Skeletal Muscle Deoxygenation Following the Onset of Moderate Exercise Suggests Slowed Microvascular Blood Flow Kinetics in Type 2 Diabetes

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Received for publication 1 May 2007 and accepted in revised form 16 July 2007.
Abstract

**Objective**: People with type 2 diabetes (T2DM) have impaired exercise responses even in the absence of cardiovascular complications. One key factor associated with the exercise intolerance is abnormally slowed oxygen uptake (\(\dot{V}O_2\)) kinetics during submaximal exercise. The mechanisms of this delayed adaptation during exercise are unclear but likely relate to impairments in skeletal muscle blood flow. The study was conducted to compare skeletal muscle deoxygenation ([HHb]) responses and estimated microvascular blood flow (Qm) kinetics in T2DM and healthy subjects following the onset of moderate exercise.

**Research design and Methods**: Pulmonary \(\dot{V}O_2\) kinetics and [HHb] (using near infrared spectroscopy) were measured in 11 T2DM and 11 healthy subjects during exercise transitions from unloaded to moderate cycling exercise. Qm responses were calculated using \(\dot{V}O_2\) kinetics and [HHb] responses via rearrangement of the Fick principle.

**Results**: \(\dot{V}O_2\) kinetics were slowed in T2DM compared with controls (43.8±9.6s vs. 34.2±8.2s, P<0.05), and the initial [HHb] response following the onset of exercise exceeded the steady state level of oxygen extraction in T2DM compared with controls. Mean response time of estimated Qm increase was prolonged in T2DM compared with healthy subjects (47.7±14.3s vs. 35.8±10.7s, P<0.05).

**Conclusions**: T2DM skeletal muscle demonstrates a transient imbalance of muscle O\(_2\) delivery relative to O\(_2\) uptake following exercise onset, suggesting a slowed Qm increase in T2DM muscle. Impaired vasodilatation secondary to vascular dysfunction in T2DM during exercise may contribute to this observation. Further study of mechanisms leading to impaired muscle oxygen delivery may help explain the abnormal exercise responses in T2DM.
Exercise is highly recommended as a cornerstone of treatment for people with type 2 diabetes (T2DM). However, reduced peak exercise tolerance is common in T2DM (1-3) and linked to mortality in persons with T2DM as well as healthy people (4). Submaximal exercise responses are also affected in persons with T2DM, as demonstrated by an abnormally slowed increase of oxygen uptake ($\dot{V}O_2$ kinetics) following the onset of exercise, and appears potentially related to abnormal cardiovascular responses (1,2). Clinically, these findings observed during submaximal exercise testing are significant as they indicate a greater perturbation of intramuscular homeostasis in response to any exercise challenge, potentially contributing to the premature muscular fatigue (5) and hence the reduced exercise tolerance in T2DM individuals (1,2). However, insight into the mechanisms of impaired muscle oxygen delivery and/or oxidative metabolism responsible for the abnormal exercise responses in T2DM remains unclear.

Recent work in rodent models of T2DM has demonstrated impaired skeletal muscle capillary hemodynamics (6) and abnormal capillary $\dot{P}O_2$ responses during exercise (7). These findings demonstrated a transient impairment of oxygen delivery relative to muscle oxygen uptake following the onset of exercise that may limit oxygen transfer and utilization (7,8). It is unknown whether a similar defect exists in people with T2DM. Such an impairment of microvascular oxygen delivery and exchange in human skeletal muscle could similarly contribute to the observed limitation of $\dot{V}O_2$ and exercise performance in T2DM. Indeed, reductions in exercising leg blood flow (9) and baseline metabolic defects (10,11) are known to occur in human T2DM skeletal muscle. Given that skeletal muscle plays an important role in the pathophysiology of insulin resistance and T2DM, the investigation of oxygen delivery and blood flow at the level of the exercising skeletal muscle in human T2DM would provide unique insight into the exercise limitations observed in this patient population.

Near infrared spectroscopy (NIRS) is a non-invasive technique that offers functional insight into the changes in skeletal muscle oxygen status (12). This technique utilizes the absorption characteristics of NIR light directed into tissue to determine the concentration changes of oxygenated and deoxygenated hemoglobin/myoglobin in the small vessels (arterioles, capillaries, venules) and skeletal muscle. Thus, similar to capillary $PO_2$, the time course of muscle deoxygenated hemoglobin/myoglobin ([HHb]) increase following exercise onset reflects the local balance of $O_2$ delivery and $O_2$ uptake within the muscle region studied. Prior studies have demonstrated NIRS to be highly sensitive to muscle changes due to exercise, hypoxemia, and aging (13-15), and thus, NIRS [HHb] provides a non-invasive surrogate of muscle oxygen extraction. Moreover, the measurement of [HHb] in parallel with $\dot{V}O_2$ during exercise can provide useful inferences regarding regional blood flow and allows for the estimation of the increase in muscle microvascular blood flow ($Q_m$) via the Fick principle (16,17).

The present study examined whether skeletal muscle [HHb] responses and estimated $Q_m$ kinetics are altered in people with T2DM compared with sedentary healthy control subjects. We hypothesized that people with T2DM would have altered skeletal muscle oxygen extraction responses and concordantly slowed estimates of microvascular blood flow ($Q_m$) kinetics compared with healthy subjects following the onset of moderate constant work rate exercise. If confirmed, these observations would further explain the potential mechanisms of exercise limitation and intolerance in people with T2DM as related to changes in skeletal muscle blood flow and oxygen delivery.

**Research Design and Methods**

Eleven subjects with T2DM (5 male, 6 female) and 11 healthy control subjects (6 male, 5 female) between the ages of 30 and
55 years volunteered to participate in this study (Table 1). The study was approved by the University of Colorado Multiple Institutional Review Board, and subjects provided informed consent prior to study participation. Subjects were sedentary, which was defined as participating in low- to moderate-intensity exercise less than 2 days per week in the preceding 3 months and confirmed using a low-level physical activity recall (LOPAR) (1). Healthy control subjects were defined as taking no medications and did not have a direct family member (parent or sibling) with T2DM. Diabetes was documented in T2DM subjects by chart review and confirmed using fasting blood glucose and HbA1c at screening. Subjects were excluded from study if they demonstrated: 1) a history of stroke, congestive heart failure, hypertension, or cardiopulmonary disease, 2) current smoking or smoking within the last 12 months, 3) autonomic or distal neuropathy, 4) LDL cholesterol >130 mg/dl, total cholesterol >200 mg/dl or triglycerides above 250 mg/dl, 5) HbA1c > 9.0%, or 6) taking exclusionary medicines: insulin, thiazolidinediones (pioglitazone or rosiglitazone), alpha-glucosidase inhibitors, beta-blockers, or calcium channel blockers. Women were included who were pre-menopausal and not taking birth control or hormone replacement therapy.

Protocol. Study participants completed three visits at the laboratory to obtain initial screening measurements, establish baseline peak exercise capacity, and perform constant work rate exercise protocols. Exercise visits were performed using a bicycle ergometer (Lode, The Netherlands), and subjects were instructed to avoid the consumption of alcohol, caffeine and food within 4 hours before each exercise visit. To assess peak exercise performance (peak \( \dot{VO}_2 \)) and provide an estimate of lactate threshold, subjects performed an incremental exercise test (10-20 watts/min) to volitional fatigue. On a separate day, subjects performed two 6-minute constant work rate (CWR) exercise tests at a work rate equivalent to approximately 85% of the individual’s estimated lactate threshold. Each CWR test was preceded by a baseline resting period and 4 minutes of unloaded cycling before a step increase to the prescribed CWR was initiated. A 30-minute period of seated rest separated each test.

Measurements. For all exercise tests, \( \dot{VO}_2 \), carbon dioxide production (\( \dot{VCO}_2 \)), minute ventilation (\( V_E \)), and other ventilatory variables were measured using a breath-by-breath metabolic system (Ultima CPX, Medical Graphics Corp., St. Paul, MN, USA). The O\(_2\) and CO\(_2\) analyzers were calibrated prior to each test, and pneumotach volumes were calibrated using a syringe of known volume (3.0 L). Heart rate was monitored continuously by 12-lead ECG (Q-stress, Quinton Instruments, Seattle, WA, USA) and recorded synchronously with the ventilatory data for offline analysis. Arterial hemoglobin saturation was monitored and recorded during rest, exercise, and recovery of all experiments by an oximeter placed on the index finger of the dominant hand (Ohmeda Corp., Louisville, CO).

Skeletal muscle [HHb] was assessed by a frequency domain multi-distance NIRS monitor (Optiplex TS, ISS, Champaign, IL, USA) during each CWR exercise test. The use and limitations of NIRS have been extensively reviewed (18,19). The NIRS monitor uses two wavelengths of NIR light (690 and 830nm) and four light source-detector distances at 2.0, 2.5, 3.0 and 3.5 cm. Local muscle oxygen extraction was determined as the change in [HHb] as previously described (13,20). The NIR data were sampled continuously and recorded at 50Hz. The device probe was positioned on the distal third of the vastus lateralis of the dominant limb, secured using a Velcro strap, and covered with a cloth bandage to exclude ambient light. The NIRS monitor was calibrated prior to each visit using a calibration phantom of known scattering and optical properties.

Data Analysis. Breath-by-breath gas exchange data for each CWR exercise transition were processed using a software
program as previously described (21). Data from the two CWR tests were time-aligned and averaged to provide a single, average kinetic response for each subject (\(\dot{V}O_2\), HR, and [HHb]). The kinetic responses were then evaluated by computerized non-linear regression (SigmaPlot 9.0, SPSS, Inc., Chicago, IL, USA) using standard techniques (20,21) to define the primary endpoint (i.e. the kinetic time constant reflecting the time to reach ~63% of the exponential response). A 1-component exponential model was used to describe the simple exponential increase in [HHb] and HR:

\[
[\text{HHb}](t) = [\text{HHb}](\text{baseline}) + A_1(1 - e^{-(t-TD)/\tau})
\]

(1)

and a 2-component model was used to describe the two phases of pulmonary \(\dot{V}O_2\) (for determination of \(\dot{V}O_{2\text{muscle}}\)) and Qm kinetics: Where for Qm or \(\dot{V}O_2 = X\)

\[
X(t) = X(\text{baseline}) + A_1(1 - e^{-(t-TD_1)/\tau_1}) \quad \text{(phase 1)}
+ A_2[1 - e^{-(t-TD_2)/\tau_2}] \quad \text{(phase 2)}
\]

(2)

In the exponential models, the curve fit provided estimates of the baseline and amplitude for each exponential phase \((A_1, A_2)\) as well as the time delays \((TD_1, TD_2)\) and time constants \((\tau_1, \tau_2)\) for each of the measured exponential phases (see figure 1E for graphical definition of 2 component model fit). Thus, the resulting curve fit described the time-course and magnitude of increase of the respective phases from baseline to steady state exercise.

Qm responses were calculated using the measured [HHb] as a surrogate of muscle oxygen extraction and the phase 2 response of pulmonary \(\dot{V}O_2\) kinetics (e.g. representing muscle \(\dot{V}O_2\) kinetics (\(\dot{V}O_{2\text{muscle}}\)) (22,23)) as previously described (16,17).

\[
Qm(t) = \dot{V}O_2(\text{phase } 2)(t)/[\text{HHb}](t)
\]

(3)

Because the absolute volume of muscle tissue represented in the NIRS signal is not known, the Qm responses were calculated in arbitrary units. However, this method has been previously compared and contrasted with that of conduit artery blood flow with qualitative agreement (17). The kinetics of Qm were then evaluated using the 2-component exponential model. The mean response time (MRT) for [HHb] was calculated as the sum of TD and \(\tau\) from the single exponential model. The MRT for Qm was calculated using a weighted model adjusting for the amplitude, time delay, and time constant of each phase as described (17).

**Statistical Analysis.** Two-tailed independent Student t-tests were used for comparison of the kinetic responses between T2DM and healthy subjects (NCSS Statistical Software, Kaysville, UT, USA). Pearson’s R was used to evaluate the correlations between Qm kinetics and \(\dot{V}O_{2\text{peak}}\). Statistical significance was declared at \(p < 0.05\).

**Results**

Subject characteristics did not differ between groups (Table 1) except for habitual physical activity scores (using the Low Level Physical Activity Recall) which were higher in the T2DM group compared with control subjects \((p<0.05)\). As expected, fasting glucose levels and HbA1c were higher in the diabetic subjects (Table 1). However, although numerically lower in T2DM, the peak \(\dot{V}O_2\) from incremental exercise testing was not different between T2DM and control subjects \((24.6 \pm 4.8 \text{ vs. } 20.9 \pm 5.1 \text{ ml kg}^{-1}\text{min}^{-1}, \text{ NS})\). In all subjects, resting arterial hemoglobin saturation was 95% or higher and revealed no changes in any subject during exercise testing.

The time constants indicating muscle \(\dot{V}O_2\) kinetics (\(\dot{V}O_{2\text{muscle}}\)) in T2DM subjects were significantly slowed compared with controls \((p<0.05, \text{ Table 2})\). Heart rate kinetics were not different between T2DM and healthy control subjects, and no differences were observed between groups for the initial kinetic parameters of [HHb] (Table 2).
Representative plots of the $\dot{V}O_{2\text{muscle}}$, [HHb], and the calculated Qm responses and curve fit for a control subject and subject with T2DM are presented in Figure 1. Following the onset of exercise, the [HHb] response profile demonstrated a noticeable excursion of [HHb] above the level observed for steady state exercise (e.g. during the first ~ 90-100s) in the majority of subjects with T2DM.

There was no difference between groups for the time constant of phase 1 of the Qm response (Table 2). However, the T2DM subjects demonstrated a significantly slower time constant for phase 2 of the Qm response ($p<0.05$), and the calculated MRT of Qm was significantly slower in the T2DM subjects compared with healthy control subjects ($p<0.05$). Correlations between Qm kinetic parameters and $\dot{V}O_{2\text{peak}}$ were not significant ($p>0.05$).

**Conclusions**

This study demonstrated differences in the pattern of skeletal muscle deoxygenation following the onset of exercise in humans with T2DM compared with healthy subjects. Given the prolonged increase of oxygen uptake during exercise in T2DM subjects, these data indicate the increase of microvascular blood flow with exercise is abnormally slow in T2DM and suggests the limitation of oxygen uptake during submaximal exercise in T2DM may be related to impaired control or maldistribution of muscle blood flow. Impaired skeletal muscle oxygen delivery in response to exercise may thus contribute to the observed exercise deficit of T2DM.

In the present study, we observed a transient excursion of [HHb] (e.g. ‘overshoot’) above the level achieved for steady state exercise in the majority of T2DM subjects. Because the response of [HHb] increase are a measure of the increase in local muscle deoxygenated hemoglobin/myoglobin concentration (and hence reflect tissue oxygen extraction), the overshoot [HHb] response observed in T2DM subjects provides evidence of an impaired increase of muscle blood flow relative to muscle oxygen uptake following exercise onset (7,24,25). This reflects an increased dependence upon oxygen extraction in T2DM muscle compared with controls that occurs early in exercise. This response is qualitatively equivalent to the capillary $P_O_2$ responses previously only observed in the exercising muscle of diabetic animals (7,8). The significance of this response is related to a transient lowering of capillary $P_O_2$ that, in turn, may impair capillary-myoocyte $O_2$ transport (via a lowered diffusion gradient) and constrain early increases in muscle oxygen uptake (7,8). Our [HHb] findings appear to support the concept that the early increase in muscle blood flow may be attenuated in T2DM and this abnormality may contribute to the slowed $\dot{V}O_2$ kinetics and exercise deficit observed in T2DM.

Consistent with previous reports (16,17) and other measures of exercise blood flow in animals (26) and humans (27), our estimated Qm demonstrated a biphasic response in all T2DM and control subjects following the onset of moderate exercise. The phases of blood flow responses after exercise onset have previously been characterized (27,28) with the first phase of blood flow increase generally considered to result from muscle contractions (e.g. muscle pump) and rapid vasodilatation, although the precise factors responsible for the latter mechanism remain unclear (28,29). The second phase of blood flow increase is closely matched with metabolic demand, resulting from metabolic feedback control (e.g. $H^+$, $K^+$, prostaglandins, nitric oxide, and others). While we found similar phase 1 kinetics of estimated Qm in T2DM and controls, the time constants for phase 2 of Qm were significantly longer in T2DM compared with healthy subjects. We acknowledge that our estimate of Qm is qualitative in nature and dependent upon assuming homogenous muscle [HHb] characteristics; however, there is evidence to support the assumption that the sampled muscle (vastus lateralis) reflects the predominant active muscles during cycling as a whole (30) and, therefore, the relative kinetics of estimated Qm responses should be preserved. Thus, the slower phase 2 time
constant and MRT of Qm demonstrates the plausible notion that metabolic feedback control during exercise may be altered in T2DM. Indeed, there is evidence for macro- and microvascular dysfunction in T2DM (31,32) that could explain impaired microvascular blood flow responses during exercise.

It is well established that NO-dependent endothelial function is impaired in the conduit arteries in T2DM (33,34), and this mechanism has been associated with reduced steady state leg blood flow in T2DM subjects during submaximal exercise (9). However, it is unclear whether conduit artery blood flow dynamics following exercise onset are altered in T2DM or to what extent vascular dysfunction or changes in microvascular architecture in T2DM may impair macro- or microvascular blood flow dynamics and the distribution of muscle blood flow following the onset of exercise. We have previously observed prolonged heart rate responses in subjects with T2DM (2). Thus, it is plausible that a central impairment of cardiac output could undermine blood flow and oxygen delivery to the exercising skeletal muscle vascular beds. However, cardiac output during submaximal exercise appears normal in T2DM (35) and we observed no differences in heart rate kinetics between groups. Therefore, the putative Qm abnormality observed during submaximal exercise in T2DM subjects may likely be specific to the control of blood flow of the exercising legs.

The role of skeletal muscle in the impaired submaximal exercise response of T2DM has not been elucidated. However, the likelihood of an integral role is suggested by the available data. For example, capillary density appears reduced in T2DM skeletal muscle, (36) and basement membrane structures are altered (37). These structural changes could directly contribute to alterations in microvascular hemodynamics, exacerbate potential mis-matching of muscle blood flow-to-oxygen uptake, and impair O2 exchange from capillary to myocyte as suggested by the work in rodent models (6,8,38). However, the skeletal muscle of T2DM patients also demonstrates reduced mitochondrial content (10) and increased potential for mitochondrial dysfunction compared with healthy counterparts (5,10,11,39), although the functional evidence for this notion is controversial (40). Whether the changes observed in the skeletal muscle of people with T2DM are related to altered muscle fiber type composition (greater type IIb fibers relative to type I fibers) (41), detraining, or other factors is unclear. However, it appears that both the ability to deliver oxygen to the skeletal muscle and for the muscle to utilize oxygen during exercise may be compromised in T2DM. The findings of the present study appear to support the importance of impaired skeletal muscle oxygen delivery as an important determinant of the submaximal exercise impairment of T2DM. However, given the similar (and not faster) [HHb] kinetics in T2DM subjects compared with control subjects, these findings could also indicate, to a lesser extent, the potential contribution of muscle oxidative dysfunction or other factors in limiting the submaximal exercise response.

In contrast to the long appreciated defects in peak exercise capacity (VO2peak) observed in T2DM, defects in the response at the onset of submaximal exercise represent a challenge that will be encountered during routine activities. Thus, the finding of impaired submaximal exercise responses in otherwise uncomplicated T2DM is clinically relevant. In the present study, we showed slowed VO2 kinetics in T2DM compared with healthy subjects, consistent with previous reports (2,42) which may provide a mechanism for the T2DM exercise intolerance. The significance of slowed VO2 kinetics is that it indicates a prolonged period of adaptation to any acute submaximal exercise demand, such as is regularly encountered during daily life. Importantly, the prolonged VO2 kinetics results in a greater oxygen deficit and, hence, greater dependence upon substrate level phosphorylation (PCr degradation and glycolysis) to support even low and moderate levels of exercise. This is significant since the activities of daily life are
carried out at these low levels of physical activity. Thus, the accumulated oxygen deficit that occurs with the initiation of exercise may ultimately affect the ability or willingness to sustain the activity, resulting in the limited exercise tolerance and reduced peak exercise capacity observed in T2DM.

In conclusion, skeletal muscle [HHb] responses are altered in T2DM during the transition from light to moderate exercise indicating a slowed increase of microvascular blood flow in response to exercise in T2DM patients. The prolonged kinetics of estimated Qm suggests that muscle VO₂ during exercise may be constrained by an impairment of muscle oxygen delivery in T2DM skeletal muscle, potentially leading to diminished submaximal exercise function in T2DM.

Impairments in skeletal muscle oxygen delivery due to abnormal vascular control or other abnormalities of T2DM skeletal muscle may explain, in part, the observed exercise deficit observed in persons with T2DM.

Acknowledgements
This work was supported by an American Diabetes Association Award to Dr. Regensteiner and by MO1 #RR000051. Dr. Bauer was supported by NIH T32 HL007822-10. Dr. Reusch is supported by VA Merit Review (JEBR) and NIH DK064741. The authors thank ISS, Inc. for use of the Optiplex TS™ spectrometer. In addition, we thank Vermed, Inc for the donation of ECG electrodes.
Reference List


Table 1: Subject Characteristics

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<th>Control</th>
<th>T2DM</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47 ± 6</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.5 ± 18.5</td>
<td>84.0 ± 8.8</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>28.0 ± 3.0</td>
<td>30.8 ± 4.3</td>
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<tr>
<td>HbA1c (%)</td>
<td>5.4 ± 0.2</td>
<td>6.75 ± 1.2 **</td>
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<tr>
<td>Fasting Insulin (µU/ml)</td>
<td>8.0 ± 3.6</td>
<td>13.6 ± 12.1</td>
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<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>96 ± 9</td>
<td>128 ± 42 *</td>
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<tr>
<td>Body Fat (%)</td>
<td>32.6 ± 7</td>
<td>32.7 ± 7</td>
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</table>

Values are mean ± SD. BMI, body mass index; HbA1c, glycosylated hemoglobin; Body Fat, calculated from DEXA scan; LOPAR, Low Level Physical Activity Recall. *p<0.05, ** p<0.01 T2DM versus Control.
Table 2: Kinetic Parameters for $\dot{V}O_2$, HHb, HR and Qm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>T2DM</th>
</tr>
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<tbody>
<tr>
<td>$\tau_{O_2}$ (s)</td>
<td>34.2 ± 8.2</td>
<td>43.8 ± 9.6 *</td>
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<td>$\tau_{HR}$ (s)</td>
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<td>$\tau_{HHb}$ (s)</td>
<td>10.2 ± 4.4</td>
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<td>MRT HHb (s)</td>
<td>17.8 ± 5.5</td>
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<td>$\tau_1$ Qm Phase 1 (s)</td>
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<tr>
<td>MRT Qm (s)</td>
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<td>47.7 ± 14.3 *</td>
</tr>
<tr>
<td>$\tau_{O_2}$ (s)</td>
<td>34.2 ± 8.2</td>
<td>43.8 ± 9.6 *</td>
</tr>
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</table>

Values are mean ± SD. Refer to text for calculation of $\tau$, $\tau_1$, $\tau_2$, and mean response time (MRT). $\tau_{O_2}$, time constant for $\dot{V}O_2$ kinetics; $\tau_{HR}$, time constant for heart rate; $\tau_{HHb}$, time constant for increase in HHb; $\tau_1$ Qm, time constant for phase 1 of Qm; $\tau_2$ Qm, time constant for phase 2 of Qm. *p<0.05 T2DM versus Control.
Figure 1. Representative example of $\dot{V}O_2_{\text{muscle}}$ derived from pulmonary $\dot{V}O_2$ kinetics (A, B), deoxygenated hemoglobin/myoglobin ([HHb]) responses (C, D), and estimated microvascular blood flow (Qm) responses (E, F) during the transition from unloaded to moderate cycling in a healthy control (A, C, E) and T2DM subject (B, D, F). Data are presented as a function of end exercise response. Note transient ‘overshoot’ of [HHb] response in T2DM subject. Estimated Qm profile was calculated from $\dot{V}O_2_{\text{muscle}}$ divided by [HHb] in arbitrary units (a.u.). Loaded cycling exercise begins at time $= 0$. Qm kinetic model parameters: BSL, baseline. TD, time delay. $\tau_1$, $\tau_1$, time constants. A1, A2, response amplitudes. $\tau$, time constant of $\dot{V}O_2_{\text{muscle}}$. MRT, Qm mean response time. Long dash lines represent curve-fit of Qm kinetic response.