been observed following an acute exercise bout, potentially enhancing O_2 delivery to active muscles (23). However, the PE-augmented oxidative phosphorylation may also be related to a combination of enhanced muscle perfusion and O_2 delivery with the upregulation of rate-limiting mitochondrial oxidative enzymes (20, 21).

Limitations

The inclusion of pre- and post-menopausal women should be acknowledged as whilst the magnitude of impairments in $\dot{V}O_2$ does not appear to be affected by menopausal status (30), the same is unknown for muscle oxygenation & deoxygenation. In an attempt to minimise this our study matched groups on this characteristic (pre-menopausal: 2 T2D and 2 ND vs. post-menopausal: 3 T2D and 3 ND). We also acknowledge functional limitations pertaining to the NIRS technology utilised herein. Firstly, only one superficial muscle was investigated, thus interpretation of NIRS-derived data is limited to the examined region. Secondly, the established heterogeneity extant within a single muscle in terms of vascularity and fibre type

(25), fibre recruitment, vascular control and blood flow (5, 35, 42), likely extends to the vastus lateralis herein. In addition, identified variances both, between muscles (specifically vastus lateralis and rectus femoris) and between deep and superficial segments within a muscle (particularly rectus femoris) during constant load cycling need to be acknowledged (10, 34, 51, 57, 63), although this appears not to be the case between the distal and proximal portions of the vastus lateralis during ramp incremental exercise (6, 31). Thirdly, we did not correct for adipose tissue thickness at the site of measurement. However, the thickness of the skin and adipose tissue measured at the site of the interrogation via B-mode 2D ultrasound were not different between groups. Finally, the present findings are limited to middle-aged participants, so future studies should examine other populations (i.e. older adults).

Perspectives and Significance

This study demonstrated that a single heavy-intensity warm-up or priming exercise bout elicits a faster adaptation of the $\dot{V}O_2$ kinetics response in middle-aged individuals with uncomplicated T2D, by way of enhancing blood flow distribution at the level of the muscle microvasculature and better matching O_2 delivery to utilisation. Such favourable manipulation of the $\dot{V}O_2$ kinetics response via heavy-intensity priming exercise in diabetes is promising, given that a faster provision of aerobic metabolism reduces muscle fatigue during light-, moderate-intensity transitions carried out during routine everyday tasks (7, 40). This is important given that individuals with T2D perceive light to moderate exercise as being more difficult than healthy counterparts (24). Thus, exercise training interventions designed to benefit $\dot{V}O_2$ control and functional independence in T2D should also focus on microvascular O_2 delivery. Moreover, exercise training protocols should incorporate heavy-intensity warm-up exercise to maximise the oxidative capacity of muscles and increase the effectiveness of the therapeutic effect of exercise in this all too prevalent condition.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Author contributions

J.R., N.G., M.E., D.O'S. and S.G. conception and design of research; J.R. and N.G. performed experiments; N.G., J.R. and M.E. analyzed data; J.R., N.G., S.G. and M.E. interpreted results of experiments; N.G., J.R. and M.E. prepared figures; J.R., N.G. and M.E. drafted manuscript; N.G., J.R., S.G. and M.E. edited and revised manuscript; N.G., J.R., D.O'S., S.G. and M.E. approved final version of manuscript.

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Figure captions

Figure 1 Schematic representation of the protocol. Cycling exercise at moderate-intensity without priming (Mod A) and with priming (Mod B), performed at a power output corresponding to 80% of each participant's first ventilatory threshold (VT). Priming exercise (PE) consisted of a high-intensity cycling exercise (Δ50%; the sum of the power output at VT and 50% of the difference between the power output at VT and $\dot{V}O_{2peak}$). All step transitions, each lasting 6 min, were preceded by 3 min of cycling at 10 W (i.e. 'baseline' cycling). Mod A and PE step transitions were followed by 12 min of passive rest. The 3 step transitions (Mod A, PE and Mod B) were repeated following 45 min of passive rest within the same laboratory visit.

Figure 2. Oxygen uptake ($\dot{V}O_2$) responses for a representative individual with type 2 diabetes (A) and a healthy control (B) during moderate-intensity cycling transitions without priming exercise (Mod A, open circles) and with priming exercise (Mod B, solid circles). The continuous lines of best fit (black for Mod A; grey for Mod B) illustrate the primary phase of the oxygen uptake ($\dot{V}O_2$) response. Note the relatively slower response of the primary phase of the $\dot{V}O_2$ response in the unprimed compared with the primed bouts.

Figure 3. Normalised τ'Δ[HHb+Mb] (solid circles, panels A, B, D & E) and $\dot{V}O_2$ adjustment (open circles, panels A, B, D & E) at the onset of moderate-intensity cycling transitions for a representative individual with type 2 diabetes (panel A, Mod A; panel B, Mod B) and a healthy control (panel D, Mod A; panel E, Mod B). Profiles of the $\Delta[HHb+Mb]/\Delta\dot{V}O_2$ index are shown in panels C (individuals with T2D) and F (healthy control) where the cycling

731 transitions without priming exercise (Mod A open circles) and with priming exercise (Mod B,
732 solid circles) are plotted as a function of time.
733
734 **Figure 4:** Relationships between changes in $\tau\dot{V}O_2$ (%) from Mod A to Mod B and changes in
735 Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio (%) in participants with T2D and ND controls.
736

Figure 1

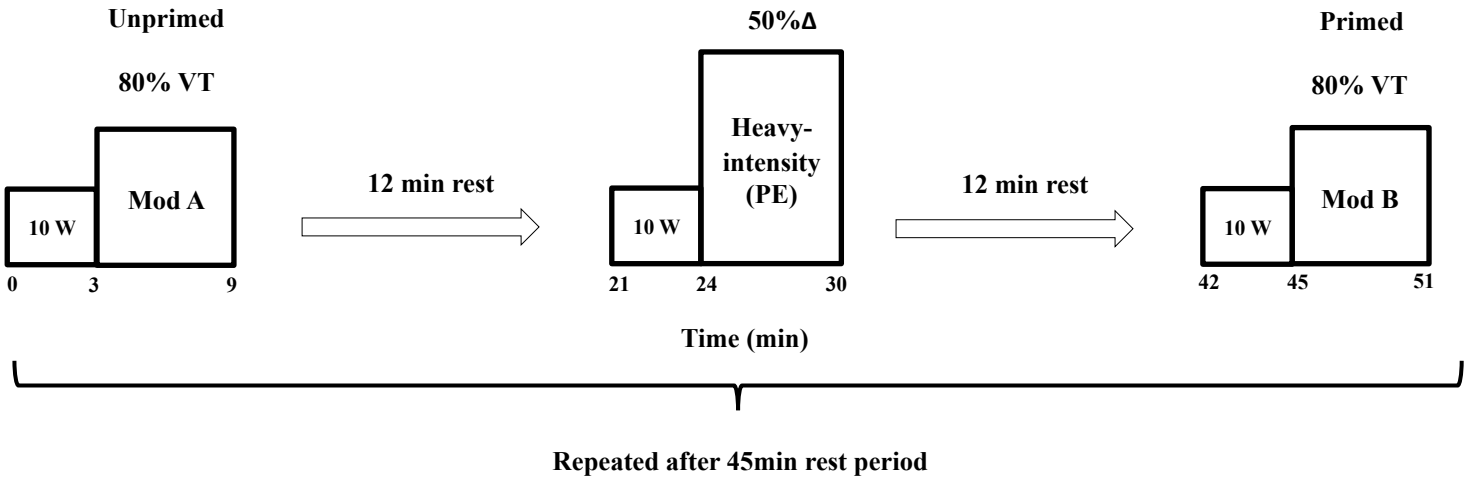
Unprimed
80% VT
10 W
Mod A
0
3
9
12 min rest
50%Δ
Heavy-intensity (PE)
10 W
21
24
30
12 min rest
Primed
80% VT
10 W
Mod B
42
45
51
Time (min)
Repeated after 45min rest period

Figure 2

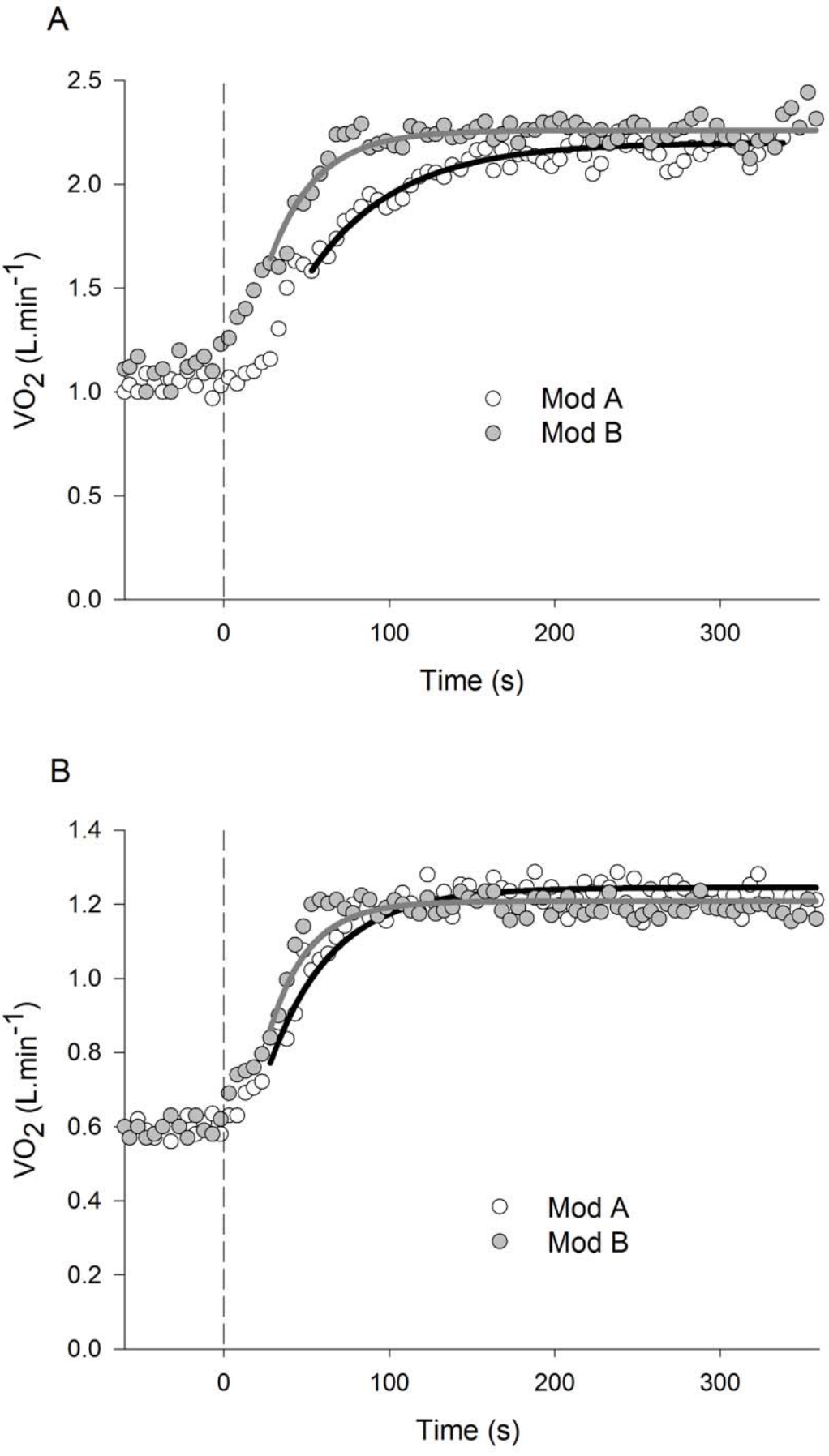
A
2.5
2.0
1.5
1.0
0.5
0.0
VO_2 (L.min^{-1})
0
100
200
300
Time (s)
Mod A
Mod B
B
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
VO_2 (L.min^{-1})
0
100
200
300
Time (s)
Mod A
Mod B

Figure 3

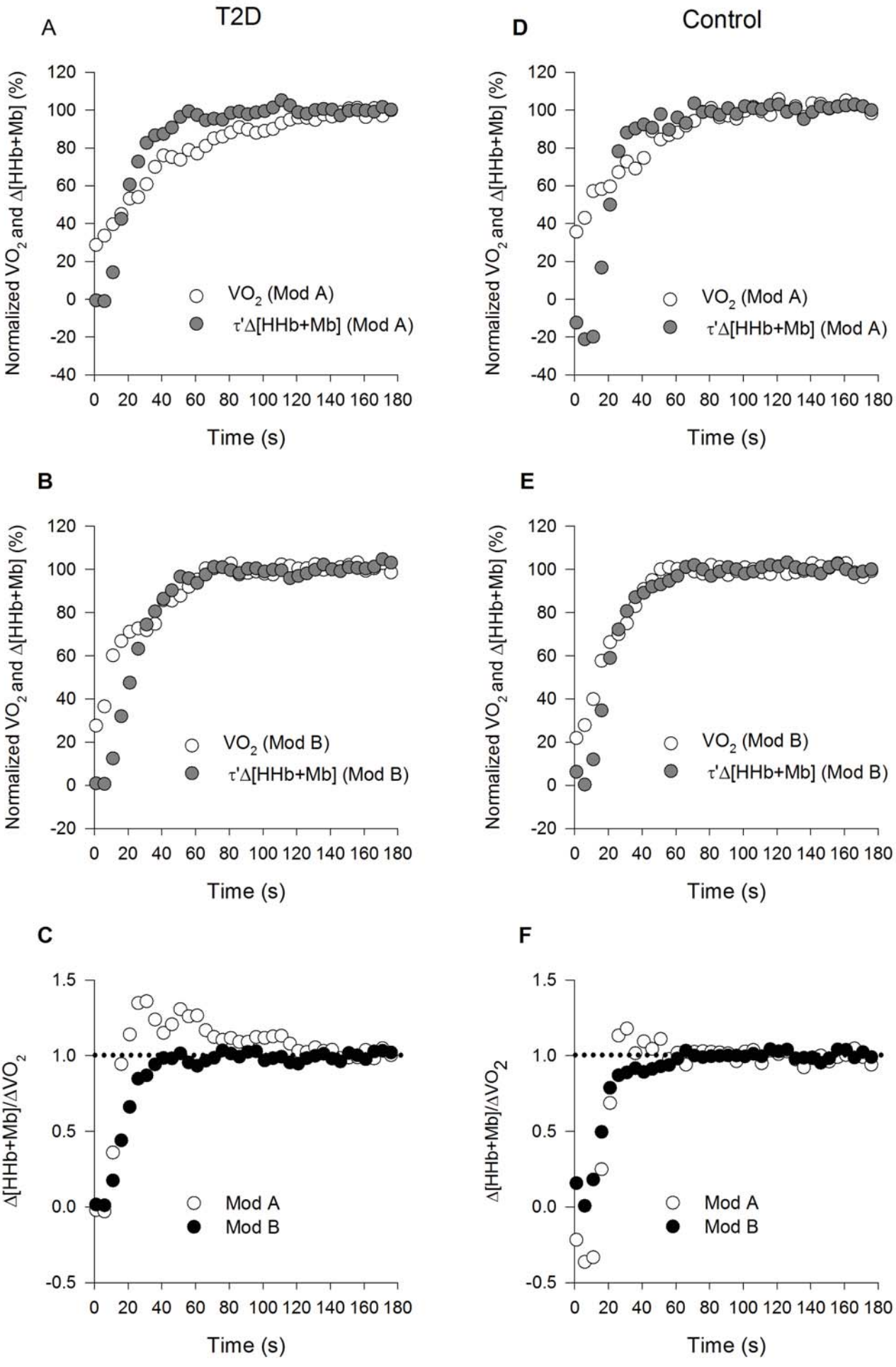
T2D
Control
A
B
C
D
E
F
Normalized VO2 and Δ[HHb+Mb] (%)
VO2 (Mod A)
τ'Δ[HHb+Mb] (Mod A)
VO2 (Mod B)
τ'Δ[HHb+Mb] (Mod B)
Time (s)
Δ[HHb+Mb]/ΔVO2
Mod A
Mod B

Figure 4

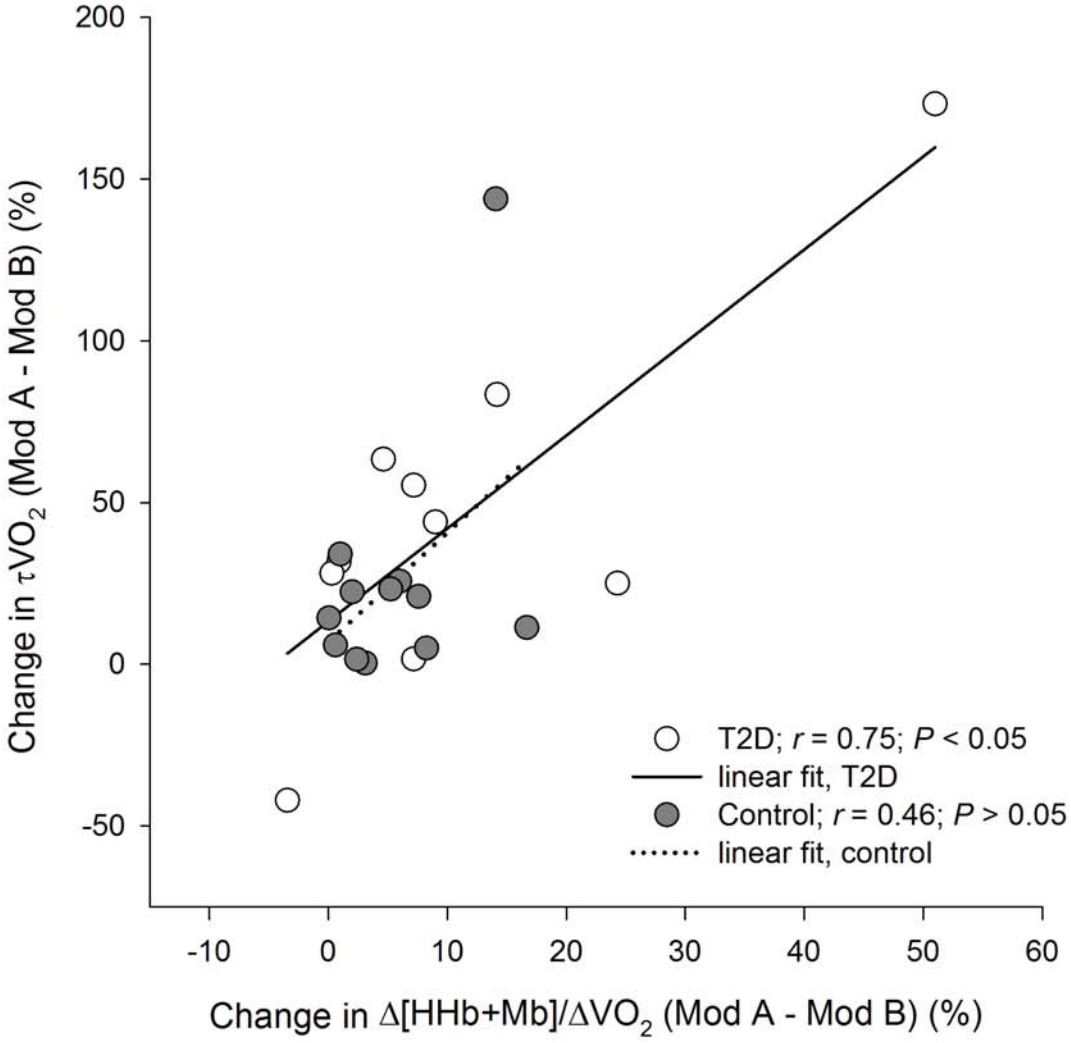
200
150
100
50
0
-50
-10
0
10
20
30
40
50
60
Change in τVO2 (Mod A - Mod B) (%)
Change in Δ[HHb+Mb]/ΔVO2 (Mod A - Mod B) (%)
T2D; r = 0.75; P < 0.05
linear fit, T2D
Control; r = 0.46; P > 0.05
linear fit, control

Table 1. *Physical characteristics and activity levels.*

	ND	T2D	*P* value
n	12	12	
Physical characteristics			
Sex (male, female)	7, 5	7, 5	
Age (yr)	44 ± 9	48 ± 9	0.23
Stature (m)	1.68 ± 0.07	1.69 ± 0.08	0.85
BMI ($kg.m^{-2}$)	30.4 ± 4.1	32.1 ± 5.6	0.35
Body Mass (kg)	86.0 ± 12.0	91.7 ± 18.6	0.38
Fat layer VL (mm)[a]	8.3 ± 4.5	6.5 ± 1.7	0.21
HbA1c (%)[b]	*5.1 (0.5)*[*]	*6.8 (1.4)*	<0.001
FPG ($mmol.L^{-1}$)[c]	*4.0 (0.7)*[*]	*7.3 (4.1)*	<0.001
Time since diagnosis (yr)		5.9 ± 4.2	
Total cholesterol ($mmol.L^{-1}$)[d]	*3.4 (1.6)*[*]	*4.7 (1.2)*	0.03
LDL-C ($mmol.L^{-1}$)[e]	1.6 ± 1.3	2.3 ± 1.3	0.11
HDL-C ($mmol.L^{-1}$)[e]	1.3 ± 0.3	1.2 ± 0.2	0.26
Triglycerides ($mmol.L^{-1}$)[e]	*1.1 (2.5)*[*]	*1.8 (0.7)*	0.03
Habitual physical activity			
Inactive ($h.day^{-1}$)[f]	19.2 ± 1.2[*]	17.8 ± 0.8	0.05
Light ($h.day^{-1}$)[f]	*4.3 ± 1.1*[*]	*6.0 ± 2.0*	0.05
Moderate ($h.day^{-1}$)[f]	0.6 ± 0.4	0.7 ± 0.6	0.95
Vigorous ($h.day^{-1}$)[f]	*0.2 (0.3)*	*0.1 (0.2)*	0.35

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. *n*, no. of participants; BMI, body mass index; HbA_{1c}, glycosylated haemoglobin; FPG, fasting plasma glucose; VL, vastus lateralis; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2D ($P \leq 0.05$).

[a] = 12 (ND) and 10 (T2D); [b] = 7 (ND) and 11 (T2D); [c] = 9 (ND) and 10 (T2D); [d]=8 (ND) and 9 (T2D); [e] = 9 (ND) and 7 (T2D); [f] = 9 (ND) and 5 (T2D).

Table 2. *Dynamic response characteristics of oxygen uptake ($\dot{V}O_2$).*

	Mod A		Mod B	
	Controls	Type 2 diabetes	Controls	Type 2 diabetes
n	12	12	12	12
Baseline $\dot{V}O_2$, L/min^{-1}	0.78 ± 0.10	0.91 ± 0.25	0.82 ± 0.10*	0.98 ± 0.23*
$\dot{V}O_2$ A, L/min^{-1}	0.68 ± 0.31	0.55 ± 0.25	0.72 ± 0.35	0.50 ± 0.20*
$\dot{V}O_2$ end A, L/min^{-1}	1.46 ± 0.35	1.46 ± 0.42	1.54 ± 0.39*	1.48 ± 0.39
$\dot{V}O_2$ τ, s	33.5 ± 7.4	42.1 ± 12.2	28.3 ± 8.7*	32.2 ± 9.1*
$\dot{V}O_2$ gain, mL.min^{-1}.W^{-1}	9.3 ± 2.1	9.4 ± 2.3	9.9 ± 2.1*	8.6 ± 1.3

Values are means ± SD; *n*, no. of participants. A, amplitude; τ, time constant; end A, steady-state $\dot{V}O_2$ response.

* $P < 0.05$ vs. Mod A within same diabetes status group (i.e. within controls or within Type 2 diabetes). † $P < 0.05$ vs. participants with type 2 diabetes within same condition (i.e. within Mod A or Mod B).

Table 3 *Dynamic response characteristics Δ[HHb+Mb] & TOI, and Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio index*

	Mod A		Mod B	
	Controls	Type 2 diabetes	Controls	Type 2 diabetes
n	12	11	12	11
Baseline Δ[HHb+Mb], μMol*cm	-68.8 ± 45.2†	-15.0 ± 40.8	-43.1 ± 65.6†	5.2 ± 56.0
Δ[HHb+Mb] A, μMol*cm	57.4 ± 48.0	92.6 ± 61.7	57.7 ± 43.1	112.2 ± 63.4*
Δ[HHb+Mb] τ, s	14 ± 7	15 ± 8	18 ± 7	16 ± 6
Δ[HHb+Mb] TD, s	11 ± 3	13 ± 5	11 ± 2	13 ± 3
Δ[HHb+Mb] τ', s	26 ± 5	29 ± 6	29 ± 8	29 ± 6
Baseline TOI, %	76.3 ± 5.1†	69.6 ± 3.5	78.4 ± 5.3†*	73.3 ± 4.3*
TOI A, %	4.5 ± 2.5	6.7 ± 3.7	4.9 ± 2.5*	8.1 ± 4.1*
TOI τ, s	11 ± 8	12 ± 4	13 ± 4	13 ± 4
TOI TD, s	12 ± 4	12 ± 4	12 ± 3	13 ± 4
TOI τ', s	23 ± 5	24 ± 4	25 ± 5	26 ± 6
Normalized Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio	1.04 ± 0.12	1.17 ± 0.17	1.01 ± 0.12	1.05 ± 0.14

Values are means ± SD; *n*, no. of participants. A, amplitude; τ, time constant; TD, time delay; τ', effective response time (τ + TD); [HHb+Mb], deoxygenated haemoglobin; TOI, tissue oxygenation index.
* $P < 0.05$ vs. Mod A within same diabetes status group (i.e. within controls or within Type 2 diabetes). † $P < 0.05$ vs. participants with type 2 diabetes within same condition (i.e. within Mod A or Mod B).