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TITLE

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ABSTRACT

We tested the hypothesis that type 2 diabetes (T2D) alters the profile of muscle fractional oxygen (O_2) extraction (near-infrared spectroscopy) during incremental cycle exercise. Seventeen middle-aged individuals with uncomplicated T2D and 17 controls performed an upright ramp test to exhaustion. The rate of muscle deoxygenation (i.e. deoxygenated haemoglobin and myoglobin concentration, Δ[HHb+Mb]) profiles of the vastus lateralis muscle were normalised to 100% of the response, plotted against % power output (PO) and fitted with a double linear regression model. Peak oxygen uptake was significantly (P<0.05) reduced in individuals with T2D. The %Δ[HHb+Mb]/%PO slope of the first linear segment of the double linear regression function was significantly (P<0.05) steeper in T2D than controls (1.81±0.61 vs 1.35±0.43). Both groups displayed a near-plateau in Δ[HHb+Mb] at an exercise intensity (%PO) not different among them. Such findings suggest that a reduced O_2 delivery to active muscles is an important underlying cause of exercise intolerance during a maximum graded test in middle-aged individuals with T2D.

Keywords: near-infrared spectroscopy, oxygen extraction, cycling, exercise tolerance, type 2 diabetes

1. Introduction

Individuals with uncomplicated type 2 diabetes mellitus (T2D) demonstrate impairments in peak exercise capacity ($\dot{V}O_{2peak}$), an established clinical predictor of cardiovascular and all-cause mortality (Kodama et al., 2009; Swift et al., 2013), in the region of 20% (Baldi et al., 2003; Kiely et al., 2015; Mac Ananey et al., 2011; O'Connor et al., 2015; O'Connor et al., 2012; Regensteiner et al., 1998). Importantly, this impairment is independent of obesity and age, and present in the absence of clinically apparent cardiovascular disease (Green et al., 2015). Whilst the precise mechanisms for this diminished exercise capacity remain to be elucidated, it is likely the consequence of a complex array of pathophysiological changes at a central and/or peripheral level (Green et al., 2015; Poitras et al., 2018). Maximum $\dot{V}O_2$, representative of the integration of the pulmonary, cardiovascular and muscular systems to uptake, transport and utilise O_2 respectively, is governed by the oxygen cascade from the environment to the muscle mitochondria (Poole, 1997; Wagner et al., 1997), and is thus, consequent to the product of whole-body perfusive and diffusive O_2 conductance. However, most commonly, the Fick relationship is determined either at the pulmonary level, or across the exercising limb(s), and is representative of pooled fractional O_2 extraction across multiple compartments which may not necessarily reflect the discrete adjustments of O_2 exchange within the microvasculature of the active muscle (Iannetta et al., 2017; Okushima et al., 2016; Spencer et al., 2012). As such, considering the matching of O_2 delivery (QO_2)-to- $\dot{V}O_2$ and diffusive O_2 conductance at the level of the active muscle vasculature during exercise is of great relevance when exploring the mechanistic bases for the decreased exercise tolerance observed in T2D.

Substantial evidence exists to suggest that peripheral O_2 delivery in the lower limbs is impaired in individuals with uncomplicated T2D. For instance, the maximum leg haemodynamic and vasodilatory responses during an incremental calf plantar-flexion exercise (Kiely et al., 2014) as well as steady-state femoral artery blood flow measurements during cycling (Kingwell et al.,

2003) and knee extension exercise (Lalande et al., 2008) are reduced in men and women with uncomplicated T2D. Additionally, leg vascular conductance kinetics at the onset of heavy-intensity plantar-flexion exercise (Kiely et al., 2014; MacAnaney et al., 2011), and quadriceps muscle microvascular blood flow kinetics during moderate cycling (Bauer et al., 2007) are impaired (i.e. slowed/blunted) in individuals with T2D free from cardiovascular disease. In contrast, Poitras et al. (2015) recently reported unaffected leg blood flow kinetics during knee extension/flexion exercise in individuals with T2D; although participants had a more advanced diabetes and history of cardiovascular disease, with their control group also having a similar history of cardiovascular disease/comorbidities (Poitras et al., 2015). In agreement with Poitras et al. (2015), Copp et al. (2010) found that locomotory muscle(s) blood flow during running was not decreased in the rat GK model of type 2 diabetes (Copp et al., 2010) despite grossly impaired microvascular perfusion at rest (Padilla et al., 2006).

It is therefore plausible that the maldistribution of active muscle blood flow in individuals with uncomplicated T2D (Kiely et al., 2014; MacAnaney et al., 2011), and subsequently a decreased microvascular partial pressure of O_2 ($P_{mv}o_2$) (Padilla et al., 2007), would mandate an increased reliance on fractional O_2 extraction in the exercising muscle in an effort to achieve a given increase in $\dot{V}O_2$. The use of near-infrared spectroscopy (NIRS) during exercise permits a non-invasive assessment of microvascular O_2 extraction (DeLorey et al., 2003). By measuring the concentration changes in deoxygenated haemoglobin and myoglobin (Δ[HHb+Mb]), an estimate of fractional O_2 extraction is possible. NIRS, therefore, provides insights into the dynamic balance between regional QO_2 and $\dot{V}O_2$ at the level of the microvasculature (Spencer et al., 2012), the determining factor for $P_{mv}o_2$. Accordingly, investigating the dynamic response of [HHb+Mb] within the microcirculation of the exercising muscles during a ramp incremental test may offer insight into pathophysiological mechanisms potentially implicated in the reduced exercise capacity in T2D. In the present study the profile of %Δ[HHb+Mb] during a ramp

incremental test was characterized using a function including two linear segments; the 'double-linear model' (Vieth, 1989) as it has been proffered to best characterise this profile (Spencer et al., 2012). In the first segment, a linear increase in %Δ[HHb+Mb] relative to changes in work rate occurs, representing the increasing reliance on O_2 extraction relative to metabolic demand. This culminates at a 'breakpoint' (Δ[HHb+Mb] – *BP*), from which a "plateau-like" response ensues despite the continued increase in work rate. The breakbpoint has been associated with transitions in exercise intensity domains between heavy to severe-intensity exercise (Bellotti et al., 2013; Keir et al., 2015). This plateau in the [HHb+Mb] signal does not indicate the upper limit of O_2 extraction during incremental tests, and it seems to be connected to the re-distribution of blood flow towards the active tissues once this upper boundary of exercise is achieved (Inglis et al., 2017).

The aim of the present study was to explore the influence of T2D on the profile of local muscle fractional O_2 extraction, as indicated by the NIRS-derived Δ[HHb+Mb] response. We hypothesized that individuals with T2D would display an accelerated muscle deoxygenation response throughout the ramp incremental exercise bout. This would be depicted by a steeper primary slope of the double linear equation, thereby signifying an increased dependence on O_2 extraction for providing adequate $\dot{V}O_2$ at a given work rate. To avoid the potential effects of aging on the T2D-related impairments on exercise tolerance previously established in men (O'Connor et al., 2015; Wilkerson et al., 2011) we limited the age of participants to < 55 yr.

2. Methods

2.1. Participants

Thirty four individuals, 17 with uncomplicated T2D (12 males, 5 females), and 17 age- and BMI-matched controls (ND) (12 males, 5 females) volunteered to participate in this study. The age range of all participants was between 36 and 55 yr. (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. Five female participants were premenopausal (2 T2D, 3 ND) and 5 were postmenopausal (3 T2D, 2 ND) not undergoing hormone replacement therapy. All participants were non-smokers and had not smoked during the 12-month period preceding the study. Individuals with T2D had a clinical history of diabetes of between 2 to 9.5 years, with adequately controlled HbA_{1c} levels (<10%) (Table 1) and were not taking insulin or beta-blockers. Two of the controls were on prescriptive medications (statins, n = 2), and with the exception of one participant, all participants with T2D were taking oral (n = 15) and/or subcutaneous (n =1) hypoglycaemic prescription medications (metformin monotherapy, n = 9; metformin & sulphonylurea, n = 3; metformin & thiazolidinedione, n = 1; glucagon-like peptide 1, n = 1; sodium glucose cotransporter 2 inhibitors, n = 4). In addition, a subgroup of individuals with T2D were taking antihypertensive prescription drugs (angiotensin converting enzyme inhibitor, n = 4; angiotensin II receptor blocker, n = 2; calcium channel blocker, n = 5) and statins (n =6).

At the commencement of the present study, individuals with T2D displayed no clinical evidence of ischemic heart disease (normal ECG during treadmill stress test following the Bruce protocol), peripheral arterial disease (0.9 < ABI < 1.3), kidney dysfunction (urine protein < 200mg/dl), or liver dysfunction (urine creatinine levels < 2.2 mg/dl). Participants were classified as physically inactive 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 accelerometery (Stayhealthy Inc, CA) in a subset of participants (Table 1) (Rowlands et al., 2004). All participants provided written informed consent before commencement, and 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 Declaration of Helsinki (2008).

2.2. Study Protocol

2.2.1. Overview.

Following a satisfactory completion of the 12-lead ECG stress test, participants were tested on one occasion either at St. Columcille's Hospital or the cardiovascular laboratory in Trinity College Dublin. Premenopausal participants were tested during the mid-follicular phase (days 5-12) of the menstrual cycle. All participants refrained from consuming alcohol, caffeine and non-prescribed nutritional supplements in the 24 hours prior to testing and constrained their exercise to normal activities of daily living. All participants performed a ramp incremental cycling test to exhaustion to determine $\dot{V}O_{2peak}$.

2.2.2. Ramp incremental cycling tests to exhaustion. The ramp incremental cycling test to exhaustion was performed in an upright position on an electrically braked cycle ergometer (Excalibur Sport; Lode B.V., Groningen, The Netherlands). Exercise was performed at an initial workload of 10 W for 2 min. This was followed by 10-15 $W.min^{-1}$ increments in PO in females or 10-25 $W.min^{-1}$ increments in males (depending on stated activity levels), until volitional exhaustion. Pedal frequency was held constant at an individually selected cadence between 60-75 revolutions per minute (rpm). Failure in a test was determined as a drop in cadence exceeding 10 rpm for >5 s. Peak workload was determined according to the point of termination of the test. $\dot{V}O_{2peak}$ was determined by identifying the highest 15-s mean $\dot{V}O_2$ value recorded before the participant's volitional termination of the test. The ventilatory threshold (VT) was determined as the exercise level at which $\dot{V}_E/\dot{V}O_2$ exhibited a systematic exponential increase without a concomitant increase in $\dot{V}_E/\dot{V}CO_2$ (Wasserman et al., 1973), and the deflection point of carbon dioxide output ($\dot{V}CO_2$) versus O_2 uptake ($\dot{V}O_2$; V-slope method) (Amann et al., 2006;

Beaver et al., 1986). The respiratory compensation point (RCP) was estimated by identifying the second non-linear increase of $\dot{V}_E$ and $\dot{V}CO_2$, whereby an increase in $\dot{V}_E/\dot{V}O_2$ was accompanied by an increase of $\dot{V}_E/\dot{V}CO_2$ (Wasserman and McIlroy, 1964).

2.3. Measurements

During exercise participants wore a facemask to continuously collect expired air using an online metabolic system (Innocor, Innovision A/S, Odense, Denmark). Analysis of expired air allowed determination of pulmonary O_2 uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$) and the respiratory exchange ratio (RER) breath by breath. Heart rate was recorded every 5 s (Polar S610i, Polar Ltd, Finland), with peak HR defined as the highest heart rate attained within the last 15 s of the point of termination of the test. Beat-to-beat systolic and diastolic blood pressure was continuously monitored throughout the exercise protocol using the volume clamp method at the level of the finger (Finometer, Finepress Medical Systems B.V. the Netherlands). MAP was calculated from systolic and diastolic pressures (MAP: 0.33 systolic BP + 0.66 diastolic BP). Peak BP was expressed as the highest 15-second mean pressure obtained before the participant's volitional termination of the test.

A continuous wave NIRS system (Hamamatsu Niro 200Nx; Hamamatsu Photonics, Hamamatsu, Japan), was used to non-invasively determine the oxygenation status of the right quadricep's *vastus lateralis* (VL) muscle. This was determined using the spatially resolved spectroscopy (SRS) technique and modified Beer-Lambert (MBL) principle with three wavelengths of emitting light (λ = 735, 810, and 850 nm). The theoretical basis of NIRS and its use in exercise measurements have been described in detail elsewhere (Ferrari et al., 2011). Briefly, this technique estimates the optical density changes of deoxygenated haemoglobin and myoglobin (HHb+Mb) based on the O_2 dependency of absorption changes for near-infrared light in these proteins. As the VL muscle is a dominant locomotor muscle during cycling

196 (Laplaud et al., 2006), the present study examined the Δ[HHb+Mb]) profiles of the right VL
197 muscle. After shaving the skin, the probes were placed on the belly of the muscle (5-8 cm above
198 the lateral femoral condyle), parallel to the major axis of the thigh with a 3 cm spacing between
199 the emitter and receiver. The probes were housed in a black rubber holder and secured on the
200 skin surface with bi-adhesive tape and then covered with a dark elastic bandage, which
201 minimised extraneous movement and the intrusion of stray light throughout the exercise
202 protocol. Since the depth of the measured area is estimated to be between one-half and one-
203 third of the distance between the emitter and the receiver (~1.5 cm) (Ferrari et al., 2004; Van
204 Beekvelt et al., 2001), the thickness of the skin and adipose tissue at the site of the probe
205 placement was measured via 2D ultrasound operating in B-mode (Zonare Ultra Smart Cart,
206 Software version 4.7, USA). This was to ensure that data largely represented absorption of near-
207 infrared light in muscle tissue and not in subcutaneous fat.
208
209 *2.4. Data analysis*
210 *2.4.1. Muscle deoxygenation*. The NIRS-derived signal was normalised whereby the unloaded
211 exercise baseline value was adjusted to zero ('zero set'). Thus the NIRS data are presented as a
212 relative change from the baseline- to the end-exercise values. As such 0% represents the mean
213 steady-state value of the last 30 s of the unloaded cycling and 100% represents the highest mean
214 value of the last 30 s of any work rate. This was done given the uncertainty of the optical path
215 length in the VL at rest and during exercise, so, data are presented as normalised delta units
216 Δ[HHb+Mb]. Prior to analysis, NIRS data were averaged to give 1 s intervals. The second-by-
217 second [HHb+Mb] data was averaged by applying a five-point moving average and then
218 normalised to the peak amplitude of the response (%Δ[HHb+Mb]). The [HHb+Mb] response
219 dynamics were expressed in relation to relative power output (%PO) prior to curve fitting.
220 Therefore, individual profiles were plotted as a function of %PO and characterised by a linear
221 function with two terms to establish the slope of increase of deoxygenation ($Slope_1$), plateau as

maximal exercise was approached ($Slope_2$), and the break point (*BP*) located between the increasing deoxygenation and its plateau. The double linear function was applied using TableCurve 2D (Systat Software, USA) as:

$$y = a + b * x - c * (x-d)*f$$

$$f = if\ (x<d,\ 0,\ 1)$$

where *a* and *b* represent the y-intercept and slope of the first linear function ($slope_1$), *d* is the time delay or *BP* where the segments intersect, with the slope of the second linear function ($slope_2$) being calculated from the parameter estimates of *b* and *c* ($slope_2 = b - c$).

2.4.2. $\Delta\dot{V}O_2/\Delta PO$

The rate of change $\dot{V}O_2$ relative to PO during ramp incremental exercise reflects the capacity of aerobic metabolism to adjust to the non-steady state conditions incurred during a ramp incremental protocol. Initially, the mean response time (MRT) of ramp incremental exercise was estimated using the approach recently described by Iannetta et al. (2019). Briefly, we determined the average steady-state $\dot{V}O_2$ corresponding to three separate bouts of moderate-intensity constant-power outputs (performed on a separate visit), and we then compared the ramp-derived power output associated with that $\dot{V}O_2$ to the constant-power output that elicited that $\dot{V}O_2$ (Iannetta et al., 2019). The difference between these power outputs was converted to the time to retrieve the time-interval corresponding to MRT. The breath by breath $\dot{V}O_2$ data were averaged over 15 s intervals and plotted as a function of work rate after applying the MRT to reflect the increase in aerobic metabolism ($\Delta\dot{V}O_2$) for each increase in power output (ΔPO). From this the $\Delta\dot{V}O_2/\Delta$PO slope was calculated over the same range of PO as used to determine the first %Δ[HHb+Mb]/%PO slope (i.e parameter *b* or $slope_1$) as described above.

248 *2.5. Statistical analyses*

249 Statistical analysis was performed using the software SigmaPlot version 12.5 (Systat Software,
250 Point Richmond, CA). Prior to analysis, normal Gaussian distribution of the data was assessed
251 using the Shapiro-Wilk's test. Physical characteristics and NIRS-derived muscle deoxygenation
252 responses between groups were compared using unpaired 2-tailed Student's t-test for
253 parametric analyses, or the Mann-Whitney U test for non-parametric analyses. Based on *a*
254 *priori* evidence on the pre-determined reduced functional exercise capacity in individuals with
255 uncomplicated T2D, the peak physiological responses between groups were compared using
256 unpaired 1-tailed Student's t-test for parametric analyses, or the Mann-Whitney U test for non-
257 parametric analyses. Correlations between variables were established using the Peaarson
258 product-moment correlation coefficient (Pearson r). Statistical significance was accepted at a P
259 ≤ 0.05. All values are expressed as means ± standard deviation (SD) or as median and
260 interquartile ranges for data that were deemed not normally distributed.

261

262 **3. Results**

263 *3.1. Physical characteristics and activity levels.*

264 Participants' physical characteristics and activity levels are shown in Table 1. Both groups were
265 well matched according to sex, age, body mass and BMI. Inactivity levels did not differ between
266 groups, but individuals with T2D recorded higher light intensity activity levels. As expected,
267 participants with T2D displayed higher HbA_{1c} and fasting plasma glucose levels. They also had
268 higher total cholesterol than the controls.

269

270 *3.2. Performance data from ramp incremental cycling test*

271 Relative $\dot{V}O_{2peak}$ (mean difference = 6.14 $mL.kg^{-1}.min^{-1}$), absolute $\dot{V}O_{2peak}$ (mean difference =
272 0.42 $L.min^{-1}$) and peak PO were significantly ($P < 0.05$) reduced in individuals with T2D

compared with controls (Table 2). In addition, $\dot{V}O_2$ at VT and $\dot{V}O_2$ at RCP were also significantly lower in T2D ($P < 0.05$) compared with controls (Table 2).

3.3. NIRS-derived [HHb+Mb] response dynamics and correlations

Group mean parameter estimates from the double linear model of the %Δ[HHb+Mb] profile as a function of normalised power output (%PO) are displayed in Table 3. Individual representative profiles of the modelled [HHb+Mb] response dynamics as a function of %PO are displayed in Fig 1, while group mean responses are shown in Fig 2. Due to a technical error with the NIRS data (i.e. the entire [HHb+Mb] responses were negative instead of positive), data from 6 participants (3 controls: 2 males, 1 female; and 3 participants with T2D: 2 males, 1 female). were excluded from the analyses. The slope of the first linear regression function ($slope_1$) used to establish the dynamic adjustment of [HHb+Mb] was significantly steeper ($P < 0.05$) in participants with T2D than the controls (Table 3, Fig 3). In addition, in T2D $slope_1$ was significantly correlated with absolute $\dot{V}O_{2peak}$ (r = -0.67, $P = 0.009$), relative $\dot{V}O_{2peak}$ (r = -0.64; $P = 0.013$) and peak PO (r= -0.74; $P = 0.003$); whereas $slope_1$ was not correlated with these variables in ND controls (r = 0.132, $P = 0.65$; r = 0.01, $P = 0.97$; r = 0.155, $P = 0.60$; respectively). Correlations between $slope_1$ and absolute $\dot{V}O_{2peak}$ for both groups are shown in Fig 4. The exclusion of these 6 participants did not affect the physical characteristics of each group (i.e. they were matched in terms of age, body composition and activity levels) or the peak exercise responses between groups.

3.4. $\Delta\dot{V}O_2/\Delta PO$

The rate of change in $\dot{V}O_2$/PO was not significantly different during the ramp incremental exercise between the T2D and ND groups with no observed differences in slopes (9.3 ± 3.4 vs. 9.6 ± 1.1 $mL.min^{-1}.W^{-1}$ respectively, $P = 0.23$).

4. Discussion

The principal original finding of the present investigation was that individual with T2D demonstrated a significantly steeper primary slope of the bi-linear regression used to establish the dynamic adjustment of [HHb+Mb] during a ramp incremental exercise compared with controls. Concomitant with the reduced (~21%) $\dot{V}O_{2peak}$ responses observed in individuals with T2D compared with controls herein and previously (Baldi et al., 2003; Kiely et al., 2015; Mac Ananey et al., 2011; O'Connor et al., 2015; O'Connor et al., 2012; Regensteiner et al., 1998), such adjustment of [HHb+Mb] provides further insight into pathophysiological mechanisms potentially responsible for the reduced functional capacity in this clinical population. Given that overall, the objectively measured physical activity levels did not differ between groups, the exaggerated exercise intolerance is likely not affected by differences in activity levels. Therefore, in agreement with our hypothesis, the present study suggests that T2D alters the profile of muscle fractional O_2 extraction during ramp incremental cycle exercise. Specifically, T2D induced a greater reliance on normalized O_2 extraction for a given normalized PO up to the [HHb+Mb]-BP (i.e. larger $slope_1$), and importantly, $slope_1$ was inversely correlated with peak exercise capacity in participants with T2D.

Accordingly, the accelerated muscle deoxygenation revealed by the steeper primary %Δ[HHb+Mb]/%PO slope of the bi-linear regression indicates a reduced capacity to increase peripheral O_2 delivery to meet increasing O_2 demands. The expression of this response in relation to the absolute workload may provide misleading conclusions given a diseased population with an established exercise intolerance (i.e. lower peak PO) is being compared to a healthy, albeit obese, population. A steeper adjustment of Δ[HHb+Mb] would be expected in participants with T2D given their lower peak PO during the ramp incremental test. Thus, it is warranted to make comparisons amongst these populations in the context of relative intensity (i.e. as a function of PO%) (Murias et al., 2013). A reduced $PmvO_2$ and intracellular PO_2 will

radically impact muscle metabolism by reducing [phosphocreatine] and elevating $[ADP]_{free}$, [Pi], $[H^+]$ and [NADH]. This increased glycolysis will rely on finite glycogen stores culminating in premature muscular fatigue and ultimately increased exercise intolerance in this clinical population. It should be noted that owing to the generation of noisy $\dot{V}O_2$ data in some of the participants, in the present study we were unable to assess the relationship of %Δ[HHb+Mb] with $\%\dot{V}O_2$ responses.

These findings are in accordance with studies whereby O_2 availability during incremental exercise is deliberately compromised. Specifically, where O_2 delivery was manipulated via exercising in the supine posture and subsequently reducing perfusion pressure (DiMenna et al., 2010; Egaña et al., 2013). In particular, DiMenna et al. (2010) demonstrated a significantly steeper slope of the %Δ[HHb+Mb]/%PO sigmoidal response profile in the supine compared with upright posture during a ramp incremental exercise, implying a greater reliance on O_2 extraction for the same PO (DiMenna et al., 2010). Similarly, Behnke et al. (2002) reported $P\overline{mv}o_2$ reductions at a given muscle stimulation intensity in the GK rodent model of T2D compared with healthy controls predicating either a reduced O_2 diffusion across the capillary-myocyte space to the mitochondria or a lowered intramyocyte PO_2 which would impair muscle metabolism and function (Behnke et al., 2002). Thus, the findings of the present study combined with the previously reported blunted microvascular blood flow responses at the onset of moderate-intensity cycling exercise in individuals with uncomplicated T2D (Bauer et al., 2007), strengthens the argument for reduced O_2 delivery as a likely source of impairment in $\dot{V}O_2$ control in this population.

With evidence of an imbalance in QO_2 relative to PO within the microvasculature during ramp incremental exercise in T2D, and the resultant lowered $P\overline{mv}o_2$, an impaired haemodynamic response can be posited as a potential mechanistic basis for the diminished exercise capacity

herein. Indeed, the significant correlations observed between the initial slope of muscle deoxygenation with $\dot{V}O_{2peak}$ and peak PO in the group with T2D support this notion. In this regard, the attenuated hyperaemic and haemodynamic response during maximum graded calf plantar flexion exercise demonstrated by this clinical population (Kiely et al., 2014) is of relevance. Specifically, Kiely et al. (2014) demonstrated that peak leg blood flow and the slope of leg blood flow relative to percentage peak force during an incremental calf exercise were significantly blunted in men and women with T2D. These reductions were accompanied by significantly lower (magnitude of ~15%) peak force relative to MVC during the calf graded test, which also coincided with a significant (~15%) reduction in $\dot{V}O_{2peak}$ during a graded cycling test in the same participants (Kiely et al., 2014). Therefore, the demonstration in the present study of a faster rise in the primary linear %Δ[HHb+Mb]/%PO signal ($slope_1$) in T2D compared with controls, combined with a similar rate of increase in $\dot{V}O_2$ relative to PO (i.e. $\Delta\dot{V}O_2/\Delta PO$) extends the findings of a dampened hyperaemic response previously observed in isolated muscle groups to that of whole body exercise in uncomplicated T2D.

Although the mechanisms responsible for the altered profile of muscle fractional O_2 extraction observed in individuals with T2D were not directly explored in this study, the impaired vascular function extensively evidenced in T2D is a likely culprit. For instance, attenuated endothelium-dependent vasodilation of resistance vessels in both, the resting forearm (McVeigh et al., 1992; Williams et al., 1996), and the lower limb during cycle exercise (Kingwell et al., 2003) have been reported in individuals with uncomplicated T2D compared to controls. In addition tempered vasodilator responses of the vascular smooth muscle elicited subsequent to exogenous, direct-acting nitric oxide (NO) donors in the form of glyceryl trinitrate (McVeigh et al., 1992) and sodium nitroprusside (Kingwell et al., 2003; Williams et al., 1996) have also been reported in the respective T2D cohorts. It is pertinent to acknowledge, however, that in the absence of cardiac output (CO) data, we cannot exclude the possibility that impairments in

377 cardiac function (Joshi et al., 2010; Regensteiner et al., 2009; Wilson et al., 2017a; Wilson et
378 al., 2017b) could induce subsequent regional O_2 delivery impediments; although peak CO is
379 not significantly reduced in uncomplicated T2D (Baldi et al., 2003; Regensteiner et al., 2009).
380 Moreover, factors beyond convective and diffusive O_2 delivery may also be involved given that
381 structural changes in the skeletal muscles of individuals with T2D havae been observed.
382 Specifically, reductions in mitochondrial content (~30%) (Boushel et al., 2007; Ritov et al.,
383 2005) and functional capacity (~40%) (Kelley et al., 2002; Ritov et al., 2005), as well as
384 alterations in muscle fibre type (Marin et al., 1994), having an approximate 2-fold increase in
385 type IIb fibres relative to type I (Mogensen et al., 2007) have been reported in T2D, although
386 the functional evidence for this notion is unclear (Rabol et al., 2006).
387
388 Limitations of the present study should be acknowledged. Firstly, given the functional
389 limitations of the NIRS technology utilised herein, we were unable to make direct comparisons
390 of absolute concentration and changes in Δ[HHb+Mb] between individuals with and without
391 T2D. However, [HHb+Mb] possesses a time course similar to fractional O_2 extraction (Koga et
392 al., 2012). Secondly, the present findings relate to the evaluation of a single muscle, the VL,
393 and as such, cannot wholly represent the skeletal muscle blood flow response to exercise. Also,
394 the heterogeneity within an individual muscle is recognised; structurally, pertaining to
395 vascularity and fibre type (Johnson et al., 1973), and functionally, relating to fibre recruitment,
396 vascular control and blood flow (Behnke et al., 2003; Koga et al., 2011; McDonough et al.,
397 2005). Thirdly, adipose tissue thickness at the site of measurement has the potential to influence
398 NIRS measurements through its effect on the scattering properties of the tissue. As such, the
399 thickness of the skin and adipose tissue was measured at the site of the interrogation via 2D
400 ultrasound operating in B-mode, with no differences revealed between groups. The current
401 findings are applicable to individuals <55 yr, so, future studies should assess if these effects are
402 also apparent in older people with T2D.

5. Conclusions

The findings from the present study offer an insight into potential contributory mechanisms for the consistently observed reduction in exercise capacity in T2D. The demonstration of a greater rate of O_2 extraction for a given increase in PO suggests that a reduced O_2 delivery within the microvasculature is an important underlying cause of exercise intolerance during a maximum graded test in T2D. This observation strengthens the notion that factors beyond central control also contribute to the diminished exercise tolerance of this clinical population. Such factors are most likely attributed to impairments in active muscle microvascular perfusion. Thus, exercise training interventions designed to benefit exercise tolerance in T2D should also focus on microvascular O_2 delivery.

Author contribution statement

NG, JR, ME, DO'S and SG designed the study. NG, JR and AMcD contributed to data collection. NG, JR, SG and ME performed the data analysis. NG, JR and ME performed the statistical analyses. NG and ME wrote the manuscript. All authors commented on the manuscript and approved the final version of the manuscript.

Declarations of interest

None.

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Figure captions

Figure 1: Representative profiles of the modelled [HHb+Mb] response dynamics during ramp incremental exercise for an individual without, and an individual with T2D when expressed as a function of relative power output (PO%). Double-linear regression models are superimposed on the data. The first %Δ[HHb+Mb]/%PO slope of the double linear regression is indicated beside each curve. Note the relatively larger slope in the participant with T2D compared with the control participant.

Figure 2: Group mean ± SD normalised [HHb+Mb] responses as a function of relative power output (PO%). Data are shown at 10% PO intervals. Note the relatively steeper increase in [HHb+Mb] in the group with T2D compared with the control group.

Figure 3: Individual and mean ± SD (*bar graph*) responses of the first %Δ[HHb+Mb]/%PO slope ($Slpoe_1$) of the double linear regression in the T2D and control groups.

Figure 4: Relationships between first %Δ[HHb+Mb]/%PO slope ($Slpoe_1$) of the double linear regression and $\dot{V}O_{2peak}$ ($mL.kg^{-1}.min^{-1}$) in participants with T2D and ND controls.

Figure 1

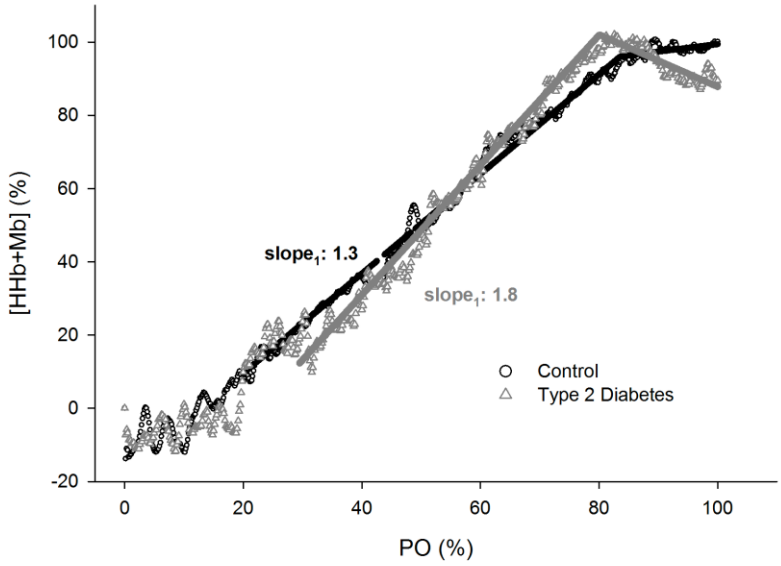

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Figure 2

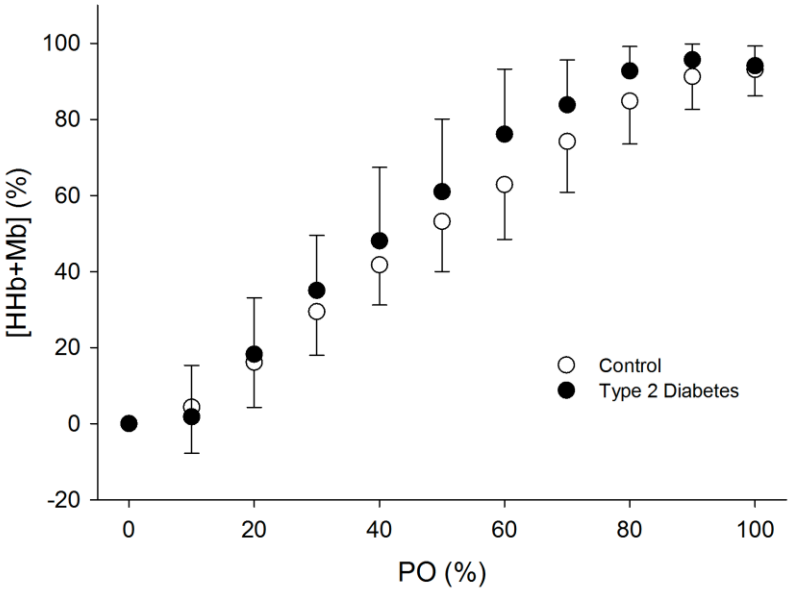

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Figure 3

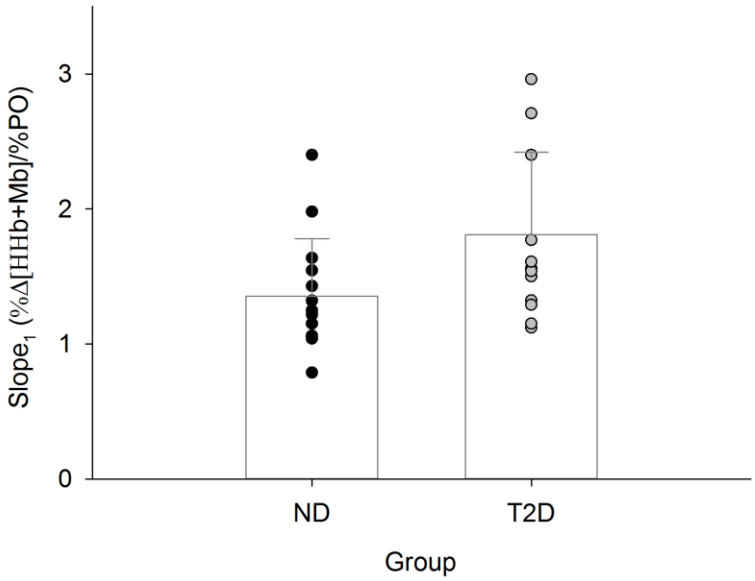

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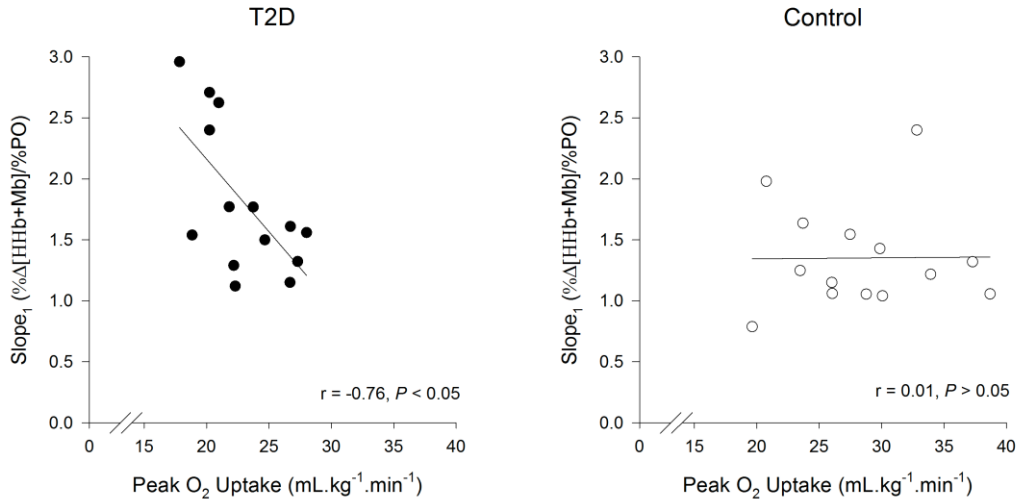
Figure 4



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623 Alternative Fig 4 (no regression line in control

Figure 4

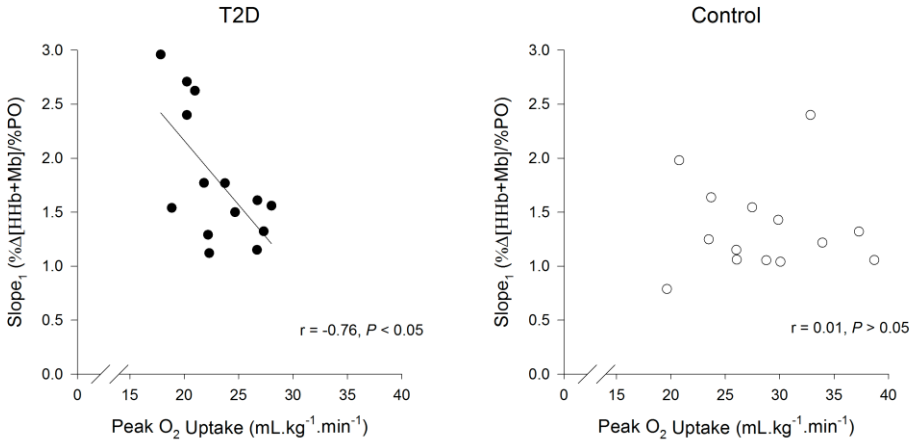

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Table 1. *Physical characteristics and activity levels.*

	ND	T2D	*P* value
n	17	17	
Physical characteristics			
Sex (male, female)	12, 5	12, 5	
Age (yr)	44 ± 8	48 ± 7	0.13
BMI ($kg.m^{-2}$)	30.8 ± 3.5	31.9 ± 4.8	0.46
Body Mass (kg)	91.1 ± 13.8	95.8 ± 18.3	0.40
HbA1c (%)[a]	*5.1 (0.5)*[*]	*6.8 (0.9)*	<0.001
FPG ($mmol.L^{-1}$)[b]	*4.0 (0.4)*[*]	*7.4 (2.9)*	<0.001
Fat layer VL (mm)[c]	7.8 ± 4.5	5.9 ± 1.6	0.14
Time since diagnosis (yr)		5.7 ± 3.7	
Total cholesterol ($mmol.L^{-1}$)[d]	3.6 ± 0.9[*]	4.4 ± 0.7	0.03
LDL-C ($mmol.L^{-1}$)[e]	2.0 ± 0.7	2.2 ± 0.7	0.50
HDL-C ($mmol.L^{-1}$)[d]	1.2 ± 0.2	1.35 ± 0.3	0.62
Triglycerides ($mmol.L^{-1}$)[f]	*1.1 (0.9)*[†]	*1.5 (1.3)*	0.08
Habitual physical activity			
Inactive ($h.day^{-1}$)[g]	18.9 ± 1.3	18.0 ± 1.0	0.15
Light ($h.day^{-1}$)[g]	4.2 ± 1.0[*]	5.3 ± 1.1	0.05
Moderate ($h.day^{-1}$)[g]	0.7 ± 0.4	0.6 ± 0.6	0.69
Vigorous ($h.day^{-1}$)[g]	*0.2 (0.2)*	*0.1 (0.1)*	0.17

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. 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 ($P \leq 0.05$). †Tendency towards a difference than T2D ($P \leq 0.10$).

[a] = 7 (ND) and 15 (T2D); [b] = 10 (ND) and 13 (T2D); [c] = 13 (ND) and 15 (T2D); [d]=10 (ND) and 12 (T2D); [e] = 10 (ND) and 10 (T2D); [f] = 10 (ND) and 13 (T2D); [g] = 13 (ND) and 6 (T2D).

Table 2. *Physiological responses to the ramp incremental test.*

	ND	T2D	*P* value
n	17	17	
$\dot{V}O_{2peak}$ (mL.kg^{-1}.min^{-1})	28.62 ± 5.50*	22.48 ± 3.65	<0.001
$\dot{V}O_{2peak}$ (L.min^{-1})	2.60 ± 0.58*	2.18 ± 0.65	0.03
Peak PO (W)	*196 (108)**	*186 (106)*	*0.04*
Peak HR (beats.min^{-1})	*175 (27)**	*165 (26)*	0.04
Peak RER (a.u.)	*1.2 (0.1)*	*1.1 (0.1)*	*0.20*
Peak MAP (mmHg)[a]	126 ± 17	137 ±24	0.14
Peak SBP (mmHg)[a]	170 ± 24*	187 ± 19	0.05
Peak DBP (mmHg)[a]	103 ± 16	103 ± 23	0.48
$\dot{V}O_2$ at VT (W)	1.78 ± 44*	1.55 ± 0.47	0.02
$\dot{V}O_2$ at RCP (W)	2.25 ± 0.49*	1.93 ± 0.57	0.04

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. $\dot{V}O_2$, volume of oxygen uptake; PO, power output; HR, heart rate; RER, respiratory exchange ratio; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; VT, ventilatory threshold; RCP, respiratory compensation point. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2D ($P \leq 0.05$).

[a] = 9 (ND) and 12 (T2D).

Table 3. *Parameter estimates for the %Δ[HHb+Mb] profile for both groups plotted as a function of normalised PO (%) during the ramp incremental test.*

	ND	T2D	*P* value
n	14	14	
b (slope$_1$)	1.35 (0.43)*	1.81 (0.61)	0.02
b - c (slope$_2$)	0.15 ± 0.67	-0.21 ± 0.57	0.14
BP (%)	81.2 ± 11.9	75.2 ± 12.5	0.20

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. *Slope$_1$* and *Slope$_2$* of linear regression before and after breakpoint (*BP)* respectively.*Significantly different than T2D ($P < 0.05$).