Independent effect of type 2 diabetes beyond characteristic comorbidities and medications on immediate but not continued knee extensor exercise hyperemia

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Type 2 diabetes is one of the most common chronic diseases, with growth in prevalence and incidence continuing at unprecedented rates (6). Exercise is widely recognized as a critical component of the management of type 2 diabetes (T2D) (40, 42), with positive effects on blood glucose and lipid levels, blood pressure, cardiovascular events, mortality, and quality of life (6). However, exercise intolerance is considered a major complication of T2D (1, 15, 34), which may contribute to poor exercise adherence. Indeed, 60 to 80% of adults with T2D do not exercise sufficiently (33). This limits the use of exercise as a treatment modality in the long-term management of the disease.

Recent evidence indicates that this exercise intolerance may be due at least in part to impaired muscle blood flow (MBF) and compromised oxygen delivery (17, 19), factors that are known to hasten the development of fatigue (45). However, key knowledge gaps currently exist.

First, in terms of the dynamics of the oxygen delivery response (i.e., adjustment of MBF to a steady state), an impairment has been inferred from observations of slowed VO2 kinetics (3, 34) and a transient undershoot in muscle oxygenation at the onset of exercise in T2D (1, 29). However, these observations could also indicate impaired oxygen consumption, which could potentially occur in T2D [e.g., due to mitochondrial dysfunction (31)]. Understanding the mechanistic basis of impairment is critical for the development of effective intervention strategies. In addition, the dynamics of the oxygen delivery response per se have been examined only in one study, and this was carried out in terms of vascular conductance (vs. blood flow specifically), and only in the transition from rest-to-exercise at a single intensity (21). Although this was an important initial investigation, our understanding remains limited because 1) there is some evidence that VO2 kinetic impairment is intensity-dependent in T2D (34); 2) in young healthy persons some kinetic response characteristics are sensitive to basal exercise intensity (36, 37), and thus propensity for impairment may be different in rest-to-exercise vs. exercise-to-exercise transitions in T2D; and 3) much of daily living involves transitioning between different intensities of physical activity, rendering exercise-to-exercise transitions particularly relevant. Impairment at the onset of exercise (or in the transition from one intensity to another) would be important because the dynamics of oxygen delivery establish intramyocellular oxygenation such that a slower adjustment of oxygen delivery can result in a greater oxygen deficit, which contributes to increased fatigue (7).

Second, whether T2D has an effect on exercising muscle oxygen delivery within the constellation of comorbidities and medications characteristically taken by this population has not been investigated. T2D is typically comorbid with obesity (39), hypertension (38), and dyslipidemia (4), and patients routinely require associated medications (18, 41). However, previous studies investigated exercising muscle oxygen delivery in persons with T2D who were free from comorbidities and medi-
cations vs. age- and weight-matched controls (1, 19), or who withdrew medications prior to testing (17, 23, 50), or the studies did not have control groups matched for comorbidities and their associated medications (16, 21, 43, 50). Therefore previous findings, although potentially isolating an impact of T2D specifically, lack ecological validity for this population. Mechanistically, impaired steady-state MBF could result from peripheral vascular dysfunction, but it could also be due to vasoconstriction secondary to impaired cardiac function (26, 53); therefore, assessment of peripheral vascular control specifically requires the use of a small muscle mass modality that would not be limited by central hemodynamic responses. Impaired MBF at steady state would result in a lower myocellular oxygenation at a given submaximal $V\dot{O}_2$ and thereby increase the rate of fatigue progression (47).

With this as a foundation, the objective of the present study was to characterize the MBF response during exercise in persons with T2D and to determine whether and how this is impaired relative to controls individuals matched for age, body mass index (BMI), aerobic fitness, comorbidities, and common non-T2D medication use. We aimed to bridge the current gaps in the literature by 1) enrolling representative participants with T2D (i.e., with common comorbidities and medications) and matching controls for these and other important characteristics; 2) characterizing the MBF response directly in terms of $a)$ the dynamics of the rest-to-exercise response, $b)$ the dynamics of a low- to moderate-intensity exercise transition, and $c)$ the steady-state response at each intensity; and 3) utilizing a single-leg isometric exercise model to better isolate local peripheral contributions during exercise. It was hypothesized that the T2D group would have reduced MBF (rate and amount) per workload compared with the control group.

### METHODS

#### Participants

Thirteen men with T2D and 11 matched control participants took part in this study. Participants were recruited via the Cardiac Rehabilitation Centre at Hotel Dieu Hospital (HDH; Kingston, ON, Canada), and all testing was completed between their screening for and commencement of this program. Inclusion criteria were 1) BMI of 24-35 kg/m², 2) achievement of 4 to 10.0 metabolic equivalents of task (METs) on a graded treadmill exercise test, 3) baseline femoral artery diameter of $\geq 0.79$ cm (11), and 4) clear presence or absence of T2D (T2D: fasting plasma glucose $\geq 7.0$ mmol/l and/or oral glucose tolerance test (OGGT) 2-h plasma glucose $\geq 11.1$ mmol/l; controls: fasting plasma glucose $< 6.1$ mmol/l and OGGT 2-h plasma glucose $< 7.8$ mmol/l). Exclusion criteria were 1) presence of Stage 3 (or more advanced) renal disease, 2) current smoker or having smoked within the past 12 mo, or 3) usage of exclusionary medications ($\beta$-blockers, nitroglycerine, or other nitric oxide donors). Participants taking $\beta$-blockers were not excluded if they withdrew these medications for 48 h prior to testing under medical supervision (Dr. Stephen LaHaye, Cardiologist at HDH) ($n = 1$ in each group). Participants completed testing while taking all other medications (Table 1).

Before the day of testing, participants visited the laboratory to be screened for clear blood velocity signals (Doppler ultrasound) and images (echo ultrasound) of the femoral artery, and to practice the exercise for purposes of familiarization. The study protocol was approved by the Health Sciences Human Research Ethics Board at Queen’s University, and individuals gave written consent to participate on forms approved by the board.

### Experimental Protocol

Participants were instructed to abstain from taking unsupervised anti-inflammatory medications within 48 h of the study (excluding daily low-dose aspirin, $n = 8$ in the T2D group, $n = 9$ in the control group), from exercising within 24 h, and from consuming alcohol or caffeine for 12 h and food for 3 h before the testing session (44). Data collection was performed in a quiet, temperature-controlled room (19–23.5°C).

Participants were positioned in a semireclined position (~30 to 35° from horizontal) with their knees bent at 90° on a custom leg-ergometer. The left ankle was secured to a custom-built force system using a nylon strap. The system consisted of a force transducer (Interface model SM-2000N Load Cell; Durham Instruments) that was mounted on a steel plate running horizontally behind the participant’s leg and connected to a data acquisition system (Powerlab, ADInstruments). Participants performed isometric knee-extension exercise, and the force produced during the exercise was digitized, sampled at 200 Hz, and recorded on a personal computer with live visual feedback provided to the participant. Isometric (vs. dynamic) exercise was chosen to reduce the potential for any movement artifact, which could increase femoral artery Doppler ultrasound measurement error.

#### Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group</th>
<th>T2D Group</th>
<th>$P$</th>
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<tbody>
<tr>
<td>$n$</td>
<td>11</td>
<td>13</td>
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<tr>
<td>Age, yr</td>
<td>62.7 ± 11.2</td>
<td>63.2 ± 9.5</td>
<td>0.921</td>
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<tr>
<td>Diabetes duration, y</td>
<td>5.9 ± 5.4</td>
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<td></td>
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<tr>
<td>Height, cm</td>
<td>178.6 ± 6.2</td>
<td>172.7 ± 6.8</td>
<td>0.039*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>97.4 ± 19.6</td>
<td>95.8 ± 15.1</td>
<td>0.823</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.4 ± 5.0</td>
<td>32.1 ± 4.9</td>
<td>0.404</td>
</tr>
<tr>
<td>WC, cm</td>
<td>105.2 ± 12.5</td>
<td>110.2 ± 12.8</td>
<td>0.349</td>
</tr>
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<td>Albumin/creatinine ratio, mg/mmol</td>
<td>1.37 ± 0.31</td>
<td>2.68 ± 4.71</td>
<td>0.439</td>
</tr>
<tr>
<td>Fence plasma glucose, mmol/l</td>
<td>5.1 ± 0.3</td>
<td>8.2 ± 2.1</td>
<td>&lt;0.001*</td>
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<tr>
<td>HDL-C, %</td>
<td>5.6 ± 3.6</td>
<td>7.3 ± 1.4</td>
<td>0.001*</td>
</tr>
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<td>Fasting triglycerides, mmol/l</td>
<td>1.8 ± 2.2</td>
<td>1.5 ± 0.9</td>
<td>0.700</td>
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<td>Total cholesterol, mmol/l</td>
<td>3.5 ± 1.2</td>
<td>3.5 ± 1.2</td>
<td>0.986</td>
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<td>HDL cholesterol, mmol/l</td>
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<td>1.0 ± 0.2</td>
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<td>LDL cholesterol, mmol/l</td>
<td>1.5 ± 0.6</td>
<td>1.7 ± 1.0</td>
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<td>Baseline GXT, METs</td>
<td>8.3 ± 0.8</td>
<td>8.2 ± 1.8</td>
<td>0.817</td>
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<td>Leg MVC, kg</td>
<td>33.4 ± 8.1</td>
<td>36.4 ± 13.6</td>
<td>0.525</td>
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<tr>
<td>% MVC at 6 kg workload</td>
<td>19.1 ± 5.6</td>
<td>18.2 ± 5.5</td>
<td>0.693</td>
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<td>% MVC at 12 kg workload</td>
<td>38.3 ± 11.2</td>
<td>36.5 ± 10.9</td>
<td>0.693</td>
</tr>
<tr>
<td>Number of non-T2D medications</td>
<td>4.2 ± 1.1</td>
<td>4.6 ± 1.8</td>
<td>0.484</td>
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<td>Medication use, n (%)</td>
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<tr>
<td>COX inhibitor</td>
<td>9 (82%)</td>
<td>8 (62%)</td>
<td></td>
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<tr>
<td>ACE inhibitor</td>
<td>7 (64%)</td>
<td>13 (100%)</td>
<td></td>
</tr>
<tr>
<td>HMG-CoA reductase inhibitor</td>
<td>10 (91%)</td>
<td>13 (100%)</td>
<td></td>
</tr>
<tr>
<td>(statin)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thienopyridine (or derivative; antiplatelet)</td>
<td>4 (36%)</td>
<td>4 (31%)</td>
<td></td>
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<tr>
<td>Cholesterol absorption inhibitor</td>
<td>0 (0%)</td>
<td>3 (23%)</td>
<td></td>
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<tr>
<td>Biguanide</td>
<td>0 (0%)</td>
<td>9 (69%)</td>
<td></td>
</tr>
<tr>
<td>Insulin/analogue</td>
<td>0 (0%)</td>
<td>1 (8%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>9 (82%)</td>
<td>7 (54%)</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular health history, n (%)</td>
<td>3 (27%)</td>
<td>3 (23%)</td>
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<td>Acute myocardial infarction</td>
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<td></td>
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<tr>
<td>Angioplasty</td>
<td>5 (45%)</td>
<td>6 (46%)</td>
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<tr>
<td>Coronary artery bypass surgery</td>
<td>5 (45%)</td>
<td>3 (23%)</td>
<td></td>
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<tr>
<td>History of cardiomyopathy</td>
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<td>1 (8%)</td>
<td></td>
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<tr>
<td>Heart valve repair/ replacement</td>
<td>1 (9%)</td>
<td>4 (31%)</td>
<td></td>
</tr>
<tr>
<td>Transient ischemic stroke</td>
<td>1 (9%)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. ACE, angiotensin converting enzyme; COX, cyclo-oxygenase; GXT, graded exercise test (treadmill); HDL, high-density lipoprotein; HMG-CoA reductase, 3-hydroxy-3-methyl-glutaryl-coA reductase; LDL, low-density lipoprotein; METs, metabolic equivalents; MVC, maximum voluntary contraction; WC, waist circumference. *$P < 0.05$ compared with control group.
After 1 min of resting baseline measurements, participants performed single-leg isometric knee-extension exercise at an absolute intensity equivalent to 6 kg, with live computer feedback displaying force output, in time with a metronome having a 1:2-s work-rest duty cycle. After 5 min at this intensity, the workload was increased to 12 kg for an additional 5 min. This series of rest followed by step increases in workload was repeated two additional times, separated by at least 10 min of rest to allow hemodynamic parameters to return to baseline. These workloads were shown in pilot work to 1) achieve an adequate response magnitude such that the kinetics of the muscle blood flow response could be quantified, and 2) be moderate enough to avoid cumulative fatigue over repeated trials.

Measurements

Participant characteristics. Anthropometric measures (height, weight, BMI, waist circumference) and blood samples for determination of fasting plasma glucose; HbA1c; triglycerides; and total high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were taken during the participants’ screening process for the Cardiac Rehabilitation Program at HDH. Participants also completed a graded treadmill exercise test, and their maximal METs achieved were estimated on the basis of treadmill speed and grade. The maximum voluntary contraction (MVC) in the exercising (left) leg was measured during the screening visit to the laboratory by having participants perform three maximal-effort single-leg isometric knee extensions, each separated by 1 min of rest. The largest value was taken to represent the MVC.

Mean arterial blood pressure. Mean arterial pressure (MAP) was measured continuously with finger photoplethysmography on the participants’ right hand positioned at heart level via an arm sling (Finometer MIDI; Finapres Medical Systems).

Femoral artery diameter and mean blood velocity. Images of the common femoral artery on the exercising (left) leg were obtained at rest using a 10-MHz linear echo ultrasound probe (Vivid 12; GE Medical Systems) operating in two-dimensional B-mode. Images were recorded in Digital Imaging and Communications in Medicine (DICOM) format for later analysis. Mean blood velocity (MBV) was measured approximately 2–3 cm proximal to the femoral artery bifurcation at the site imaged with the echo probe with a 4-MHz Doppler ultrasound probe (500B Transcranial Doppler; Multigon Industries). The corresponding voltage output was recorded continuously at 200 Hz in the data acquisition software program LabChart (ADInstruments) for later analysis. To quantify absolute MBV, voltage-to-velocity calibrations were performed with known flow velocities at a range of insonation angles (in 1° increments to achieve a range of ±15° from the 70° angle of insonation inherent to the probe). For each participant, the femoral artery was imaged at the site of Doppler probe placement to quantify the actual angle of insonation (using an on-screen protractor), and the appropriate voltage-to-velocity calibration was applied prior to data analysis. This enabled accurate absolute blood velocity measurements allowing for between-participant comparisons.

Leg force. Leg force was obtained using a force transducer (Interface model SM-2000N Load Cell; Durham Instruments) connected to a data acquisition system (Powerlab; ADInstruments) and recorded on a personal computer (LabChart; ADInstruments).

Data Analysis

MBV and MAP. After applying the appropriate voltage-to-velocity calibration to the 200-Hz MBV data, a frequency-domain filtering procedure was used to eliminate higher-frequency noise due to heart rate and muscle contraction [i.e., a low-pass filter with a cutoff frequency of 0.2 Hz (lowpas.s.xfm, SigmaPlot 11.0, Systat Software)] (10). The data were then resampled to retrieve every 20th datum to reduce the total number of data points (from ~132,000 to 6,600) to enable curve-fitting on a personal computer. Blood flow kinetic parameter estimates have been found to be unaffected by this resampling procedure and are superior to parameter estimates via beat-by-beat or contraction-relaxation cycle averaging (10). MAP data were similarly sampled at 200 Hz, filtered, and resampled.

Femoral artery diameter. Vessel diameter was quantified at baseline using automated wall tracking via an updated version of the software package described by Woodman et al. (51) as previously described (30), and manual caliper measurements. Several values were averaged to obtain a single femoral artery diameter value to minimize the effect of random diameter measurement error on calculated blood flow. Resting femoral artery diameter values were used to calculate leg blood flow because previous work has shown that femoral arteries of a baseline diameter of 0.79 cm or greater do not dilate at any time point of an exercise transient (11).

Leg blood flow, leg vascular conductance, and MAP. Leg blood flow (LBF) was used as a surrogate for oxygen delivery and was calculated according to the formula:

\[ LBF = MBV \times 60 \times \frac{1}{\pi} \times \left( \frac{\text{diameter}}{2} \right)^2 \]

where LBF is measured in ml/min, MBV in cm/s, and femoral artery diameter in centimeters. Leg vascular conductance (LVK) was calculated as:

\[ LVK = \left( \frac{LBF}{\text{MAP}} \right) \times 100 \]

where LVK is measured in ml/mm⁻¹/min⁻¹, 100 mmHg such that the values for LVK are quantitatively similar to those for LBF.

Data for all three trials were time-aligned to the onset of exercise and averaged together. In one T2D participant there was evidence of measurement error (i.e., compromised signal) in one of the three trials; this trial was excluded and the remaining two were averaged together. LBF and LVK for the transitions from rest-to-6 kg and 6 kg-to-12 kg were fit with one-, two-, or three-component exponential models (SigmaPlot 11.0; Systat Software; details in Kinetics analysis), whereas MAP data were fit with linear or one- or two-component exponential models as appropriate. The regression equations were used to calculate the average values for LBF, LVK, and MAP at rest (baseline, 1-min average) and in the last 30 s at each workload (i.e., steady-state at 6 and 12 kg). The change from baseline to steady-state values was calculated (i.e., LBFSS, LVKSS, and MAPSS) where the baseline for the 12-kg workload was the steady state at 6 kg. For the 12-kg workload, the change from the overall baseline was also calculated (ΔLBFSS-12, ΔLVKSS-12, and ΔMAPSS-12).

Kinetics analysis. As mentioned, the time-course plots for LBF and LKV from rest-to-6 kg and 6 kg-to-12 kg were fit with one-, two-, or three-component exponential models for analysis of dynamic response characteristics. The models have a baseline component (G₀) and one or more amplitude terms (G₁, G₂, and/or G₃), time constants (τ₁, τ₂, and/or τ₃), and time delays (TD₁, TD₂, and/or TD₃) consistent with the number of phases of the response (36). G represents the magnitude of the response, τ represents the time it takes to achieve 63% of the response magnitude (describing the rate at which LBF or LKV increases), and TD gives a measure of the delay in onset of response (i.e., how quickly the vascular control systems can begin to respond following the onset of muscle contraction or increase in intensity). The model is described by the following equation:

\[ Y(t) = G₀ + G₁\left[1 - e^{-(t-TD₁)τ₁}\right] μ₁ + G₂\left[1 - e^{-(t-TD₂)τ₂}\right] μ₂ + G₃\left[1 - e^{-(t-TD₃)τ₃}\right] μ₃, \]

where

\[ μ₁ = 0 \text{ for } t < TD₁ \text{ and } μ₁ = 1 \text{ for } t ≥ TD₁ \]

\[ μ₂ = 0 \text{ for } t < TD₂ \text{ and } μ₂ = 1 \text{ for } t ≥ TD₂ \]

\[ μ₃ = 0 \text{ for } t < TD₃ \text{ and } μ₃ = 1 \text{ for } t ≥ TD₃ \]

where t is time in seconds and Y(t) is the time-dependent variation in LBF or LVK.
The mean response time (MRT) quantifies the time to reach 63% of the overall amplitude of the response from baseline, and was calculated as a weighted sum of the time delay and time constant of each response phase (22):

\[
MRT = \left[ \frac{G_1}{G_1 + G_2 + G_3} \right] 
\cdot \left( TD_1 + \tau_1 \right) + \left[ \frac{G_2}{G_1 + G_2 + G_3} \right] 
\cdot \left( TD_2 + \tau_2 \right) + \left[ \frac{G_3}{G_1 + G_2 + G_3} \right] 
\cdot \left( TD_3 + \tau_3 \right)
\]

Adjustment amplitude. Measures of amplitude at different time points during the dynamic increase were also used to compare the adjustment of the response. This was carried out via 1) the change from baseline to the peak initial response (PIR) of LBF and LVK (defined as the greatest 1-s average (11 data points) within the first 10 s of exercise at each intensity; \(\Delta LBF_{PIR}\) and \(\Delta LVK_{PIR}\), respectively) and 2) the change from baseline to the on-transient (ON) of LBF, LVK, and MAP (mean of 1-s averages from curve fit at 15, 45, and 75 s of exercise; \(\Delta LBF_{ON}\), \(\Delta LVK_{ON}\), and \(\Delta MAP_{ON}\), respectively; see Fig. 3A). In other words, the initial amplitude (PIR) and amplitude of the response at discrete time points in the exercise transient (obtained from the best fit of the data) are indicative of the dynamics of the response in that a greater magnitude equates to a greater speed (either within the first 10 s or averaged at 15, 45, and 75 s). For MAP, the PIR (\(\Delta MAP_{PIR}\)) was calculated at the time that corresponded with the change from baseline (PIR, ON, and SS) and workload transitions (rest-to-6 kg and 6-to-12 kg) on Fi (absolute and relative) and 38% MVC. As anticipated, the T2D subjects had greater fasting plasma glucose \((P < 0.001)\) and HbA1c \((P = 0.001)\). The 6- and 12-kg workloads represented intensities of \(\sim 19\%\) MVC and \(38\%\) MVC (not different between groups; both \(P = 0.693\)).

Force Impulse

The average \(F_i\) at each of the target workloads was not significantly different between groups (5.7 \(\pm\) 0.8 vs. 5.9 \(\pm\) 0.8 kg/s and 11.4 \(\pm\) 2.1 vs. 12.2 \(\pm\) 1.7 kg/s for the 6- and 12-kg targets in T2D vs. control groups, respectively; \(P = 0.343;\) Fig. 1). \(\Delta F_i\) was not different between T2D and control groups at any time point (\(P = 0.316, P = 0.476, P = 0.436\) for change from baseline to PIR, ON, and SS, respectively) nor was it different between 6- and 12-kg workloads at any time point (\(P = 0.213, P = 0.070, P = 0.934\) at PIR, ON, and SS, respectively), confirming that the exercise was performed as prescribed.

Hemodynamic Parameters

Hemodynamic parameters at rest and during exercise for a representative participant are shown in Figure 2. LBF, LVK, and MAP were not different between groups at the overall resting baseline (\(P = 0.148, P = 0.361,\) and \(P = 0.095\), respectively). Baseline values for LBF, LVK, and MAP in T2D vs. control groups were, respectively, as follows: 319.7 \(\pm\) 83.2 vs. 404.2 \(\pm\) 182.4 ml/min, 419.2 \(\pm\) 128.2 vs. 489.3 \(\pm\) 233.2 ml\(^{-1}\)min\(^{-1}\)100 mmHg, and 78.5 \(\pm\) 7.5 vs. 84.9 \(\pm\) 10.6 mmHg.

Dynamic Adjustment of Hemodynamic Parameters

Mean response time. For LBF, the MRT was not different between groups (\(P = 0.856\)) or between rest-to-6 kg and 6-to-12 kg workload transitions (\(P = 0.881\) (values for rest-
to-6 kg and 6-to-12 kg workload transitions in T2D vs. control groups were 39.8 ± 41.1 vs. 43.4 ± 47.7 s and 49.3 ± 17.8 vs. 49.7 ± 35.0 s, respectively). Similarly for LVK, the MRT was not different between groups (P = 0.442) or between workloads (40.6 ± 40.6 vs. 44.8 ± 49.0 s; 61.3 ± 53.2 vs. 37.2 ± 29.4 s for 6- and 12-kg workloads in T2D vs. control groups, respectively; P = 0.616). Note that although the difference between groups at 12 kg may appear to be large (~24 s), this can be attributed to an outlier participant in the T2D group whose LVK response was triphasic and who had a calculated MRT of 214 s (and a z-score of 2.9). Removal of this participant’s datum from analysis did not alter the result, and therefore it is included in the overall group mean. Dynamic response characteristics for LBF are shown in Table 2.

Peak initial response. $\Delta LBF_{PIR}$ was significantly lower in the T2D group vs. the control group (P = 0.037), but it was not different between rest-to-6 kg and 6-to-12 kg workload transitions (P = 0.305) (Fig. 3, Bi, Ci, and Di). Similarly, $\Delta LVK_{PIR}$ tended to be lower in the T2D group vs. the control group (P = 0.063), but it was not different between workload transitions (P = 0.421). $\Delta MAP_{PIR}$ was not different between groups (P = 0.731) and was significantly smaller at the 6-to-12 kg transition than the rest-to-6 kg transition (P < 0.001). The proportion of steady-state LBF achieved with PIR (%$\Delta LBF_{PIR}$-ss) was not different between groups (P = 0.287), but it was significantly lower at the 6-to-12 kg vs. the rest-to-6 kg workload transition (71.6 ± 32.5% vs. 75.5 ± 31.0% and 47.6 ± 19.6% vs. 63.1 ± 21.2% for rest-to-6 kg and 6-to-12 kg in T2D vs. control groups, respectively; P = 0.009). Similarly, %$\Delta LVK_{PIR}SS$ was not different between groups (P = 0.392), but it was significantly lower at 6-to-12 kg vs. rest-to-6 kg (73.7 ± 36.8% vs. 79.6 ± 37.1%, and 52.2 ± 29.5 vs. 66.3 ± 28.2% for rest-to-6 kg and 6-to-12 kg in T2D vs. control groups, respectively; P = 0.027).

On-transient. $\Delta LBF_{ON}$ and $\Delta LVK_{ON}$ were not different between groups (P = 0.150 and P = 0.237, respectively) or across workload transitions (P = 0.167, P = 0.346) (Fig. 3, Bi, Ci, and Di). $\Delta MAP_{ON}$ was not different between groups (P = 0.638) but it was significantly lower at 6-to-12 kg vs. rest-to-6 kg (P < 0.001).

Steady-State Hemodynamic Responses

Change from baseline to steady state, where baseline for the 12-kg workload was at a steady state for 6 kg. $\Delta LBF_{SS}$ and $\Delta LVK_{SS}$ were not different between groups (P = 0.204, P = 0.347) but they were significantly greater at the 6-to-12 kg vs. rest-to-6 kg workload transition (P = 0.006, P = 0.034) (Fig. 3, Bi, Ci, and Di). $\Delta MAP_{SS}$ was not different between groups (P = 0.821), but it was significantly smaller at the 6-to-12 kg vs. rest-to-6 kg workload transition (P < 0.001).

Change from overall resting baseline to 12 kg steady state. $\Delta LBF_{SS-12}$, $\Delta LVK_{SS-12}$, and $\Delta MAP_{SS-12}$ were not different between T2D and control groups (P = 0.204, P = 0.347, and P = 0.82, respectively) (Fig. 4).

Change in Hemodynamic Parameters Over Time

Control group. In the control group, there was a main effect of time for $\Delta LBF$ (P < 0.001) such that $\Delta LBF_{SS}$ was greater than both $\Delta LBF_{ON}$ and $\Delta LBF_{PIR}$ (both P < 0.001), but $\Delta LBF_{PIR}$ and $\Delta LBF_{ON}$ were not different (P = 0.962). Likewise for $\Delta LVK$, $\Delta LVK_{SS}$ was greater than both $\Delta LVK_{ON}$ and $\Delta LVK_{PIR}$ (both P < 0.001), but $\Delta LVK_{PIR}$ and $\Delta LVK_{ON}$ were not different (P = 0.970). In contrast, $\Delta MAP$ was not different across PIR, ON, and SS (P = 0.123), but it was greater at rest-to-6 kg vs. 6-to-12 kg (P = 0.002).
**Table 2. LBF dynamic response characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Phase 1 T2D n = 13, Control n = 11</th>
<th>Phase 2 T2D n = 13, Control n = 9</th>
<th>Phase 3 T2D n = 0, Control n = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>rest-to-6 kg</td>
<td>$G_0$</td>
<td>$G_1$ TD$_1$ $\tau_1$</td>
<td>$G_2$ TD$_2$ $\tau_2$</td>
<td>$G_3$ TD$_3$ $\tau_3$ MRT</td>
</tr>
<tr>
<td>T2D</td>
<td>319.7 ± 83.2</td>
<td>229.1 ± 154.2</td>
<td>11.1 ± 0.9</td>
<td>15.2 ± 2.2</td>
</tr>
<tr>
<td>Control</td>
<td>404.2 ± 182.4</td>
<td>320.5 ± 189.1</td>
<td>4.2 ± 9.0</td>
<td>2.6 ± 3.1</td>
</tr>
<tr>
<td>6 kg-to-12 kg</td>
<td>$G_0$</td>
<td>$G_1$ TD$_1$ $\tau_1$</td>
<td>$G_2$ TD$_2$ $\tau_2$</td>
<td>$G_3$ TD$_3$ $\tau_3$ MRT</td>
</tr>
<tr>
<td>T2D</td>
<td>759.6 ± 325.0</td>
<td>236.1 ± 169.3</td>
<td>2.1 ± 1.5</td>
<td>3.4 ± 8.6</td>
</tr>
<tr>
<td>Control</td>
<td>935.3 ± 405.5</td>
<td>341.9 ± 122.6</td>
<td>1.3 ± 1.1</td>
<td>3.9 ± 4.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. Gains (G) are expressed in ml/min, and time-based parameters (TD, $\tau$, MRT) are expressed in seconds. The number of participants included in each phase of the response (i.e., as determined by presentation with a mono-, bi-, or triphasic response) is indicated (n).

**T2D group.** In the T2D group, for $\Delta$LBF there was a significant time × workload interaction ($P = 0.003$) such that $\Delta$LBF$_{SS}$ was significantly greater at 12 kg vs. 6 kg ($P = 0.003$) but it was not different between workloads at $\Delta$LBF$_{PIR}$ ($P = 0.173$) or $\Delta$LBF$_{ON}$ ($P = 0.839$). For both workloads, $\Delta$LBF$_{SS}$ was greater than both $\Delta$LBF$_{PIR}$ and $\Delta$LBF$_{ON}$ (all $P < 0.05$), but $\Delta$LBF$_{PIR}$ and $\Delta$LBF$_{ON}$ were not different ($P > 0.05$). Similarly for LVK, there was a significant time × workload interaction ($P = 0.023$), with a tendency for $\Delta$LVK$_{SS}$ to be greater at 12 kg vs. 6 kg ($P = 0.059$), but no differences between workloads at $\Delta$LVK$_{PIR}$ ($P = 0.339$) or $\Delta$LVK$_{ON}$ ($P = 0.900$) were observed. At both workloads, $\Delta$LVK$_{SS}$ was greater than $\Delta$LVK$_{PIR}$ and $\Delta$LVK$_{ON}$ (all $P < 0.05$), but $\Delta$LVK$_{PIR}$ and $\Delta$LVK$_{ON}$ were not different from one another (both $P > 0.05$). For $\Delta$MAP, there was a main effect of time ($P = 0.003$) and a main effect of workload ($P < 0.001$) such that $\Delta$MAP$_{SS}$ tended to be greater than $\Delta$MAP$_{ON}$ ($P = 0.061$), it was significantly greater than $\Delta$MAP$_{PIR}$ ($P = 0.003$), and was greater at rest-to-6 kg than at 6-to-12 kg ($P < 0.001$).

**Linear Regressions**

Within the T2D group, $\Delta$LBF and HbA1c were not related at any point in the exercise transient (i.e., PIR, ON, SS) in either the rest-to-6 kg or 6-to-12 kg workload transition (all $P > 0.300$, data not shown).

**DISCUSSION**

The objective of this study was to test the hypothesis that T2D, when present in the characteristic constellation of comorbidities and medications, slows the dynamic adjustment of exercising MBF and blunts the steady state, relative to controls matched for comorbidities and non-T2D medications. The major findings of this study were as follows: 1) the change from baseline to the peak initial LBF response ($\Delta$LBF$_{PIR}$) was blunted in the T2D group relative to the control group, irrespective of whether the transition was from rest to low-intensity or low-to-moderate intensity exercise; 2) regardless of group, the proportion of the total gain (i.e., at steady state) achieved with the peak initial response ($\%\Delta$LBF$_{PIR\_SS}$) was significantly smaller in the 6-to-12 kg transition than the rest-to-6 kg transition; 3) the dynamic adjustment of LBF was not different between groups at either rest-to-exercise or low-to-moderate intensity exercise, as quantified via both the MRT and change from baseline to discrete time points in the ON ($\Delta$LBF$_{ON}$) of the response; and 4) the change from baseline to steady-state exercising LBF ($\Delta$LBF$_{SS}$) was not different between groups at either intensity [low (6 kg) or moderate (12 kg)]. These results demonstrate that individuals with T2D and characteristic comorbidities and medications exhibit an immediate amplitude impairment in the LBF response at the onset of exercise (or at the transition between exercise intensities) but that the overall dynamic adjustment and eventual steady state during low- and moderate-intensity submaximal exercise are not impaired in this population relative to matched controls.

**Dynamic Adjustment of Exercising LBF**

With a step increase in exercise intensity, MBF increases in an exponential manner and reaches a plateau that is in proportion to the metabolic demand, such that steady-state blood flow is linearly related to exercise intensity (12, 32). The initial rapid increase (phase I) is an immediate but incomplete response under feed-forward control that reaches a plateau in approximately 5–10 s (22). This increase may be followed by a transient reduction before a second slower feedback-mediated increase (phase II) begins (within approximately 15–30 s of the increase in contraction intensity) that adjusts blood flow to its steady state during low- and moderate-intensity exercise (22, 36). During high-intensity exercise there is a third and very slow component (phase III) that begins around 1.5 to 2 min (36). Thus each phase of the response is governed by different mechanisms and, because each phase can affect myocellular oxygenation, both the initial adjustment and the eventual steady state are important with regards to a potential oxygen delivery impairment.

**Initial Response Amplitude**

In the present study, the change in LBF from baseline to the PIR (i.e., highest 1-s average in the first 10 s) was significantly reduced in T2D vs. control groups. This was due to a tendency for a blunted $\Delta$LVK$_{PIR}$ (with $\Delta$MAP$_{PIR}$ being similar between groups) and it occurred irrespective of whether the transition was from rest to low-intensity or low-to-moderate intensity exercise (see Fig. 3). Mechanisms capable of contributing to the rapid initial response include mechanical compression of blood vessels (5), the muscle pump (48), venous emptying-mediated vasodilation (46), and rapid release of vasodilatory factors [K+, nitric oxide (NO), prostaglandins, and adenosine acting together] (9, 35).

Because our participants were matched for BMI (and presumably, crudely, leg size) and leg strength (MVC, Table 1) it is unlikely that there were any differences in mechanical compression or muscle pump function at the same absolute
workloads. Although the evidence for venous-emptying-mediated vasodilation is quite strong (46, 49), the mechanism by which emptying of congested veins evokes vasodilation is unknown, and thus it is uncertain whether and/or how this might be impaired in T2D. With regard to rapid vasodilatory factors, at present it is also unknown how these mechanisms might be compromised in individuals with T2D. Impaired endothelial function and overall reduced bioavailability of NO are consistently observed in T2D (25), making reduced NO at the onset of exercise a potential candidate. Future investigation will be required to uncover the mechanistic basis for the blunted initial response.

For the 12-kg workload, the baseline is the 6-kg steady state for each variable. Data are means ± SD. Symbols represent individual participant data; gray bars and closed symbols indicate the control group, white bars and open symbols indicate the T2D group. *Main effect of group. †Main effect of workload (P < 0.05).
of the same magnitude initiated from rest (37). This baseline dependency may result from the absence of any additional contribution of the muscle pump and/or venous emptying-mediated vasodilation in the transition from low-to-moderate exercise intensity, because the muscle pump is already at its capacity for flow enhancement during low-intensity contractions (20) and because venous-congestion-mediated vasodilation occurs in proportion to the degree of venous congestion (49), which would not be anticipated to change during a workload transition.

**Speed of Adjustment and Steady State**

Despite the initial difference in the amplitude of the response, the overall speed of the LBF adjustment was not different between groups. This was confirmed by characterizing the dynamics in two different ways: the MRT and the average amplitude change from baseline to three time points during the ON (15, 45, and 75 s). As previously observed (22), the ON was characterized by a rapid initial increase followed by a brief reduction and then a continued adjustment to the steady state. Within both T2D and control groups, the ΔLBF_{PR} and ΔLBF_{ON} were not significantly different from each other in magnitude, signifying that the ON rapidly re-established flow following its brief reduction after the peak initiatory response (Figs. 3A and 4A). The change in LBF from baseline to the steady state was not different between groups at either the low- or moderate-exercise intensity, and for the 12-kg workload this was true whether it was examined as two successive increments (i.e., rest-to-6 kg and 6-to-12 kg, Fig. 3 Bi) or as the total change from rest to the 12-kg steady state (Fig. 4A). This suggests that despite the initial disturbance to oxygen delivery in T2D, feedback control mechanisms are able to adjust LBF appropriately to the steady state. These findings are in agreement with previous work in an animal model in which reduced partial pressure of oxygen in the microvasculature at the initiation of exercise (but not at the steady state) in type 2 diabetic vs. healthy skeletal muscle suggests a muscle blood flow deficit at the onset of exercise (2, 29). Within the T2D group there was no relationship between the degree of glycemic control (i.e., as indicated by HbA1c) and ΔLBF at any time point (PIR, ON, SS).

**Current Understanding of Potential T2D-Mediated Impairment**

The present study is the first investigation of the impact of T2D on the dynamic adjustment of exercising MBF when accompanied by the typical constellation of comorbidities and most associated medications in this population. In the only other investigation of oxygen delivery dynamics in T2D, MacAnaney et al. (21) recruited a healthier cohort; only a small proportion of the T2D group also had dyslipidemia (33%) and hypertension (11%) as evidenced by medication use, and those in the control group were not matched for these characteristics. MacAnaney and colleagues (21) quantified leg vascular conductance during the transition from rest to intermittent calf contractions (at 70% MVC) and found that the phase 1 amplitude tended to be ~20% lower in the T2D group (nonsignificant), in agreement with the present findings of a blunted ΔLVK_{PR}. They also observed that the time constant (τ) of the second phase of the LVK response was significantly greater in T2D vs. control groups, resulting in a greater MRT (median values of ~31 vs. 16 s). Our findings of no difference in the MRT between T2D vs. control groups suggests that T2D in a constellation of comorbidities still affects the rapid initial response, but not the overall kinetics. The difference in findings between our study and that of MacAnaney et al. (21) may be due to the cohort that was examined, whereby the comorbidity and medication status typical of this population already has an impairment effect that is not further compromised by T2D, as evidenced by the considerably slower MRT of LVK in our control and T2D groups (~46 s) compared with theirs. In other words, it is possible that T2D exerts an independent effect in isolation, but that this effect is superseded by the greater combined influence of comorbidities. If this is the case, this explanation could provide important mechanistic insight. However, given that persons with T2D and no other health problems represent a minority of patients, the practical utility of this suggestion is limited. Alternately, because control participants in the previous study were not matched for comorbid conditions, perhaps the finding of impairment should not be solely attributed to T2D per se. Our findings thus extend those of MacAnaney et al. (21) by 1) evaluating LBF responses within the typical clustering of comorbidities and most medications in this population and matching control participants for these characteristics to achieve ecological validity, 2) providing direct measures of LBF (in addition to LVK) kinetics, and 3) evaluating the kinetic response during both rest-to-exercise and exercise-to-exercise transitions.

With respect to steady-state exercising LBF, investigations by Kingwell et al. (17) and Lalande et al. (19) are frequently cited as evidence for reduced steady-state exercising LBF in persons with T2D, a finding that was not confirmed in the
present study. However, Lalonde et al. (19) enrolled only comorbidity- and medication-free participants, whereas Kingwell et al. (17) included only two participants with T2D who took antidiabetic agents but completed testing after a 24-h drug-free period. As previously mentioned, persons with T2D typically take antidiabetic agents to manage their disease (18) as well as antihypertensive and antihyperlipidemic medications (4, 41). Importantly, antihypertensive and antidiabetic agents are known to improve endothelial function (24, 28) and may increase exercising MBF (13, 52). Thus these medications may have contributed to the conserved LBF responses in the T2D group at the steady state in the present study. Accordingly, whereas the previous approaches of medication exclusion or withdrawal also have merit (i.e., perhaps better reflecting endogenous vascular function in T2D), should these medications be responsible for the preserved LBF response, the implication is that in the real world, persons with T2D (who are taking these medications) do not experience impairment that might otherwise be endogenous to the disease. Notably, however, others have observed impaired blood flow in T2D when medications were continued during testing [only if HbA1c $\geq$8 (16)] or preserved blood flow was when medications were discontinued (23). This suggests that differences in medication status cannot fully explain disparate findings across studies, and we cannot estimate the magnitude of effect the medications may have exerted in the present study.

Perspectives

The workloads employed in the present study represent low-to-moderate exercise intensities and reflect metabolic requirements encountered during activities of daily living (e.g., walking or climbing a flight of stairs). The current findings indicate that although muscle oxygen delivery may be briefly blunted at the onset of exercise (or in the transition between exercise intensities) in persons with T2D in the context of comorbidities and common medications, the overall rate of adjustment and eventual steady state are not different from those in matched control participants. The functional significance of the initial blunting of LBF is unknown, particularly because it is transient (i.e., occurring within the first 10 s of exercise, with unimpaired flow relative to controls restored shortly thereafter). Indeed, a recent investigation in our laboratory found no impairment to small muscle mass exercise tolerance in this population (unpublished observations), and others have demonstrated no impairment to exercising muscle blood flow in T2D (8, 23, 43). However, it remains possible that when superimposing multiple transitions during activities of daily living, sequential impairments in the initiating response could accrue to affect exercise tolerance. Future work will be required to ascertain the effect (if any) on exercise tolerance and to identify the mechanisms responsible for the attenuation of LBF at the onset of exercise.

Limitations

In the present study, a small muscle mass modality was used to isolate peripheral vascular contributions to exercising muscle blood flow. Although no compromise to the overall speed or magnitude of the LBF adjustment was evident, it remains possible that a cardiac-mediated limitation to oxygen delivery could be present during whole-body exercise [i.e., heightened activity of the cardiac sympathetic afferent reflex (53)]. In addition, we cannot exclude the possibility of compromised spatial matching of blood flow to metabolic demand because a recent investigation found that microvascular perfusion was impaired in persons with T2D and microvascular complications in the absence of impaired conduit artery flow (50). Findings cannot be generalized to the minority of persons with T2D who do not have comorbidities or who are treated by diet and exercise alone. The present findings are also limited to men, although sex differences are unlikely given that $V_O_2$ kinetics have been shown to be similar in men and women with T2D (27). Similarly, results can be generalized only to the range of exercise intensities employed in the present study (i.e., low to moderate), although if anything, impairment would be expected to be less likely during high-intensity exercise since in a previous investigation $V_O_2$ kinetics were found to be slowed at workloads below but not above the lactate threshold in persons with T2D (34). Finally, we assessed exercising hemodynamics during fasting conditions; LBF may have been differentially affected in the postprandial state since exercise hyperemia has been found to be reduced in insulin-resistant individuals when exercise is performed in hyperinsulinemic conditions (14).

Conclusions

This is the first study to systematically characterize the dynamic and steady-state LBF responses during both rest-to-low-intensity and low-to-moderate-intensity exercise transitions in persons with T2D within the common cluster of comorbidities and medications that accompany this disease. As anticipated, the initial amplitude of the LBF response was significantly blunted in T2D vs. control groups, but this was not intensity-dependent (i.e., the attenuation occurred irrespective of whether the transition was from a resting or exercising baseline). Contrary to our hypothesis, however, there was no robust or consistent impact of T2D on top of the comorbidities and medications typical of this population, on the overall dynamic adjustment of LBF, or the steady-state levels ultimately achieved. These data demonstrate that impaired adjustment of oxygen delivery may have a limited contribution to exercise intolerance in persons with T2D, and that a reduction in exercising muscle oxygen delivery at the steady state, beyond that which may already be present in the context of comorbidities, is not an obligatory consequence of this disease. Future work will be required to uncover the one or more mechanisms underlying the initial amplitude impairment and the impact (if any) on exercise tolerance.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES


