Older type 2 diabetic males do not exhibit abnormal pulmonary oxygen uptake and muscle oxygen utilization dynamics during sub-maximal cycling exercise

Running head: Oxygen uptake kinetics in older Type 2 diabetic patients

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Abstract

There are reports of abnormal pulmonary oxygen uptake ($\dot{V}_{O_2}$) and deoxygenated hemoglobin ([HHb]) kinetics in individuals with Type 2 diabetes (T2D) below 50 years of age with disease durations of <5 years. We examined the $\dot{V}_{O_2}$ and muscle [HHb] kinetics in 12 older T2D patients with extended disease durations (age: 65 ± 5 years; disease duration 9.3 ± 3.8 years) and 12 healthy age-matched control participants (CON; age: 62 ± 6 years). Maximal oxygen uptake ($\dot{V}_{O_2}$ max) was determined via a ramp incremental cycle test and $\dot{V}_{O_2}$ and [HHb] kinetics were determined during subsequent sub-maximal step exercise. The $\dot{V}_{O_2}$ max was significantly reduced ($P<0.05$) in individuals with T2D compared to CON (1.98 ± 0.43 vs. 2.72 ± 0.40 L·min$^{-1}$, respectively) but, surprisingly, $\dot{V}_{O_2}$ kinetics was not different in T2D compared with CON (phase II time constant: 43 ± 17 vs. 41 ± 12 s, respectively). The $\Delta$[HHb]/$\Delta\dot{V}_{O_2}$ was significantly higher in T2D compared to CON (235 ± 99 vs. 135 ± 33 AU·L·min$^{-1}$; $P<0.05$). Despite a lower $\dot{V}_{O_2}$ max, $\dot{V}_{O_2}$ kinetics is not different in older T2D compared to healthy age-matched control participants. The elevated $\Delta$[HHb]/$\Delta\dot{V}_{O_2}$ in T2D individuals possibly indicates a compromised muscle blood flow that mandates a greater O$_2$ extraction during exercise. Longer disease duration may result in adaptations in the O$_2$ extraction capabilities of individuals with T2D thereby mitigating the expected age-related slowing of $\dot{V}_{O_2}$ kinetics.

Keywords: Exercise tolerance, deoxygenated haemoglobin, senescence
Introduction

The exercise intolerance characteristic of patients with Type 2 diabetes has been attributed substantially to dysfunction at multiple steps of the O2 transport pathway. Thus, Type 2 diabetes impairs cardiac function (16, 38, 43) reducing maximal O2 transport which, combined with peripheral dysfunction, decreases total and fractional O2 extraction (3) and maximal pulmonary O2 uptake (VO2 max; 3, 39, 40). Sentinel changes in vascular function and skeletal muscle include blunted endothelium-dependent vasodilation and blood flow increases (22, 24, 31, 32, 50), elevated plasma concentrations of endothelin-1 (46), reduced capillary density (27, 28) and dysfunctional capillary hemodynamics (34, 47) as well as decreased mitochondrial volume density (41) and function (21, 26). There may also be a relative propensity for a greater fraction of Type IIb (highly glycolytic) muscle fibres in these patients (27, but see also 1).

A key determinant of exercise tolerance is the rate at which oxygen uptake (VO2) rises to meet the ATP turnover requirements of the exercise (i.e., VO2 kinetics). VO2 kinetics are usually determined via pulmonary measurements of VO2, which has been shown to be indicative of muscle VO2 kinetics once the transit delay from muscle to lung has been accounted for (15, 23). Faster VO2 kinetics are beneficial because this increases the contribution of oxidative relative to non-oxidative metabolism to energy turnover and helps minimize intracellular perturbations (i.e., Δ[Phosphocreatine], Δ[ADP]free,Δ[H+], Δ[lactate]) that are associated with exercise intolerance (rev. 20). There are several reports of slower VO2 kinetics in Type 2 diabetic patients compared to healthy age-matched controls (4, 8, 39, 40). In healthy humans, during moderate or heavy intensity cycling, VO2 kinetics is thought to be limited by intramuscular energetics rather than O2 delivery per se (rev. 36). In contrast, Padilla at al. (35, electrically-stimulated rat spinotrapezius muscle, phosphorescence quenching) and Bauer et al. (4, human quadriceps during cycling) recently reported a transiently increased skeletal muscle deoxygenation (as assessed using near infrared spectroscopy; NIRS) following the onset of contractions in Type 2 diabetic vs. healthy control individuals. These results were interpreted as evidence for a relative mismatch in muscle O2 delivery-to-VO2 during the early stages of exercise such that, in the diabetic condition as opposed to the healthy condition, the VO2 kinetics limitation might be related in part to a compromised O2 delivery.

Previous work examining VO2 kinetics in Type 2 diabetic individuals has focused predominantly on pre-menopausal females under 50 years of age (disease duration <5 years, 8, 39, 40) which may have been inspired by the observation that this disease has a more profound impact on the VO2 max of females compared to males (39). Considering that the prevalence of Type 2 diabetes is approximately equal among genders (53), it is important to examine the VO2 kinetic responses of Type 2 diabetic males as it is currently unknown if their response is similar to that found in females. In addition, as the prevalence of Type 2 diabetes increases substantially with age it is also important to resolve whether similarly impaired VO2 kinetics and muscle deoxygenation profiles are found in older individuals in whom the disease duration may have been far longer. It would seem reasonable to predict that extended disease duration may lead to greater disruption in the individual’s ability to transport and/or utilise O2.

Our hypotheses were that older Type 2 diabetic males would have: (1) a significantly lower VO2 max and maximal work rate, (2) slower pulmonary VO2 kinetics, and (3) an abnormal muscle deoxygenation profile compared to healthy age-matched control participants. Further, as the patients with Type 2 diabetes that were the focus of this investigation were older and were >5 years from presentation of diabetes, we also hypothesised that the difference in the VO2
kinetic responses between the two groups would be greater compared with previous studies (8, 39, 40).

Methods

Participants

Twelve male participants with Type 2 diabetes and 12 healthy control subjects volunteered to participate in this study (Table 1). All participants provided their written informed consent to take part in this research, which had been approved by the Exeter and North Devon Local Medical Research Ethics Committee. All participants were sedentary, which was defined as participating in low-to-moderate intensity exercise less than 2 days/week in the preceding 3 months. The inclusion criteria for the patient group were as follows: Type 2 diabetes diagnosed at least 5 years prior to the commencement of the study at 33 years or older, no ketones at time of diagnosis, treated with diet alone or oral hypoglycaemic agents. The healthy control participants were defined as taking no medication, with no immediate family member with Type 2 diabetes, and confirmed glucose tolerant with an oral glucose tolerance test. Participants were excluded from the study if they had suffered a stroke or myocardial infarction, systemic vasculitis, uncontrollable hypertension (>160/90 mmHg), unstable angina, a pacemaker, or an abnormal ECG.

Blood samples

Glycated haemoglobin (HbA$_{1c}$), glucose and insulin were measured following an overnight fast. HbA$_{1c}$ was measured using HPLC (Tosoh G7 HPLC System; Tosoh Bioscience, Redditch, UK). Plasma glucose was measured using a Roche modular analyser (Roche Diagnostics, Lewes, UK), and insulin was measured using an immunoenzymometric assay (Appligene Oncor/Lifescreen, Uxbridge, UK) calibrated against IRP66/304 with no detectable cross-reactivity with intact proinsulin.

Experimental overview

Following a screening session (conducted on a different day), participants completed three bouts of exercise on a cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) at a cadence of 60-65 rpm. Participants were asked to arrive at the laboratory rested and fully hydrated, having avoided the consumption of food and caffeine in the preceding 2 h. The first bout of exercise was a ramp incremental test (15 W.min$^{-1}$) which resulted in volitional exhaustion within 8-12 minutes. The participants then rested seated for a minimum of 45 minutes. It has been shown that 45 minutes is a sufficient rest period to allow the restoration of a ‘normal’ $\dot{V}_{O_2}$ response following prior high-intensity exercise in young healthy participants (9). In the present study, we were careful to ensure that sufficient recovery time was allowed such that $\dot{V}_{O_2}$ during the ‘unloaded’ cycling baseline which preceded subsequent exercise bouts was restored. The second and third tests were identical and consisted of cycling at the lowest available power output on the ergometer (20 W) for 3-minutes, followed by an immediate ‘step’ increment in power output to a power that was 50% of the maximum achieved in the ramp incremental test for 6 minutes. Participants rested for 10 minutes before completing the second of the ‘step’ tests. Two step exercise tests were completed to improve the signal-to-noise ratio and enhance the underlying response characteristics of the $\dot{V}_{O_2}$ response (25). It was decided a priori to compare the groups at the same relative as opposed to absolute exercise intensity. As mentioned previously, it is widely reported that individuals with Type 2 diabetes
have a lower $\dot{V}O_2$ max than aged matched healthy control participants (e.g. 39), thus exercise conducted at the same absolute work rate would not provide a fair comparison between the groups (i.e. the groups would likely be in different exercise intensity domains, known to elicit distinctly different metabolic and pulmonary gas exchange responses; rev. 20).

Measurements

Pulmonary gas exchange was measured breath by breath throughout all exercise tests (Jaeger Oxycon Alpha, Hoechberg, Germany). The volume transducer was calibrated before each test with a 3-liter calibration syringe, and the analysers were calibrated with gases of known concentration. Heart rate (HR) was recorded every 5-s using short range telemetry (Polar PE 4000, Kempele, Finland).

The oxygenation status of the *m. vastus lateralis* of the right leg was monitored using a commercially available NIRS system (model NIRO 300, Hamamatsu Photonics KK, Hiugashiku, Japan). The system consists of an emission probe that irradiates laser beams and a detection probe, which is positioned several centimetres from the emission probe in an optically dense rubber holder. Four different wavelength laser diodes provided the light source (776, 826, 845 and 905 nm) and the light returning from the tissue was detected by a photomultiplier tube in the spectrometer. The intensity of incident and transmitted light was recorded continuously at 2 Hz and used to estimate concentration changes from the resting baseline for oxygenated, deoxygenated and total tissue haemoglobin. The device was secured to the skin with adhesive at 20 cm above the fibular head. The validity and utility of the use of NIRS during exercise has been reviewed (7).

Data Analysis Procedures

The breath-by-breath data were linearly interpolated to provide second-by-second values and, for each individual, the two bouts were time-aligned to the start of exercise and ensemble-averaged. The first 20 s of data after the onset of exercise (i.e., the phase I response) were deleted and a nonlinear least-square algorithm was used to fit the data. The data were adequately fit with a mono-exponential model (eqn 1) in most cases, although there were 4 subjects in each group for whom a bi-exponential model was necessary (eqn 2). For exercise above the gas exchange threshold (GET) a bi-exponential model is required to account for the presence of the $\dot{V}O_2$ slow component. That 4 subjects in each group were likely above their GET has minimal impact upon the interpretation of our data as the ‘fundamental phase’ $\dot{V}O_2$ kinetics are not different between moderate (<GET) and heavy (>GET) intensity exercise (51). When statistical analyses were carried out with these subjects removed from each group respectively, it made no difference to the principal findings, and thus all 12 subjects in each group were included in the analyses presented.

\[
\dot{V}O_2(t) = \dot{V}O_2_{\text{baseline}} + A_p(1-e^{-(t-TD_p)/\tau_p}) \quad (1)
\]

\[
\dot{V}O_2(t) = \dot{V}O_2_{\text{baseline}} + A_p(1-e^{-(t-TD_p)/\tau_p}) + A_s(1-e^{-(t-TD_s)/\tau_s}) \quad (2)
\]

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time $t$; $\dot{V}O_{2\text{baseline}}$ represents the mean $\dot{V}O_2$ in the baseline period; $A_p$, $TD_p$, and $\tau_p$ represent the amplitude, time delay, and time constant, respectively, describing the increase in $\dot{V}O_2$ above baseline. $A_s$, $TD_s$, and $\tau_s$ represent the
amplitude, time delay before the onset of, and time constant describing the development of, the $\dot{V}O_2$ slow component, respectively. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 seconds of exercise. The absolute fundamental component amplitude (absolute $A_p$) was defined as the sum of $\dot{V}O_{2\text{baseline}}$ and $A_p$. In addition, the functional ‘gain’ of the fundamental $\dot{V}O_2$ response was computed by dividing $A_p$ by the $\Delta$ work rate.

To provide information on muscle oxygenation, we also modelled the [HHb] response to exercise. A mono-exponential model similar to that described in Eq. 1 above was used, with the exception that the fitting window commenced at the onset of exercise (i.e., at $t = 0$). In addition to the [HHb] $\tau$ and TD derived from the mono-exponential fit, we also used the [HHb] amplitude to determine the $\Delta[\text{HHb}]/\Delta\dot{V}O_2$ during this phase of the response. This ratio indicates the degree of $O_2$ extraction required for a given increment in $\dot{V}O_2$ and can therefore provide insight into the dynamic balance between $O_2$ delivery and utilization. Finally, the ‘gain’ of the [HHb] response was calculated by dividing the steady state amplitude by the $\Delta$ work rate.

HR responses were modelled using a nonlinear least-square mono-exponential model without time delay, with the fitting window commencing at $t = 0$.

Statistics

Independent samples $t$ tests were used to test for significant differences in the parameters derived from the modelling of the $\dot{V}O_2$, [HHb] and HR data between the patients with Type 2 diabetes and healthy control participants with significance declared when $P<0.05$. Results are reported as means ± S.D unless otherwise stated.

Results

The participants’ demographic data are presented in Table 1, along with information regarding disease status and responses to the ramp incremental test. Note that the subjects were well-matched for age and BMI. As expected, the Type 2 diabetic patients had a significantly elevated fasting glucose and HbA1c levels compared to the healthy controls. The diabetic patients exhibited a significantly lower $\dot{V}O_2$ max, which was attained at a significantly lower power output than their healthy counterparts (i.e. time to exhaustion during the ramp incremental test was significantly reduced). The $\Delta\dot{V}O_2 /\Delta\text{WR}$ slope was not significantly different between the groups.

There were no significant differences between the groups for the time-course of the $\dot{V}O_2$ response during the transition from unloaded to sub-maximal exercise (Figure 1 and Table 2). There was a significant difference between the two groups for the steady state $\dot{V}O_2$ amplitude, which was elevated in the control subjects as a function of this group exercising at a higher absolute power output. The ‘gain’ of the $\dot{V}O_2$ response was not different between the groups.

There were no significant differences between the groups for the time course of the [HHb] response ($\tau$, or TD; Table 3). An ‘overshoot’ in [HHb] (28) was not a consistent feature of the response in the diabetic patients. Indeed, this behaviour ([HHb] ‘overshoot’ (i.e. an initial excursion of [HHb] above the level attained during the steady state within the first 60-100 s of exercise) was observed in only four individuals, two of whom were in the healthy control group (Figure 2). Despite the lack of any difference between groups with respect to an
overshoot of [HHb] there was a markedly higher [HHb] gain, which was approximately 50% greater in Type 2 diabetic patients, such that $\Delta[\text{HHb}]/\Delta\dot{V}_\text{O}_2$ was significantly elevated compared with healthy controls. The time constants of the heart rate kinetics was not different between Type 2 diabetic patients and healthy control participants (61 ± 15 vs. 59 ± 21 s, respectively) and was, in both instances, slower than $\dot{V}_\text{O}_2$ kinetics.

**Discussion**

To our knowledge, this is the first investigation to examine the pulmonary $\dot{V}_\text{O}_2$ kinetics and muscle deoxygenation dynamics of older males with longstanding Type 2 diabetes (i.e., disease duration exceeding 5 years). The main findings were that: (1) Type 2 diabetic individuals had a significantly reduced $\dot{V}_\text{O}_2$ max and maximal work rate compared to healthy controls (consistent with our first hypothesis and previous reports); (2) the time constant of the pulmonary $\dot{V}_\text{O}_2$ kinetics was not different between Type 2 diabetic patients and healthy controls (inconsistent with our second hypothesis) such that there was a dissociation between $\dot{V}_\text{O}_2$ max and $\dot{V}_\text{O}_2$ kinetics; and (3) the pattern of skeletal muscle deoxygenation was not different between patients and controls (i.e. no consistent [HHb] overshoot in the patients – in disagreement with our third hypothesis). However, [HHb] increased more per unit work and $\dot{V}_\text{O}_2$ in the Type 2 diabetic patients suggesting a greater relative mismatch in muscle O$_2$ delivery-to-$\dot{V}_\text{O}_2$ in this population.

These results are surprising from several perspectives. Specifically, the $\dot{V}_\text{O}_2$ kinetics and skeletal muscle HHb dynamics in these patients were not different from healthy age-matched controls despite the patients reduced $\dot{V}_\text{O}_2$ max and maximal work rate, and the extended duration of disease (>5 years). Putative explanations for the variance from previous research include gender differences, older age and duration of disease. Accordingly, these current data indicate that either males have a fundamentally different response compared with females, or that the perturbations to $\dot{V}_\text{O}_2$ and [HHb] dynamics seen in younger patients with Type 2 diabetes do not worsen ($\dot{V}_\text{O}_2$ kinetics), and indeed may even subside ([HHb] dynamics), with aging.

$\dot{V}_\text{O}_2$ max

The aging associated reduction in $\dot{V}_\text{O}_2$ max is well documented and is presumably associated with reduced muscle O$_2$ supply [blood flow] (37, 49), capillarity (12, 42; but see also 18, 29), endothelial function (10, 33), O$_2$ diffusing capacity (17), fractional O$_2$ extraction (30) and mitochondrial enzyme activity (12, 48). However, in the current study, despite similar ages, the control participants had a significantly higher $\dot{V}_\text{O}_2$ max than the type 2 diabetic participants (consistent with previous studies which examined younger participants; 3, 39, 40), despite no differences in the sub-maximal $\dot{V}_\text{O}_2$ kinetic response. It is difficult to explain how such a profound reduction in $\dot{V}_\text{O}_2$ max does not also result in significantly slower $\dot{V}_\text{O}_2$ kinetics in the Type 2 diabetic group, but it is presumably related to the pernicious effects of the disease on skeletal muscle structure and function. Individuals with type 2 diabetes have been shown to have a reduced mitochondrial volume density (41) and function (21, 26) compared to healthy control participants. Our data suggest that the influence of Type 2 diabetes on skeletal muscle structure and function has a greater impact (over and above the deleterious impact of aging/detraining) on the $\dot{V}_\text{O}_2$ max than on the sub-maximal $\dot{V}_\text{O}_2$ kinetic response.
Gender Differences in \( V_{O_2} \) kinetics

It has been previously noted that Type 2 diabetes has a greater impact upon the \( V_{O_2} \) max of females compared to males (unpublished observations noted in 39). In contrast to the notion that males might be less or even minimally impacted by this disease, the present results demonstrate a substantial lowering of \( V_{O_2} \)max (27%), and maximal work rate and time-to-exhaustion (35%) on the incremental exercise test compared to healthy control participants. In addition, Bauer et al (4) found that \( V_{O_2} \) kinetics was slowed in both male and female Type 2 diabetic patients (average age 47 years, disease duration not reported) with no sexual dimorphism noted. In addition, to our knowledge, and equating aerobic fitness (\( V_{O_2} \)max) across genders, there is no a priori reason to support the concept that those elements of the \( O_2 \) transport system that are impacted by Type 2 diabetes should be differently impacted in males compared to females (20). Whilst the current study demonstrates that older male diabetic individuals do not have abnormally slow \( V_{O_2} \) kinetics compared to their healthy counterparts, it is currently unknown if the same is true for younger Type 2 diabetic males.

Age-related Effects and Duration of Type 2 Diabetes and \( V_{O_2} \) kinetics

It is well known that aging and the accompanying reduction in physical activity manifested as individuals approach senescence result in slowed \( V_{O_2} \) kinetics at exercise onset (2, 6, 11, 45). In this regard, it is noteworthy that the mean \( V_{O_2} \) \( \tau \) of 41 s for our healthy controls herein was substantially slower than that found in younger populations (i.e., 20-24 s rev. 44). This slowing of \( V_{O_2} \) kinetics in aged individuals has been attributed to a reduced muscle \( O_2 \) supply (37, 49), capillarity (12, 42; but see also 18, 29), endothelial function (10, 33), \( O_2 \) diffusing capacity (17), fractional \( O_2 \) extraction (30) and \( V_{O_2} \)max (possibly linked to reduced mitochondrial enzyme activity; 12, 48). In addition, in aged rodent muscles, a transient \( O_2 \) delivery-to-\( V_{O_2} \) mismatch following the onset of contractions causes muscle microvascular PO2 to fall below the subsequent steady-state values (5). Thus, Type 2 diabetes (see Introduction) and aging have substantial commonality with respect to their impact on multiple elements of the \( O_2 \) transport and utilization pathway.

One important consideration is whether the effects of Type 2 diabetes and aging (and possibly duration of disease) are additive. The results of the present investigation provide evidence to support the suggestion that, at least in males, this is not the case. Rather, whereas Type 2 diabetes slows \( V_{O_2} \) kinetics in comparatively younger individuals (40-50 year-olds; 4, 8, 39, 40), the subsequent age-related slowing of \( V_{O_2} \) kinetics to the seventh decade appears to be either reduced or curtailed. This was counter to our hypothesis that older individuals who have had Type 2 diabetes for ~9 years, on average, would have had an exaggerated perturbation of \( O_2 \) delivery and utilisation capabilities, translating into appreciably slower \( V_{O_2} \) kinetics than have previously been reported for younger patients with disease durations <5 years. It is possible that the slowing seen in the \( V_{O_2} \) kinetics of individuals with Type 2 diabetes reaches a plateau within 2-3 years subsequent to disease onset, after which little or no further slowing occurs. In individuals with Type 2 diabetes, further slowing associated with aging/detraining may not accentuate the slow \( V_{O_2} \) kinetics already incurred by their disease. Thus, the similarity in the \( V_{O_2} \) dynamics between groups may be explained by the notion that the healthy controls in the present investigation (who were matched for activity levels with the diabetic patients and who were recruited from the same community) may have experienced an aging/detraining-related slowing of their \( V_{O_2} \) kinetics, whilst the Type 2 diabetic group did not. Consistent with this hypothesis and regardless of gender, \( V_{O_2} \) kinetics in the diabetic patients in the present investigation (mean Phase 2 \( \tau \) 43 s) was not different from that reported previously in younger
Type 2 diabetics (i.e., 40-50 year olds; 4, 8, 39). Rather, it was the healthy control group that evidenced a slowing of $\dot{V}_{O_2}$ kinetics when compared to previous findings in young healthy individuals (i.e., $\sim 41$ s vs. $<30$ s; 4, 8, 39). Thus it appears that the impact of aging/detraining on the $\dot{V}_{O_2}$ kinetic responses of the aged control participants in the current study may explain why no difference was observed between the groups in the dynamic $\dot{V}_{O_2}$ response at the onset of exercise. Whether there is some minimal speed of $\dot{V}_{O_2}$ kinetics that is defended in aged individuals, possibly related to the slowest value consistent with some level of ambulation, is not known at this time. This consideration is particularly interesting in light of the substantial impairment of O$_2$ transport and utilization during maximal exercise (decreased $\dot{V}_{O_2}\text{max}$ and work rate) in the present population of older Type 2 diabetic patients.

Profile of Muscle Deoxygenation

That we did not consistently observe an ‘overshoot’ in the [HHb] response in our patient group is perhaps surprising considering recent reports of this phenomenon in the Type 2 diabetic human (4) and rodent (35). The overshoot noted in Type 2 diabetics has been taken as evidence of an impaired increase of muscle blood flow relative to muscle $\dot{V}_{O_2}$. There is evidence that Type 2 diabetic individuals have both macro-vascular (22) and microvascular (34) disturbances, which might be expected to result in a greater reliance on oxygen extraction during the early stages of exercise. In the present investigation, an [HHb] overshoot was observed in four participants only (two individuals from each group; Figure 2). Thus, the [HHb] responses noted herein indicates that either: (i) the overshoot in [HHb] is not an obligatory consequence of Type 2 diabetes; or (ii) the overshoot is something that occurs predominantly during the early stages of the disease, and those that have had the disease for longer (perhaps irrespective of age) experience an adaptation in O$_2$ delivery/extraction capabilities to compensate for this perturbation. The overshoot noted in the individuals from the control group is presumably related to disruption in muscle blood flow relative to muscle $\dot{V}_{O_2}$, perhaps as a consequence of aging/detraining (14). It is noteworthy that a recent report (52) demonstrated that skeletal muscle blood flow was well preserved in patients with well controlled uncomplicated Type 2 diabetes during forearm flexor muscle exercise, but an abnormal blood flow response was noted in those with Type 2 diabetes and associated microvascular complications. Whilst the data of Womack et al. (52) are not directly comparable with those of the present study due to differences in exercise modality, the possibility remains that the patients in the present study where devoid of microvascular complications that may be necessary to elicit a detrimental impact upon the skeletal muscle blood flow.

An interesting finding regarding [HHb] was the 35% elevation in the gain of the response (i.e. increase in [HHb] per unit increase in WR) in the Type 2 diabetic individuals compared to the control participants, a corollary of this being a significantly elevated $\Delta[\text{HHb}] / \Delta\dot{V}_{O_2}$ in this group. An elevated $\Delta[\text{HHb}] / \Delta\dot{V}_{O_2}$ is indicative of a greater proportional contribution of O$_2$ extraction to satisfy a given increase in $\dot{V}_{O_2}$ (13). On first inspection it appears that the Type 2 diabetic patients have enhanced capabilities compared to the control individual to support aerobic metabolism by O$_2$ extraction. A possible explanation for the higher gain of [HHb] would be that it is a necessary adaptation to a compromised skeletal muscle blood flow in the diabetic individual, as has previously been reported (22), resulting in greater extraction from the ‘available’ blood. This may indicate that the Type 2 diabetic individual who has had the disease for an extended period has an improved O$_2$ extraction capability, something that may not be evident in shorter disease term Type 2 diabetics that have been studied previously (4, 8, 39, 40). It is noteworthy in this regard that individuals who have had Type 2 diabetes for
shorter periods of time (with similar characteristics in terms of treatment, ethnicity or complications to the patients studied in the present study) exhibit altered [HHb] dynamics (i.e. an overshoot; 4), but with a similar gain to aged matched control subjects. This [HHb] profile may be characteristic of shorter-term Type 2 diabetic individuals have not yet ‘adapted’ to the disease. This may go some way towards explaining why a slower \( \dot{V}_O_2 \) response is evident in those who have had the disease for shorter periods of time, but not in those with longer disease durations.

With respect to the limitation(s) to \( \dot{V}_O_2 \) kinetics during conventional ambulatory activities (i.e., cycling, running/walking), it is likely that healthy individuals become increasingly \( O_2 \) delivery limited with advancing age (36, 44). This would act to slow \( \dot{V}_O_2 \) kinetics at exercise onset irrespective of mitochondrial oxidative capacity. This notion has been conceptualized as a ‘tipping point’ for muscle \( O_2 \) delivery below which \( \dot{V}_O_2 \) kinetics are no longer constrained by mitochondrial energetics alone but are additionally slowed by inadequate \( O_2 \) delivery (19). Given this logic, the increased \( \Delta[HHb]/\Delta\dot{V}_O_2 \) ratio in the diabetic patients would imply that, all else being equal, \( O_2 \) delivery, and thus \( \dot{V}_O_2 \) kinetics, should be more compromised than in the healthy control participants. That this did not occur is important and suggests that other adaptations within the exercising muscles may have facilitated faster \( \dot{V}_O_2 \) kinetics than would have been expected in this population. Candidate mechanisms deserving of future investigation include altered capillary hemodynamics and improved muscle \( O_2 \) diffusing capacity.

**Exercise Tolerance**

One interpretation of the above data is that, at least at similar relative exercise intensities, older Type 2 diabetic individuals who have had Type 2 diabetes for more than several years will not have any \( \dot{V}_O_2 \) kinetics-related compromise of their exercise tolerance compared to healthy age-matched individuals. This is a potentially important observation, and one that has implications for our understanding of the exercise capabilities of older diabetic patients. However, it should be pointed out that the significantly lower \( \dot{V}_O_2 \) max of Type 2 diabetic individuals compared to control participants mandates that any absolute work-rate or exercise intensity will prove more challenging for the Type 2 diabetic patient than their age-matched healthy counterpart.

For practical reasons, the diabetic and control participants in the present study completed the ramp incremental test and the sub-maximal step tests within the same session (separated by ~ 45 minutes). This might be considered a limitation. While there is evidence that a period of 45 minutes following high-intensity exercise is sufficient for complete recovery to occur in young healthy individuals (i.e. to have no effect on \( \dot{V}_O_2 \) kinetics during subsequent exercise; 9), we cannot rule out the possibility that completion of the initial ramp incremental test impacted upon the physiological responses to subsequent step exercise in our study participants. However, this concern may be mitigated by evidence that baseline HR and \( \dot{V}_O_2 \) were not different within groups between the ramp incremental test and the subsequent sub-maximal step tests (Tables 1, 2 and 3).

**Perspectives and Significance**

In the face of decreased \( \dot{V}_O_2\)max and maximal work capacity, \( \dot{V}_O_2 \) kinetics following the onset of cycle exercise is not different in older Type 2 diabetic patients compared to healthy age-matched control participants. These similar \( \dot{V}_O_2 \) kinetic responses may be related to the healthy individuals experiencing an aging/detraining-related slowing of \( \dot{V}_O_2 \) kinetics, whereas the
patients do not. The elevated $\Delta[\text{HHb}]/\Delta\dot{V}_{O_2}$ in the Type 2 diabetic possibly indicates a compromised muscle blood flow that mandates a greater $O_2$ extraction during exercise. Interestingly, any limitations in blood flow in the transition to a higher exercise intensity did not translate into an ‘overshoot’ of [HHb] in the majority of the Type 2 diabetic individuals. It is possible that those who have had Type 2 diabetes for an extended period have experienced adaptations in their $O_2$ extraction capabilities to account for any blood flow perturbations and these adaptations mitigate the expected age-related slowing of $\dot{V}_{O_2}$ kinetics. These data enhance our understanding of exercise tolerance in older Type 2 diabetic patients by indicating that the response of these individuals to sub-maximal exercise may be less impacted by the disease than might be predicted. Future work should investigate the temporal relationships among disease duration, $\dot{V}_{O_2}$ and [HHb] kinetics, and exercise performance in Type 2 diabetic individuals, as well as muscle-specific adaptations in the $O_2$ transport pathway. Such information would be important in resolving the mechanistic bases for the impact of Type 2 diabetes (and aging) on exercise function in this burgeoning population.
References


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<th>Control</th>
<th>Type 2 diabetic</th>
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<td>Age (years)</td>
<td>62 ± 6</td>
<td>65 ± 5</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>-</td>
<td>9.3 ± 3.8*</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>87.2 ± 5.5</td>
<td>87.3 ± 8.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.4 ± 1.2</td>
<td>29.2 ± 2.5</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.6 (4.9, 5.4)</td>
<td>7.3 (7.2, 7.9)*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>135 ± 9</td>
<td>142 ± 11</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84 ± 9</td>
<td>81 ± 8</td>
</tr>
<tr>
<td>Fasting insulin (pmol.L⁻¹)</td>
<td>51 ± 21</td>
<td>100 ± 39*</td>
</tr>
<tr>
<td>Fasting Glucose (mmol.L⁻¹)</td>
<td>5.1 (4.9, 5.4)</td>
<td>8.3 (6.9, 8.6)*</td>
</tr>
<tr>
<td>Insulin sensitivity (% HOMA)</td>
<td>100 ± 39</td>
<td>49 ± 24†</td>
</tr>
<tr>
<td>Baseline $\dot{V}O_{2}$ (ramp test; L.min⁻¹)</td>
<td>0.96 ± 0.07</td>
<td>0.96 ± 0.07</td>
</tr>
<tr>
<td>Baseline heart rate (ramp test; b.min⁻¹)</td>
<td>98 ± 10</td>
<td>102 ± 11</td>
</tr>
<tr>
<td>$\dot{V}O_{2}$ max (L.min⁻¹)</td>
<td>2.72 ± 0.40</td>
<td>1.98 ± 0.43*</td>
</tr>
<tr>
<td>$\Delta \dot{V}O_{2}/\Delta WR$ slope (mL.W⁻¹.min⁻¹)</td>
<td>10.8 ± 0.50</td>
<td>10.5 ± 1.69</td>
</tr>
<tr>
<td>Time to exhaustion during ramp test (mins)</td>
<td>12.5 ± 2.5</td>
<td>8.1 ± 2.2*</td>
</tr>
<tr>
<td>Work rate for step tests (W)</td>
<td>102 ± 19.5</td>
<td>71 ± 16.3*</td>
</tr>
</tbody>
</table>

Data are means ± SD. †P < 0.01; * P < 0.05, Type 2 diabetic versus control participants.
Table 2: O₂ uptake kinetics during exercise

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Type 2 diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline $\dot{V}O_2$ (L·min⁻¹)</td>
<td>0.97 ± 0.06</td>
<td>0.91 ± 0.12</td>
</tr>
<tr>
<td>$\dot{V}O_2$ τ (s)</td>
<td>41 ± 12</td>
<td>43 ± 17</td>
</tr>
<tr>
<td>$\dot{V}O_2$ time delay (s)</td>
<td>4 ± 11</td>
<td>4 ± 11</td>
</tr>
<tr>
<td>$\dot{V}O_2$ amplitude (L·min⁻¹)</td>
<td>0.91 ± 0.19</td>
<td>0.57 ± 0.17*</td>
</tr>
<tr>
<td>$\dot{V}O_2$ gain (mL·min⁻¹·W⁻¹)</td>
<td>11.2 ± 1.2</td>
<td>11.6 ± 2.8</td>
</tr>
<tr>
<td>End-exercise $\dot{V}O_2$ (L·min⁻¹)</td>
<td>1.94 ± 0.14</td>
<td>1.52 ± 0.29*</td>
</tr>
</tbody>
</table>

Data are means ± SD. †$P$<0.01; *$P$<0.05, Type 2 diabetic versus control participants. For more information on modelling procedures please see text.
Table 3: Heart rate and deoxyhaemoglobin kinetics during exercise.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Type 2 diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline heart rate (b·min⁻¹)</td>
<td>100 ± 10</td>
<td>105 ± 15</td>
</tr>
<tr>
<td>End-exercise heart rate (b·min⁻¹)</td>
<td>136 ± 15</td>
<td>129 ± 18</td>
</tr>
<tr>
<td>Heart rate τᵣ (s)</td>
<td>59 ± 21</td>
<td>61 ± 15</td>
</tr>
<tr>
<td>[HHb] τ (s)</td>
<td>14 ± 3</td>
<td>17 ± 8</td>
</tr>
<tr>
<td>[HHb] time delay (s)</td>
<td>8 ± 2</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>[HHb] mean response time (s)</td>
<td>22 ± 6</td>
<td>25 ± 11</td>
</tr>
<tr>
<td>Δ[HHb]/ΔV̇O₂ (AU·L·min⁻¹)</td>
<td>135 ± 33</td>
<td>235 ± 99*</td>
</tr>
<tr>
<td>[HHb] gain (AU·W⁻¹)</td>
<td>1.54 ± 0.4</td>
<td>2.39 ± 0.9*</td>
</tr>
</tbody>
</table>

Data are means ± SD. * P=<0.05, Type 2 diabetic versus control participants. For more information on modelling procedures please see text.
**Figure Legends**

Figure 1: Representative examples of the pulmonary $\dot{V}O_2$ response during exercise for healthy control subjects (A; o) and patients with type 2 diabetes (B; ●). Solid lines represent the model fits to the responses with the residuals (crosses, shown below profiles) demonstrating that the data were well fit by the single exponential model. Panel C shows an overlay of the kinetic responses from A and B, by scaling the steady-state amplitude to 100%. Exercise commenced at time zero.

Figure 2: Representative examples of the [HHb] response during exercise for two healthy control subjects (A) and two patients with Type 2 diabetes (B) who either did (A: x; B:+) or did not (A:o; B:●) exhibit a [HHb] ‘overshoot’. The data are scaled such that the end-exercise values equate to 100%. The majority of subjects demonstrated no overshoot in [HHb], with an overshoot only being noted in 2/12 healthy control subjects and 2/12 patients with Type 2 diabetes; see text for more details.
Figure A shows the oxygen uptake rate ($\dot{V}O_2$) over time, with a time constant $\tau = 41$ s. Figure B displays the same with $\tau = 39$ s. Figure C illustrates the oxygen uptake rate as a percentage of the response.