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Mechanisms for the Increased Fatigability of the Lower Limb in People with Type 2 Diabetes

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ABSTRACT

Fatiguing exercise is the basis of exercise training and a cornerstone of management of type 2 diabetes mellitus (T2D), however, little is known about the fatigability of limb muscles and the involved mechanisms in people with T2D. The purpose was to compare fatigability of knee extensor muscles between people with T2D and controls without diabetes and determine the neural and muscular mechanisms for a dynamic fatiguing task. Seventeen people with T2D (10 men, 7 women: 59.6±9.0 years) and 21 age-, BMI- and physical activity-matched controls (11 men, 10 women: 59.5±9.6 years) performed 120 high-velocity concentric contractions (1 contraction/3 s) with a load equivalent to 20% maximal voluntary isometric contraction (MVIC) torque with the knee extensors. Transcranial magnetic stimulation (TMS) and electrical stimulation of the quadriceps were used to assess voluntary activation and contractile properties. People with T2D had larger reductions than controls in power during the fatiguing task (42.8±24.2% vs. 26.4±15.0%, $P<0.001$) and MVIC torque after the fatiguing task (37.6±18.2% vs. 26.4±12.1%, $P=0.04$). People with T2D had greater reductions than controls in the electrically-evoked twitch amplitude after the fatiguing task (44.0±20.4% vs. 35.4±12.1%, respectively, $P=0.01$). However, the decrease in voluntary activation was similar between groups when assessed with electrical stimulation (12.1±2.6% vs. 12.4±4.4% decrease, $P=0.84$) and TMS ($P=0.995$). A greater decline in MVIC torque was associated with larger reductions of twitch amplitude ($r^2=0.364$, $P=0.002$). Although neural mechanisms contributed to fatigability, contractile mechanisms were responsible for the greater knee extensor fatigability in men and women with T2D compared with healthy controls.

New & Noteworthy: Transcranial magnetic stimulation and percutaneous muscle stimulation were used to determine the contributions of neural and contractile mechanisms of fatigability of the knee extensor muscles after a dynamic fatiguing task in men and women with type 2 diabetes (T2D) and healthy, age-, BMI- and physical activity-matched controls. Although neural and contractile mechanisms contributed to greater fatigability of people with T2D, fatigability was primarily associated with impaired contractile mechanisms and glycemic control.

Key Words (5): muscle fatigue, diabetes mellitus, skeletal muscle, sex differences, knee extensors

Abbreviations:

ANOVA	Analysis of Variance
BMI	Body Mass Index
DEXA	Dual X-ray Absorptiometry
EMG	Electromyography
FPG	Fasting Plasma Glucose Concentration
FPI	Fasting Plasma Insulin Concentration
HbA_{1c}	Glycated Hemoglobin
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
MEP	Motor Evoked Potential
min	Minute
M_{max}	Maximal Compound Muscle Action Potential
MVCC	Maximal Voluntary Concentric Contractions
MVIC	Maximal Voluntary Isometric Contractions
SIT	Superimposed Twitch
T2D	Type 2 Diabetes Mellitus
TMS	Transcranial Magnetic Stimulation

INTRODUCTION

Type 2 diabetes mellitus (T2D) has become a global pandemic and is estimated to currently affect 8% of the world's population (34). Physical activity is a cornerstone of T2D management and along with diet is the first intervention used to treat T2D (43). Incidence of T2D can be reduced by 58% with lifestyle interventions (diet, weight loss and exercise), and these lifestyle interventions were almost twice as effective as pharmacological treatment (metformin), which reduced the incidence of diabetes by 31% (30). Fatiguing contractions of limb muscles are the foundation of exercise training and the neuromuscular adaptations that accompany regular exercise (31). However, there is minimal understanding of the mechanisms that limit a single bout of fatiguing exercise in people with T2D.

Fatigability of limb muscles is a reversible, short-term and activity-induced reduction in muscle strength or power (17, 23), and can limit performance of daily tasks that require repeated or sustained contractions (12, 42). Mechanisms that contribute to limb fatigability in healthy adults include deficits in neural drive to the muscle, impairments in neuromuscular propagation, reduced force capacity of skeletal muscle fibers, and impaired blood flow to the muscle (11, 17, 23). Few studies have examined the mechanisms of fatigability among people with diabetes. Several studies have shown that for isometric contractions with lower limb muscles (ankle dorsiflexor and knee extensor muscles), people with type 1 diabetes and diabetic polyneuropathy and people with T2D, were more fatigable than controls (3, 6, 8). The mechanisms contributing to greater fatigability in the people with type 1 diabetes who had diabetic polyneuropathy included disruption of neuromuscular transmission indicated by a concomitant decrease in the maximal compound muscle action potential (3) and slowed motor unit conduction velocities and discharge frequencies (6). The mechanisms for the greater fatigability in the lower limb muscles of people with T2D are not known.

Decrements in power during repeated dynamic fatiguing contractions are probably of greater functional significance than decrements in torque during isometric tasks in people with T2D. First, at baseline (without fatigue) the difference (reduction) in muscle power for people with T2D compared with controls is greater than for maximal isometric torque (2, 22). Second, low power and maximal velocity of limb muscles at baseline were the primary variables associated with impaired balance and gait in people with T2D (36). Whether people with T2D are more fatigable during dynamic contractions, which can further exacerbate power differences between controls and people with T2D, is relatively unexplored. One study demonstrated that after 20 moderate-velocity (120 $deg \cdot s^{-1}$) isokinetic contractions performed separately with four lower limb muscle groups, people with T2D (with and without diabetic polyneuropathy) were more fatigable than age-matched controls for the knee flexor muscles, but not the ankle plantar flexor or dorsiflexor, or knee extensor muscles (28). Another study, showed that people with T2D tended to have greater reductions in knee extensor torque over 30 isokinetic contractions at 180 $deg \cdot s^{-1}$ than healthy age-matched controls (both lean and weight-matched), although these differences in torque reductions did not reach statistical significance, possibly due to low subject numbers (n = 8) (18). There are no other known studies determining the fatigability during dynamic fatiguing tasks in people with T2D, and furthermore, the mechanisms are unknown. Lastly, despite potential differences in fatigability between men and women (24), studies of fatigability in people with T2D have been underpowered to determine whether there are sex-related differences among people with T2D (e.g. (8, 18, 28, 37).

The mechanisms for any potential increased fatigability of limb muscles in men and women with T2D may originate from both neural (supraspinal and spinal) and muscular sites. People with T2D may have impaired skeletal muscle energetics (i.e. increased inorganic phosphate and hydrogen ion within intracellular milieu) and reduced skeletal muscle blood flow during exercise compared with healthy controls (32, 39), potentially eliciting greater stimulation

of afferent feedback (Group III and IV afferents) to supraspinal and spinal centers during fatiguing exercise, further exacerbating any exercise-related reductions in neural drive to the muscle (23, 46). Furthermore, because people with T2D are at risk of neuropathy, neuromuscular transmission may contribute to differences in fatigability between people with and without T2D (2, 3). In this current study, we used non-invasive stimulation at the motor cortex and muscle to determine the contribution of neural (supraspinal and spinal) and muscular mechanisms (50, 51) to any differences in fatigability between people with T2D and controls.

The *purpose* of the study was to: 1) compare fatigability of both men and women with T2D (without clinically-evident neuropathy) with age-, BMI- and physical activity-matched controls in response to a high-velocity dynamic fatiguing task with the knee extensor muscles, and 2) determine the contribution of neural and muscular mechanisms. Our *hypotheses* were that: 1) fatigability of the knee extensor muscles would be greater in people with T2D compared with healthy controls, and 2) both neural and contractile mechanisms would contribute to the greater fatigability in people with T2D compared with healthy control participants. Because the age of onset of T2D is inversely related to disease complication risk and mortality, we enrolled participants >50 years. Additionally, because there is limited understanding of sex differences in fatigability of people with T2D, a third aim was to determine whether there were sex-related differences in fatigability and mechanisms among people with T2D. Our *hypothesis* was that there would be no sex-related differences in fatigability, as we have observed in a young and older adult population previously (42).

MATERIALS AND METHODS

Seventeen people with T2D (10 men: age, 59.7 ± 9.5 years; HbA1c, 6.92 ± 1.19%; 7 women: age, 59.6 ± 9.0 years; HbA1c, 7.20 ± 1.06%) and twenty-one healthy controls (11 men: age, 58.2 ± 10.3 years; HbA1c, 5.42 ± 0.25%; 10 women: age, 61.2 ± 8.8 years; HbA1c, 5.40 ±

0.21%) participated in the study. Prior to involvement in the study, each participant provided written informed consent and the protocol was approved by the Marquette University Institutional Review Board (HR-2402) for ethical approval in accordance with the Declaration of Helsinki for human experimentation.

Aside from glycemic control, all participants were healthy. Type 2 diabetes was physician-diagnosed and confirmed at study enrolment via fasting glucose and HbA_{1c}. Exclusion criteria included: unstable diabetes, prescribed insulin or insulin secretagogue, poor glycemic control (glycosylated hemoglobin (HbA_{1c}) >10%), diabetic neuropathy (assessed via clinical diagnosis, monofilament and tuning fork sensation tests, and sensory questionnaires), peripheral edema, severe obesity (body mass index, BMI, >45 kg/m^2), untreated hypothyroidism, epilepsy, medications that affect cortical excitability, possibility of pregnancy and any neurological, cardiovascular or musculoskeletal disease that precluded exercise testing. Any potential participants who presented with HbA_{1c} >5.7% and <6.5% (and were not diagnosed with T2D) were classified as having pre-diabetes and not included in the study; thus, all controls had an $HbA_{1c} \leq 5.6\%$.

Participants completed three sessions of testing that included a screening session to determine eligibility for the study followed by two experimental sessions. The aim of the first experimental session was to familiarize participants with experimental procedures and complete a fasting blood draw and questionnaires. The aim of the second experimental session was to complete the fatiguing task. Each session was separated by 2-7 days.

Screening Session

During the screening session, the following tests were performed: 1) lower limb sensation was assessed using a 10-gram monofilament and 128-Hz vibration sensation test, 2) autonomic nerve function was assessed using a heart rate variability test and blood pressure response to

upright posture, and 3) glycemic control was assessed using a point-of-care HbA_{1c} instrument. Skeletal muscle mass of the dominant leg and whole-body fat mass were assessed utilizing DEXA and participants were assigned a triaxial accelerometer. Then, peak aerobic capacity was estimated from a submaximal graded bicycle ergometer exercise test.

Diabetic neuropathy screening: Each participant was screened for the presence of diabetic polyneuropathy. To assess symptoms and signs of sensory neuropathy monofilament screening of the feet, vibration sensation testing (bilateral malleoli and heads of the 1st metatarsals) and Achilles tendon reflex testing were performed. Participants were excluded if impaired sensation was observed i.e., if the monofilament could not be sensed on any site on the foot; if vibrations could be sensed by the examiner for more than 10 s longer than the participant; or if the tendon jerk was absent. Participants who were suspected of having diabetic polyneuropathy (sensory or autonomic) were excluded from the study.

HbA_{1c}: HbA_{1c} was determined using blood from a fingerstick, analyzed using a point-of-care instrument assay (Siemens Healthcare Diagnostics, DCA 2000+).

Anthropometry and DEXA: Body anthropometry included measurements of height, body mass and waist circumference. Skeletal muscle mass of the dominant leg and whole-body fat mass (% body weight), were assessed utilizing DEXA (Lunar Prodigy full-body scanner, Madison, WI, USA). The scanner was calibrated prior to each scan. The analyzed data was recorded offline (Encore 2008 software by GE Health care). In the case of participants with artificial joints (n = 4), the artificial joint was excluded via encore software.

Physical Activity Monitor: Accelerometry data were collected using the Actigraph GT3X (ActiGraph, Pensacola, FL, USA) that was worn on the hip by each participant for 4 days (2 weekdays and 2 weekend days). Sixty-second epochs of data were collected and analyzed. Wear-time authentication was performed on each participant's dataset to determine whether data were

to be included in the analysis. Acceptable wear-time was set *a priori* and defined as ≥ 3 days of ≥ 9 hours (540 minutes) per day. Step count was recorded (ActiLife Software v4) and analyzed.

Submaximal, Graded Bicycle Test: Participants performed a submaximal graded exercise test (9) on a bicycle ergometer (VIAsprint 150P, CareFusion, San Diego, CA, USA) to determine estimated oxygen consumption and to screen for exercise-induced cardiac arrhythmia. Participants were required to maintain cadence of 60 revolutions per minute that was monitored via LED screen by the participant and a researcher, and the cycle load was manipulated to attain three submaximal loads that elicited incremental heart rate responses between 40% and 70% of heart rate reserve. The participant cycled at each submaximal load for four minutes to attain steady-state. During this test, a 12-lead electrocardiogram (CASE, General Electrics, Madison, WI, USA) was monitored to determine if arrhythmias were present. Participants were excluded if arrhythmia was detected, even if asymptomatic.

Experimental Session One

Participants fasted for *at least* 8 hours prior to experimental session one. Venous blood was obtained via venous draw, after which participants consumed a standardized breakfast (8 oz. fruit juice, one cereal bar, and one serving of fruit) prior to undertaking the remaining activities in the session. In conjunction with fasting, participants with T2D delayed administration of medications until after the venous draw.

Participants completed a questionnaire to determine handedness/footedness (35) to assess which leg which would be used for testing. Participants first practiced submaximal muscle contractions, maximal voluntary isometric contractions (MVICs) and maximal voluntary concentric contractions (MVCCs) of the knee extensor muscles while seated in a Biodex System 4 dynamometer (Biodex Medical, Shirley, NY). They were also habituated with electrical

stimulation of the femoral nerve, percutaneous electrical stimulation of the knee extensor muscles and transcranial magnetic stimulation (TMS) of the motor cortex.

Blood Measures: Fasting blood glucose was determined using a point of care instrument (Alere Cholestech LDX System, Alere Inc. Waltham, MA, USA). Hemoglobin concentration was determined using a point of care instrument (StatSiteM Hemoglobin Photometer, Stanbio, Boerne, TX, USA) and hematocrit was determined manually (International Micro-capillary Reader, International Equipment Company, Boston, MA, USA) per standard instruction of each instrument. Plasma insulin and thyroid-stimulating hormone concentrations were quantitatively assayed in duplicate per manufacturer instructions using enzyme-linked immunoassay kits (Quantikine Human Insulin Immunoassay (R&D Systems, Minneapolis, MN) and Human TSH (CGA) ELISA Kit (Thermo Scientific Pierce (Waltham, MA), respectively).

Questionnaires: All participants completed questionnaires to assess: clinical symptoms of fatigue using the Fatigue Impact Scale (13); sleep quality with the Pittsburgh Sleep Quality Index (10); and depression with the short form Geriatric Depression Scale (44).

Experimental Session Two

Participants consumed the same standardized breakfast as during the first experimental session; after which participants with T2D administered their diabetes medications. In this second experimental session, each participant performed baseline MVICs and MVCCs followed by a maximal-velocity fatiguing task and recovery contractions with the dominant knee extensor muscles.

Measurement of Torque, Velocity and Power

Participants performed isometric and isotonic contractions with the knee extensors muscles while seated in a dynamometer. Participants performed all contractions on their

dominant leg, unless there was any form of disease (e.g. osteoarthritis) or injury (e.g. knee reconstruction), in which case the non-dominant leg was tested (n = 2 controls, 2 people with T2D). Participants were seated with 90º of hip flexion. Padded straps mounted on the seat were securely tightened across the shoulders, the waist, and the non-dominant leg to minimize synergistic movements. The dominant leg was positioned such that the axis of rotation of the knee joint was aligned with the axis of rotation of the dynamometer. The internal goniometer of the Biodex dynamometer was calibrated using a level to measure 90º flexion of the knee joint. The analog signals corresponding to joint angle, torque, and velocity were digitized and recorded through a Power 1401 analog-to-digital (A-D) converter and Spike2 software (Cambridge Electronics Design, Cambridge, UK).

Electromyography

Electromyography (EMG) electrodes (Ag–AgCl, 8-mm diameter; 20 mm intra-electrode distance) were placed on three agonist muscles (rectus femoris, vastus lateralis and vastus medialis) in a bipolar arrangement according to recommendations (21) with reference electrodes placed over the patella of the dominant knee. The EMG signals were amplified (100×) and filtered between 13 - 1000 Hz (Coulbourn Instruments, Allentown, PA) and digitized at 2,000 Hz. Mechanical recordings from the dynamometer corresponding to torque, velocity and position were recorded online at 2,000 Hz. All analog signals were digitized using a 1401 A–D converter and Spike 2 software [Cambridge Electronics Design (CED), Cambridge, UK].

Transcranial Magnetic Stimulation (TMS)

TMS was delivered via a concave double cone coil (Magstim 200, Magstim, Whitland, UK, 11.0-cm outside diameter) over the motor cortex area to elicit motor-evoked potentials (MEPs) and torque during voluntary contractions of the dominant knee extensor muscles as described before (40). The vertex of the motor cortex was identified, and the scalp was marked

1.0 cm lateral to the vertex (over the motor area corresponding to the dominant knee extensors) to ensure repeatability of coil placement during the experimental protocol. The optimal coil position of the TMS was determined during brief contractions of the knee extensor muscles at 20% MVIC. TMS was elicited during the contractions and fine adjustments in the TMS coil position (~0.5 cm) were made to determine which site evoked the largest superimposed twitch (SIT) torque and MEP of the rectus femoris muscle. Optimal stimulator intensity was also determined with brief contractions (2-3 s) of knee extensor muscles (50% MVIC), which is the intensity that is known to elicit maximal MEPs (51). The intensity of the stimulation (% maximal of stimulator intensity) was increased by 5% increments until maximal twitch torque of the quadriceps and maximal MEP of the rectus femoris muscle were elicited. The brief contractions at 50% MVIC were separated by 30-s rest periods to avoid fatigue when establishing the intensity of TMS.

Electrical Stimulation

Single-pulse (200 μs duration, 400 V) electrical stimulation was used for femoral nerve and percutaneous muscle stimulation (DS7AH; Digitimer, Ltd., Welwyn Garden City, UK) to elicit maximal compound muscle action potentials (M_{max}) and twitch contractions at rest and during MVICs of the knee extensor muscles.

Femoral Nerve Stimulation: The femoral nerve innervating the knee extensor muscles was stimulated supramaximally (120 – 600 mA) with a single pulse to elicit the maximal compound muscle action potential (M_{max}). The cathode electrode (Ambu Neuroline electrodes, Denmark; 1.5 cm diameter) was placed over the femoral nerve within the femoral triangle and the anode was placed over the greater trochanter of the femur. The intensity of the nerve stimulation was determined by increasing the current until the twitch amplitude plateaued. The stimulation

intensity was then increased further by 20% to ensure a maximal activation of the muscles within the area of stimulation.

Percutaneous Muscle Stimulation: To assess voluntary muscle activation and twitch properties, the knee extensor muscles were stimulated with a single pulse (150 – 750 mA) via custom-made pad electrodes (6 cm × ~15 cm) placed over the quadriceps muscles. The cathode was placed near (within 10 cm) the area of the femoral triangle and the anode was placed proximal to the patella without hindering knee flexion/extension of the participant. The stimulator intensity was determined by increasing the current until the twitch amplitude plateaued, then the stimulation intensity was increased further by 20% to ensure a maximal activation of the muscles in the area of stimulation. This stimulation intensity was used for the remainder of the session. The twitch amplitude elicited via percutaneous and femoral nerve stimulation were linearly correlated (r^2 = 0.653, P < 0.001). Percutaneous muscle stimulation was used throughout the experimental protocol for assessment of voluntary activation and twitch properties, because percutaneous stimulation was more tolerable than nerve stimulation. Using the supramaximal intensity, three muscle stimulations were applied, each separated by ~15 s to assess electrically-evoked twitch contractile properties in a non-potentiated state.

Experimental Protocol

The experimental protocol entailed:

(1) *Baseline MVICs:* Participants completed at least three MVICs for ~4 seconds each with the knee extensor muscles, positioned in 90° of hip and knee flexion. Participants then performed four additional MVICs during which TMS and electrical stimulation were superimposed to estimate voluntary activation (see the 'data analysis' section for calculations). Electrically-evoked, potentiated twitch contractions were also elicited at rest immediately after each MVIC to determine contractile properties and voluntary activation of the knee extensor muscles. Each

baseline MVIC was separated by 2.5 minutes, to minimize the effect of fatigue prior to beginning the dynamic fatiguing task.

(2) *Baseline Maximal Voluntary Concentric Contractions (MVCCs):* Participants warmed-up with 10 MVCCs with a load equivalent to 20% of MVIC. These isotonic contractions were performed through an ~85º range of motion, from 90°of knee flexion until 5°of knee flexion. Participants then rested for 2.5 minutes, before initiating the dynamic fatiguing task.

(3) *Dynamic fatiguing task:* The fatiguing protocol involved 120 isotonic MVCCs of the knee extensor muscles through an ~85º range of motion (as above) with 1 MVCC performed every 3 seconds (6-minute task). Participants actively extended the knee, then the dynamometer passively returned the leg to the starting position at 90º of knee flexion after each MVCC.

(4) *Recovery Contractions:* The recovery protocol involved sets of brief contractions immediately after the fatiguing task, and then at 5 and 20 minutes of recovery. Each set of contractions involved an MVIC (with a superimposed TMS and percutaneous muscle stimulation) followed by an additional electrically-evoked twitch contraction and then five successive MVCCs.

Participants received strong verbal encouragement throughout the maximal effort contractions. During all MVCCs, participants were instructed to “kick as hard and as fast as possible” and each MVCC was initiated via strong verbal command from the authors: “KICK”. The authors provided the verbal cue each 3-s based on a visual cue from a custom-designed data collection program, and participants were encouraged to maintain maximal effort throughout the dynamic fatiguing task using several standard statements of encouragement.

Data Analysis

The torque during the MVICs was quantified as the average value over a 0.1 s interval prior to the onset of the TMS pulse. The maximum angular velocity, power and resistance torque

309 during MVCCs were quantified during the concentric phase of the contraction. The average
310 resistance torque during MVCCs was calculated as the average torque during the concentric
311 phase of the knee extension contraction. The duty cycle was calculated as: (active contraction
312 time) · (active contraction time + relaxation time)$^{-1}$. The variables from the dynamic fatiguing
313 task are presented as the average from five consecutive contractions, at baseline (contractions 1-
314 5) or the end of the fatiguing task (contractions 116-120).

315 Voluntary activation was assessed with both TMS and electrical stimulation. Voluntary
316 activation with TMS was estimated with the SIT expressed as a percentage of the total torque i.e.
317 [SIT · (MVIC + SIT)$^{-1}$ · 100%] (17). For electrically evoked contractions, voluntary activation
318 was calculated using the following equation: voluntary activation = (1 – SIT · Potentiated
319 Twitch^{-1}) × 100% (17, 50). Contractile properties of the knee extensor muscles were quantified
320 from the potentiated twitch elicited with percutaneous electrical stimulation. Variables included
321 the peak amplitude of the potentiated twitch, contraction time, and half relaxation time. Half
322 relaxation time was determined as the time interval in milliseconds (ms) elapsed from the peak
323 twitch amplitude until the torque reached 50% of the peak twitch amplitude. Post-activation
324 potentiation (PAP) from electrically-evoked twitch contractions was calculated as: (potentiated
325 twitch amplitude - non-potentiated twitch amplitude) · non-potentiated twitch amplitude^{-1} ·
326 100%.

327 Electrophysiological properties of the knee extensors were also assessed with peak-to-
328 peak amplitude of the MEPs for the agonist muscles (rectus femoris, vastus lateralis and vastus
329 medialis) elicited via TMS during MVICs. Similar results were observed for the MEP amplitude
330 and area, thus, only MEP amplitude results are presented. The duration of the silent period was
331 determined as the interval from the time of the TMS to the return of continuous EMG after the
332 MEP (47). Reduction in variables (MVIC torque, MVCC velocity, power, duty cycle, range of
333 motion, peak resistance torque, and average resistance torque, voluntary activation, twitch

amplitude, contraction time, half relaxation time, peak rate of relaxation, EMG silent period and MEP (%M_{max})) for before and after the fatiguing task, were calculated as [1 – (end value · baseline value^{-1})] × 100%. Representative traces of raw data are presented in Figure 1, for dynamic contractions (Fig. 1A) and MVCs with stimulations (Fig. 1 B-F).

[Figure 1]

Homeostatic model assessment for assessing insulin resistance (HOMA-IR) was calculated using the fasting plasma insulin concentration (FPI, mU·L^{-1}) and fasting plasma glucose (FPG, mmol·L^{-1}): HOMA-IR = (FPI × FPG) · 22.5^{-1}.

Statistics

Values are reported as mean ± SD in the text and displayed as mean ± SE in the figures. Participant characteristics and baseline muscle function (Tables 1 and 2) were compared across groups using a univariate analysis of variance (ANOVA) with two between subject factors (group: T2D vs controls, and sex: male vs. female).

To determine changes over time during the dynamic fatiguing contraction or during the 20-minute recovery period (task end, 5 mins and 20 mins post the dynamic contraction), mixed model analysis of variance with group and sex as between subject factors and repeated measures over time was used for the various dependent variables (MVIC torque, MVCC velocity, power, duty cycle, range of motion, peak applied torque, and average applied torque, voluntary activation, twitch amplitude, contraction time, half relaxation time, peak rate of relaxation, EMG silent period and MEP (%M_{max})). For each ANOVA, the sphericity of data was determined, and technical corrections were performed when necessary. If needed, post hoc analysis with Bonferroni corrections were applied when an *F* test was significant. Pearson correlation coefficients (*r*) were used to determine associations between variables including fatigability (reductions in MVIC and MVCC), participant characteristics (fasting plasma glucose, HbA1c, estimated VO2 peak, skeletal muscle mass, daily step count, and questionnaire scores), baseline

muscle characteristics (MVIC strength, MVCC power, voluntary activation, and potentiated twitch amplitude), and measurements of fatigue-related changes in the potentiated twitch and voluntary activation. Linearity of bivariate correlations was verified with visual inspection, to confirm there were no violations of the assumptions of normality, linearity, and homoscedasticity.

Significance was determined at $P < 0.05$ and all analyses were performed using IBM Statistical Package for Social Sciences (SPSS, V24).

RESULTS

Baseline Measurements

Participant and baseline characteristics are presented in Table 1. The T2D and control groups were similar in age (group effect, $P = 0.985$), BMI (group effect, $P = 0.172$), and daily physical activity (step count; group effect, $P = 0.895$). The control and T2D groups had similar body fat (group effect, $P = 0.310$), estimated VO_2 peak (group effect, $P = 0.231$) and skeletal muscle mass in the dominant leg (group effect, $P = 0.724$).

As expected, people with T2D had higher HbA_{1c} (group effect, $P < 0.001$), fasting plasma glucose (group effect, $P < 0.001$), fasting plasma insulin (group effect, $P = 0.001$) and HOMA-IR (group effect, $P < 0.001$) compared with controls (Table 1). People with T2D and controls had similar plasma thyroid-stimulating hormone concentrations (1.86 ± 0.89 vs. 1.58 ± 0.89 $mU \cdot L^{-1}$, respectively; group effect, $P = 0.306$). People with T2D and controls demonstrated no signs of anemia, hemoglobin (14.2 ± 1.8 vs. 14.6 ± 1.7 $g \cdot dL^{-1}$, respectively; group effect, $P = 0.428$) and hematocrit (42.4 ± 3.3 vs. 42.3 ± 4.0%, respectively; group effect, $P = 0.974$) concentrations were similar between the groups. Among the people with T2D, 14 people were prescribed metformin and 11 people were prescribed a statin medication. Among controls, 0 people were prescribed metformin and 4 people were prescribed a statin medication. Although

not a primary aim of the study, it is noteworthy that people with T2D prescribed to a statin medication had similar reductions in MVCC power (time × statin effect, $P = 0.458$; statin effect, $P = 0.729$) and MVIC torque (time × statin effect, $P = 0.742$; statin effect, $P = 0.571$) compared to people with T2D *not* prescribed to a statin medication. See Table 1.

[Table 1]

People with T2D and controls had similar knee extensor MVIC torque (group effect, $P = 0.421$), peak angular velocities (group effect, $P = 0.949$), peak knee extensor power (group effect, $P = 0.627$), electrically-evoked potentiated twitch amplitudes (group effect, $P = 0.667$), and post-activation potentiation (group effect, $P = 0.368$). See Table 2. Baseline levels of voluntary activation during MVICs were similar between controls and people with T2D, quantified with TMS (group effect, $P = 0.232$) and with electrical stimulation (group effect, $P = 0.715$; Table 2).

[Table 2]

For both groups, men and women were similar in age (58.9 ± 9.8 vs. 60.5 ± 8.7 years, respectively; sex effect, $P = 0.646$; group × sex, $P = 0.617$), BMI (28.9 ± 5.3 vs. 27.2 ± 6.2 $kg \cdot m^{-2}$, sex effect, $p = 0.447$; group × sex, $P = 0.205$), daily physical activity (step count: 8,690 ± 3,220 vs. 7,830 ± 3,400 $steps \cdot day^{-1}$, respectively; sex effect, $P = 0.499$; group × sex, $P = 0.608$), HbA_{1c} (6.10 ± 1.11 vs. 6.19 ± 1.15%, respectively; sex effect, $P = 0.612$; group × sex, $P = 0.568$), fasting plasma glucose (106.5 ± 25.7 vs. 102.9 ± 35.2 $mg \cdot dL^{-1}$, respectively; sex effect, $P = 0.614$; group × sex, $P = 0.786$), fasting plasma insulin (46.1 ± 21.2 vs. 45.4 ± 29.0 pMol, respectively; sex effect, $P = 0.891$; group × sex, $P = 0.118$), HOMA-IR (2.06 ± 1.14 vs. 2.17 ± 2.01 AU, respectively; sex effect, $P = 0.762$; group × sex, $P = 0.191$) and thyroid-stimulating hormone (1.75 ± 1.03 vs. 1.64 ± 0.68 $mU \cdot L^{-1}$, respectively; sex effect, $P = 0.753$; group × sex, $P = 0.520$).

Men however, had less body fat than women (28.0 ± 6.6 vs. 38.3 ± 9.6%, respectively; sex effect, $P < 0.001$, group × sex, $P = 0.142$) and greater skeletal muscle mass of the leg (9.81 ± 1.37 vs. 6.54 ± 1.24 kg, respectively; sex effect, $P < 0.001$; group × sex, $P = 0.116$). For both groups men also had a larger MVIC torque (204.6 ± 63.1 vs. 116.9 ± 34.7 Nm, respectively; sex effect, $P < 0.001$, group × sex, $P = 0.905$), similar MVCC peak angular velocity (342.3 ± 56.6 vs. 318.3 ± 41.2 deg·s^{-1}, respectively; sex effect, $P = 0.184$; group × sex, $P = 0.620$), greater MVCC peak power (329.0 ± 120.4 vs. 213.1 ± 71.1 Watts, respectively; sex effect, $P = 0.004$, group × sex, $P = 0.453$) and a larger electrically-evoked twitch amplitude (50.3 ± 21.0 vs. 31.7 ± 4.8 Nm, respectively; sex effect, $P = 0.004$, group × sex, $P = 0.670$). Baseline voluntary activation measured during the MVICs (92.6 ± 5.2 vs. 93.6 ± 2.7%, respectively; sex effect, $P = 0.529$, group × sex, $P = 0.955$) and post-activation potentiation (60.0 ± 26.7 vs. 84.6 ± 69.9%, respectively; sex effect, $P = 0.258$, group × sex, $P = 0.185$) was similar for men and women.

Men and women did not differ in estimated VO_2 peak (31.0 ± 7.2 vs. 26.0 ± 8.6 mL·kg^{-1}·min^{-1}, respectively; sex effect, $P = 0.060$; group × sex, $P = 0.063$), although there was a trend toward significance. Closer examination showed that the control men and women had similar estimated VO_2 peak (30.1 ± 7.0 vs. 30.1 ± 9.7 mL·kg^{-1}·min^{-1}; sex effect, $P = 0.99$); however, among people with T2D, men had greater estimated VO_2 peak compared to women (32.0 ± 7.7 vs. 22.0 ± 5.1 mL·kg^{-1}·min^{-1}; sex effect, $P = 0.010$).

Perception of Fatigue, Depression, and Sleep Quality: People with T2D had similar reports of perceptions of daily fatigue on cognitive function (FIS cognitive; group effect, $P = 0.216$), physical function (FIS physical; group effect, $P = 0.302$), and psychological function (FIS psychological; group effect, $P = 0.328$) compared with controls. See Table 1.

People with T2D and controls reported low but similar scores on the depression scale (group effect, $P = 0.301$), with no one reporting a clinically significant level of depression (GDS

score > 5). Sleep quality was similar in people with T2D and controls (group effect, $P = 0.415$). See Table 1. The mean scores were consistent with assessments of 'healthy control' sleepers; however, some individuals reported 'poor' sleep quality (PSQI score > 5) (10).

Fatigability and Recovery

MVCC angular power and velocity: Both the control group and people with T2D had reductions in MVCC power during the dynamic fatiguing task (time effect, $P < 0.001$), but this reduction was greater in people with T2D (time × group, $P < 0.001$; Figure 2A). Recovery, however, was similar for both groups (time effect, $P < 0.001$; group effect, $P = 0.291$; time × group, $P = 0.548$).

People with T2D demonstrated greater reductions in MVCC peak angular velocity compared with controls during the dynamic fatiguing task (time effect, $P < 0.001$; group effect, $P = 0.688$; time × group, $P = 0.03$). During recovery, both groups demonstrated increases in MVCC angular velocity after the dynamic fatiguing task (time effect; $P < 0.001$), however, people with T2D had lower MVCC angular velocity than controls throughout the recovery period (R05 & R20: group effect, $P = 0.012$) with no interaction (time × group, $P = 0.865$).

[Figure 2]

The reduction of MVCC power during the fatiguing task was not different between men and women (last 5 contractions: 29.2 ± 20.1% vs. 38.7 ± 16.8% reduction, respectively; time × sex, $P = 0.524$) for either group (time × group × sex, $P = 0.762$; sex effect, $P = 0.104$). During recovery (R05 & R20), the increase in MVCC power (time effect, $P < 0.001$) was similar for men and women (sex effect, $P = 0.634$; time × sex, $P = 0.473$; time × group × sex, $P = 0.276$).

Men and women demonstrated a similar reduction in MVCC velocity during the fatiguing task (last 5 contractions: 23.6 ± 18.8% vs. 31.9 ± 21.2% reduction, respectively; time × sex, $P =$

0.542; time × group × sex, $P = 0.621$) and similar recovery after the fatiguing task (time × sex, $P = 0.268$; time × group × sex, $P = 0.669$).

Duty Cycle: The duty cycle (work:rest ratio) was similar between people with T2D and controls during the first five dynamic contractions (group effect, $P = 0.146$). The duty cycle increased during the fatiguing task (due to slower contraction velocity), but this increase was similar between people with T2D and controls (time effect, $P = 0.031$; time × group, $P = 0.663$). The duty cycle was similar for men and women at the start of the fatiguing task (13.9 ± 1.8% vs. 14.6 ± 1.7%, respectively; sex effect, $P = 0.419$; group × sex, $P = 0.601$), and the increase in duty cycle at the end of the fatiguing task was similar (27.1 ± 19.5% vs. 28.4 ± 21.3% increase, respectively; time effect, $P < 0.001$; time × sex, $P = 0.903$).

Range of Motion: People with T2D and controls performed the concentric knee extension through a similar range of motion (baseline: 79.9 ± 9.0 vs. 80.2 ± 10.9 deg; group effect, $P = 0.898$) at the start of the fatiguing task, and the range of motion decreased similarly for both groups at the end of the fatiguing task (last 5 contractions: 74.1 ± 8.6 vs. 78.4 ± 10.8 deg; time effect, $P = 0.006$; time × group, $P = 0.137$). Men and women performed the concentric knee extension through a similar range of motion at the start of the fatiguing task (80.9 ± 8.3 vs. 79.0 ± 11.6 deg; sex effect, $P = 0.587$) and had similar reductions in range of motion (last 5 contractions: 76.5 ± 7.1 vs. 75.9 ± 12.5 deg; time × sex, $P = 0.711$; time × group × sex, $P = 0.974$).

Applied Torque: The peak applied torque during the concentric knee extension was similar for people with T2D compared with controls at the start of the fatiguing task (66.9 ± 24.8 vs. 62.7 ± 16.5 Nm, respectively; group effect, $P = 0.772$). Similarly, the average applied torque did not differ between the T2D and control groups (47.9 ± 19.0 vs. 43.4 ± 13.3 Nm, respectively; group effect, $P = 0.563$). The applied torque decreased during the dynamic fatiguing task more for people with T2D compared with healthy controls, for both the peak torque (19.5 ± 8.6% vs.

13.4 ± 10.3% reduction, respectively; time effect, $P < 0.001$; time × group, $P < 0.001$) and the average torque (17.3 ± 11.6% vs. 12.0 ± 8.9% reduction, respectively; time effect, $P < 0.001$; time × group, $P < 0.001$).

Because men were stronger than women, the peak applied torque (75.6 ± 21.6 vs. 62.7 ± 16.5 Nm, respectively; sex effect, $P = 0.001$) and the average applied torque (53.9 ± 16.9 Nm vs. 35.9 ± 8.7 Nm, respectively; sex effect, $P = 0.001$) during the concentric phase of the dynamic knee extension was greater for men at the start of the fatiguing task. Men and women had a similar reduction in both peak (14.5 ± 11.0% vs. 18.9 ± 7.8% reduction; time effect, $P < 0.001$; time × sex, $P = 0.136$) and average torque (12.9 ± 11.4% vs. 16.8 ± 9.3% reduction; time effect, $P < 0.001$; time × sex, $P = 0.236$) at the end of the fatiguing task.

MVIC Torque: The reduction in MVIC torque after the dynamic fatiguing contraction (time effect, $P < 0.001$) was greater in the T2D group than controls (time × group, $P = 0.04$; Figure 2B). MVIC torque increased during the 20 minutes of recovery (time effect, $P < 0.001$), and the increase was similar between the T2D and control groups (R05 & R20: group effect, $P = 0.120$; time × group, $P = 0.186$).

Men and women had similar reductions in MVIC torque after the dynamic fatiguing contraction (End Task: 31.5 ± 20.1% vs. 31.4 ± 9.6% reduction, respectively; time effect, $P < 0.001$; sex effect, $P = 0.917$; time × sex, $P = 0.995$; time × group × sex, $P = 0.725$). Men and women also had similar increases in MVIC torque during recovery (R05 & R20: time effect, $P < 0.001$; sex effect, $P = 0.774$; time × sex, $P = 0.951$; time × group × sex, $P = 0.110$). See Figure 2B.

Contractile Properties for the Electrically-Evoked Potentiated Twitch

Twitch Amplitude: The electrically-evoked potentiated twitch amplitude was reduced for all participants during and immediately after the fatiguing contraction (time effect, $P < 0.001$);

however, people with T2D had greater reductions than controls (time × group, $P = 0.010$). Similarly, the twitch amplitude increased during recovery (time effect, $P < 0.001$) but people with T2D recovered more slowly and the twitch was more depressed, even at 20 mins post exercise, compared with controls (R05 & R20: group effect, $P = 0.027$). See Figure 3A.

[Figure 3]

Men and women had similar reductions in potentiated twitch amplitude by the end of the fatiguing task for both the T2D and control groups (40.3 ± 27.6% vs. 39.8 ± 18.0% reduction; time effect, $P < 0.001$; sex effect, $P = 0.267$; time × sex, $P = 0.702$; time × group × sex, $P = 0.337$). During recovery, men and women demonstrated similar relative increases in potentiated twitch amplitude (R05 & R20: time effect, $P < 0.001$; sex effect, $P = 0.233$; time × sex, $P = 0.555$; time × group × sex, $P = 0.487$).

Half Relaxation Time: People with T2D and controls, both men and women, had similar increases in half relaxation time of the potentiated twitch after the fatiguing contraction (time effect, $P = 0.001$; sex effect, $P = 0.568$; time × group, $P = 0.511$; time × sex, $P = 0.368$; time × group × sex, $P = 0.982$; group effect, $P = 0.321$). During the 20-minutes of recovery (task end, and at 5 and 20 minutes post exercise), the half relaxation time decreased in all groups (time effect, $P = 0.002$; group effect, $P = 0.115$; sex effect, $P = 0.696$; time × group, $P = 0.458$; time × sex, $P = 0.440$; time × group × sex, $P = 0.747$).

Contraction Time: People with T2D and controls, both men and women, demonstrated no change in contraction time of the electrically-evoked potentiated twitch during the fatiguing task (time effect, $P = 0.377$; group effect, $P = 0.792$; sex effect, $P = 0.110$; time × group, $P = 0.564$; time × sex, $P = 0.212$; time × group × sex, $P = 0.717$), or during the 20-minute recovery (task end and at 5 and 20 minutes post exercise) (time effect, $P = 0.532$; group effect, $P = 0.717$; sex effect, $P = 0.126$; time × group, $P = 0.732$; time × sex, $P = 0.158$; time × group × sex, $P = 0.996$). See Table 2.

[Table 2]

Voluntary Activation

Voluntary Activation (Electrical Stimulation): Voluntary activation decreased in people with T2D and controls during the fatiguing contraction (End Task: 84.2 ± 9.3% vs. 86.4 ± 7.3%, respectively; time effect, $P < 0.001$), but this decrease did not differ between groups (time × group, $P = 0.840$; Figure 3B). Men and women showed similar reductions in voluntary activation by the end of the fatiguing contraction (87.5 ± 7.6% vs. 81.8 ± 8.1%, respectively; sex effect, $P = 0.456$; time × sex, $P = 0.247$; time × group × sex, $P = 0.506$). Voluntary activation remained depressed during the recovery period after the fatiguing task for all groups (time effect, $P = 0.408$; time × group, $P = 0.420$; time × sex, $P = 0.260$; time × group × sex, $P = 0.348$; sex effect, $P = 0.792$).

Superimposed Twitch Amplitude (TMS): The SIT increased (i.e. voluntary activation decreased) in both people with T2D and controls (time effect, $P = 0.015$) and this effect was similar for both groups (time × group, $P = 0.995$, Table 2) and for men and women across the groups (sex effect, $P = 0.490$, time × sex, $P = 0.625$; time × group × sex, $P = 0.717$). During the 20-minute recovery, the superimposed twitch amplitude decreased (voluntary activation increased) (time effect, $P = 0.039$), similarly for people with T2D and controls (time × group, $P = 0.600$, Table 2) and similarly for men and women (sex effect, $P = 0.944$; time × sex, $P = 0.146$; time × group × sex, $P = 0.443$).

EMG Response to Stimulation: M_{max}, MEP, Silent Period

Maximal compound muscle action potential (M_{max}): The M_{max} did not change during the fatiguing task for participants with T2D or controls for the rectus femoris (time effect, $P = 0.212$; time × group, $P = 0.176$; group effect, $P = 0.392$; group × sex, $P = 0.805$; time × sex, $P = 0.357$; time × group × sex, $P = 0.741$), vastus lateralis (time effect, $P = 0.697$; time × group, $P = 0.688$;

group effect, $P = 0.825$; group × sex, $P = 0.804$; time × sex, $P = 0.294$; time × group × sex, $P = 0.989$), or vastus medialis (time effect, $P = 0.403$; time × group, $P = 0.449$; group effect, $P = 0.885$; group × sex, $P = 0.278$; time × sex, $P = 0.187$; time × group × sex, $P = 0.503$). See Table 2.

The M_{max} did not change during the 20-minute recovery period for participants with T2D or controls for the rectus femoris (time effect, $P = 0.588$; time × group, $P = 0.628$; group effect, $P = 0.880$; group × sex, $P = 0.906$; time × sex, $P = 0.623$; time × group × sex, $P = 0.901$), vastus lateralis (time effect, $P = 0.653$; time × group, $P = 0.763$; group effect, $P = 0.727$; group × sex, $P = 0.803$; time × sex, $P = 0.830$; time × group × sex, $P = 0.973$), or vastus medialis (time effect, $P = 0620$; time × group, $P = 0.736$; group effect, $P = 0.997$; group × sex, $P = 0.254$; time × sex, $P = 0.940$ time × group × sex, $P = 0.157$). See Table 2.

Motor evoked potential (MEP): The MEP amplitude (%M_{max}) evoked during the MVC increased after the fatiguing task for the men and women with T2D and controls for the rectus femoris (time effect, $P = 0.001$; time × group, $P = 0.876$; group effect, $P = 0.422$; group × sex, $P = 0.910$; time × sex, $P = 0.955$; time × group × sex, $P = 0.142$) and vastus lateralis (time effect, $P = 0.037$; time × group, $P = 0.260$; group effect, $P = 0.949$; group × sex, $P = 0.252$; time × sex, $P = 0.324$; time × group × sex, $P = 0.231$), but not for the vastus medialis (time effect, $P = 0.139$; time × group, $P = 0.796$; group effect, $P = 0.777$; group × sex, $P = 0.747$; time × sex, $P = 0.144$; time × group × sex, $P = 0.728$). See Table 2.

The MEP amplitude (%M_{max}) reduced during recovery for men and women with T2D and controls for the rectus femoris (time effect, $P < 0.001$; time × group, $P = 0.156$; group effect, $P = 0.176$; group × sex, $P = 0.986$; time × sex, $P = 0.588$; time × group × sex, $P = 0.965$) and vastus lateralis (time effect, $P = 0.042$; time × group, $P = 0.521$; group effect, $P = 0.494$; group × sex, $P = 0.266$; time × sex, $P = 0.153$; time × group × sex, $P = 0.305$), but not for the vastus medialis

(time effect, $P = 0.126$; time × group, $P = 0.958$; group effect, $P = 0.726$; group × sex, $P = 0.859$; time × sex, $P = 0.678$; time × group × sex, $P = 0.952$).

Silent Period: The EMG silent period, assessed during the MVIC, increased during the fatiguing task for the rectus femoris (time effect, $P < 0.001$; time × group, $P = 0.615$; group effect, $P = 0.632$; group × sex, $P = 0.731$; time × sex, $P = 0.502$; time × group × sex, $P = 0.133$), vastus lateralis (time effect, $P = 0.001$; time × group, $P = 0.187$; group effect, $P = 0.393$; group × sex, $P = 0.803$; time × sex, $P = 0.406$; time × group × sex, $P = 0.245$) and vastus medialis (time effect, $P = 0.002$; time × group, $P = 0.103$; group effect, $P = 0.189$; group × sex, $P = 0.516$; time × sex, $P = 0.406$; time × group × sex, $P = 0.278$). See Table 2.

The EMG silent period decreased during recovery from the fatiguing task for men and women with T2D and controls for the rectus femoris (time effect, $P < 0.001$; time × group, $P = 0.800$; group effect, $P = 0.722$; group × sex, $P = 0.893$; time × sex, $P = 0.453$; time × group × sex, $P = 0.585$), vastus lateralis (time effect, $P = 0.002$; time × group, $P = 0.391$; group effect, $P = 0.447$; group × sex, $P = 0.660$; time × sex, $P = 0.275$; time × group × sex, $P = 0.368$), and vastus medialis (time effect, $P = 0.042$; time × group, $P = 0.249$; group effect, $P = 0.799$; group × sex, $P = 0.922$; time × sex, $P = 0.644$; time × group × sex, $P = 0.409$).

Associations

The following variables were associated with reductions in MVIC performed after the fatiguing task: the relative reduction in potentiated twitch amplitude ($r^2 = 0.364$, $P = 0.002$; Figure 4A), baseline MVIC torque ($r^2 = 0.140$, $P = 0.032$), HbA_{1c} ($r^2 = 0.145$, $P = 0.029$), fasting glucose ($r^2 = 0.130$, $P = 0.042$), and HOMA-IR ($r^2 = 0.126$, $P = 0.046$).

The following variables were associated with reductions in MVCC power at the end of the fatiguing task: estimated VO_2peak ($r^2 = 0.494$, $P <0.001$; Figure 4B), reduction in potentiated twitch amplitude ($r^2 = 0.345$, $P = 0.002$), HOMA-IR ($r^2 = 0.130$, $P = 0.042$), and HbA_{1c} ($r^2 = 0.154$, $P = 0.024$).

[Figure 4]

DISCUSSION

The novel findings of this study were that people with T2D were more fatigable for a high-velocity dynamic fatiguing task with the knee extensor muscles than healthy controls who were matched for age, BMI and physical activity, with no differences between men and women. People with T2D demonstrated greater reductions in MVCC power, MVIC torque and twitch amplitude after the dynamic fatiguing contraction compared with the healthy controls, indicating fatigability and impairments in muscle contractile properties were greater for people with T2D. Voluntary activation was reduced, and the superimposed twitch amplitude and EMG silent period increased after the dynamic fatiguing task, demonstrating reduced neural drive and possibly increased intracortical and spinal inhibition; however, these changes were similar for people with T2D and controls of both sexes. Thus, both muscular and neural mechanisms (including supraspinal fatigue) contributed to knee extensor fatigability of men and women after single limb dynamic exercise, however, contractile mechanisms were responsible for the greater fatigability of people with T2D compared with controls. Accordingly, the primary measures of fatigability, both the reduction in MVCC power and in the MVIC torque, were correlated with the reduction in potentiated twitch amplitude. Estimated maximal oxygen consumption (VO_2) at baseline and metabolic factors (HbA_{1c}, fasting plasma glucose and insulin) were also associated with reduction in MVCC power during the dynamic fatiguing task.

A strength of this study was that we designed it to understand the effects of T2D on fatigability of lower limb muscles, while controlling for confounding effects of age, diabetic polyneuropathy, daily physical activity levels, and participant anthropometrics, by excluding any patients with clinical signs of diabetic polyneuropathy and by matching groups based on age, physical activity, estimated aerobic fitness, and BMI. Additionally, people with T2D reported

similar daily levels of perceived fatigability, sleep quality and depression as the controls, indicating there was minimal influence of perceptions of fatigue that is often associated with advanced diabetes (15) and that may confound exercise-induced fatigue of the lower limb. These findings however may underestimate the group-related differences in fatigability and the contributing mechanisms may have been different if people with T2D who have diabetic polyneuropathy were included in the study. For example, after a 20-repetition, moderate-velocity (120 deg·s^{-1}) isokinetic fatiguing task with the knee extensors (28), there was a progressive, albeit not significant, increase in fatigability in people with T2D and diabetic polyneuropathy (37 ± 13% reduction of muscle work) compared with people with T2D and no signs of polyneuropathy (34 ± 13%) and healthy controls (30 ± 8%). Additionally, people with T2D and diabetic polyneuropathy demonstrated reduced motor unit number estimates, mean motor unit firing rates, and impaired neuromuscular propagation in upper and lower limb muscles compared to controls (4), which indicates impairments along the motor pathway from corticospinal centers to the interface of the nerve and muscle. These data provide a rationale for an increased role of central mechanisms contributing to fatigability of limb muscles in people with T2D and polyneuropathy, although this has not been examined.

Greater Fatigability in People with T2D

The greater fatigability of the knee extensors in people with T2D than controls was evidenced by markedly greater reductions in MVCC power (42.8% vs. 26.4% reduction) and MVIC torque at the end of the dynamic tasks (37.6% vs. 26.4% reduction) (Fig. 2). During the dynamic fatiguing task, there was a reduction in range of motion and rest time between contractions (increased duty cycle) but this was similar for both groups. However, the average applied torque declined more during the fatiguing task for people with T2D than the controls (17.3% vs. 12.0% reduction), thus, each MVCC required relatively less torque for participants

649 with T2D compared to controls at the end of the fatiguing task. Despite this, the participants with
650 T2D showed larger losses in power than controls. Thus, our study may have underestimated the
651 magnitude of the difference in loss of power between the groups by up to ~5%. These results are
652 consistent with previous research demonstrating greater fatigability for isometric contractions of
653 people with diabetes mellitus (Type 1 or Type 2) of the handgrip (37), dorsiflexor (3), and knee
654 extensor muscles (6). Importantly, our results clearly indicate that the knee extensor muscles are
655 more fatigable for dynamic contractions in people with T2D, although these results are not
656 consistent with that seen for low repetition (20 – 30 repetitions), moderate velocity (120 – 180
657 $deg \cdot s^{-1}$) isokinetic contractions for this muscle group (18, 28). The greater fatigability of people
658 with T2D in our study, but not others, could be due to faster contraction velocities or more
659 repetitions in our protocol. Close examination of the muscle power during the fatiguing task (Fig.
660 2A) demonstrates that the differences in fatigability between people with T2D and controls did
661 not become apparent until after ~60 repetitions. Thus, greater fatigability of people with T2D
662 may only occur with more repetitions or faster contraction velocities, and the magnitude of the
663 difference in fatigability between people with T2D and controls likely increases as a function of
664 exercise time.

665 A unique aspect of our study was that our cohort of T2D participants did not have
666 advanced stages of the disease, yet lower limb fatigue was greater than in controls matched for
667 age, BMI and physical activity. Many of the processes associated with advancing severity of
668 T2D will exacerbate fatigability of the lower limb even further, including diabetic
669 polyneuropathy (2) and loss of muscle mass (5), impaired microcirculation (37) and
670 cardiovascular disease. We showed however, that even prior to detectable clinical signs of
671 polyneuropathy and loss of muscle mass, people with T2D display greater fatigability of the knee
672 extensor muscles that are important for daily function, and as discussed below, was due to
673 contractile mechanisms.

674 ***Neural Mechanisms of Fatigability***

675 After the fatiguing task, there was a reduction in voluntary activation (assessed via
676 electrical stimulation) (Fig. 3B), an increase in superimposed twitch amplitude (assessed via
677 TMS), an increase in EMG silent period and a modest increase in MEP amplitude, each of which
678 were similar between the people with T2D and control (Table 2). The reduction in voluntary
679 activation elicited with electrical stimulation after the fatiguing task demonstrated a suboptimal
680 output from the motor pathway, between activation of the motor cortex and excitation of the α-
681 motor neuron (17). Because there was an increase in superimposed twitch amplitude elicited
682 with TMS during the MVIC, the reduced neural drive was in part due to a failure to generate
683 output from the motor cortex (51). However, this failure was similar across all groups, and thus
684 did not explain the difference in fatigability in the people with T2D (either the increased
685 reduction in the MVIC or power).

686 The increase in silent period reflects intracortical inhibition evoked by the TMS during
687 the maximal volitional effort which temporarily halts voluntary descending drive (49) and recent
688 data suggests the silent period may also reflect spinal inhibitory circuitry up to at least 150 ms
689 after the stimulation (52). Thus, among our participants, there was an increase in intracortical
690 inhibition, which involves the γ-aminobutyric acid ($GABA_B$) receptors (47), and possibly greater
691 spinal inhibition (52), but this increase in inhibition was similar across the groups. Although
692 there was a reduction in voluntary activation, there was a modest increase in the MEP amplitude
693 elicited by TMS observed in the rectus femoris and vastus lateralis muscles, indicating a net
694 increase in corticomotor excitability (48), in part due to an increase in cortical excitability,
695 increased spinal excitability or reduced corticospinal inhibition (29). An increase in MEP
696 amplitude is often observed with fatiguing exercise (e.g. (26)) and may reflect increased
697 descending drive despite a failure to increase the motor output. Despite the concomitant
698 increases in excitability and inhibition of the motor pathway during the fatiguing task, these

699 neural adjustments did not directly explain the greater fatigability in the men and women with
700 T2D compared with controls.

701 ***Contractile Mechanisms Primarily Explain Fatigability in People with T2D***

702 The reduction in MVCC power and MVIC torque were associated with the decline of the
703 electrically-evoked potentiated twitch amplitude, indicating muscle contractile mechanisms
704 largely explain (~35%) the greater fatigability of people with T2D. In both groups, a reduction
705 in twitch amplitude reflected fatigue in the muscle that may be due to disturbances in excitation-
706 contraction coupling, accumulation of metabolites, and/or impaired calcium handling (11, 14),
707 that ultimately reduce the torque that is able to be produced by the muscle fibers. Volitional and
708 electrically-evoked contractile function, and lean mass of the knee extensor muscles was not
709 different between the groups (T2D, control) at baseline, thus, baseline skeletal muscle
710 morphology and function likely did not contribute to greater fatigability in people with T2D.
711 However, there is evidence of contractile slowing and reduced muscle strength in people with
712 diabetes who have polyneuropathy (5).

713 There are several factors thought to affect the exercising muscle specifically in people
714 with diabetes which may contribute to the larger fatigue-related reductions in the twitch
715 amplitude, including: i) impaired neuromuscular transmission (3), ii) impaired calcium kinetics
716 and cross-bridge detachment, iii) impaired phosphorylation of myosin regulatory light chains (5),
717 and iv) motor unit loss (1, 4). Among this cohort of people with T2D who had no signs of
718 diabetic polyneuropathy, there was no reduction in M_{max} amplitude, providing evidence of
719 preserved integrity of the sarcolemma and neuromuscular junction propagation properties in our
720 cohort of men and women with T2D. There are relatively few examples of decreased M_{max}
721 amplitude after a fatiguing contraction; however, reduced M_{max} has been observed during
722 sustained isometric contractions of healthy young adults (first dorsal interosseous) (16) and in

people with type 1 diabetes and diabetic polyneuropathy (ankle dorsiflexors) (3). Additionally, there was a similar increase in half-relaxation time between groups in our study, indicating similar slowing of calcium reuptake into the sarcoplasmic reticulum and slowing of cross-bridge detachment in the skeletal muscle fibers. In addition, our data indicates that post-activation potentiation, assessed by comparing electrically-evoked twitches during a non-potentiated (no muscular effort within 30 s of the stimulation) and a potentiated state (MVIC performed within 2-s prior to evoked twitch), was similar between groups. Thus, there was probably similar phosphorylation of myosin regulatory light chains (7) between people with T2D and controls at baseline. Motor unit loss at baseline or impairments of active motor units in people with T2D may also underlie the greater impairments in contractile properties compared with controls. However, the people with T2D had no clinical signs of diabetic polyneuropathy and similar characteristics (age, strength, muscle mass and contractile properties) compared with controls, indicating no strong rationale for differences in motor unit numbers between the groups.

The greater reduction in knee extensor power across both groups, was associated with estimated fitness level, the gold standard indicator of glycemic control over the preceding two-to-three months (HbA_{1c}) and a proxy of insulin resistance (HOMA-IR). Although the T2D and controls groups were matched for fitness, participants with lower estimated fitness had greater fatigability during the dynamic fatiguing task, indicating that a lower capacity of the cardiovascular system (systemic blood flow and skeletal muscle oxygen delivery) may contribute to greater fatigability across both groups. The association of fatigability with HbA_{1c} and the HOMA-IR indicate that fatigability was greater in people with poorer glycemic control and greater insulin resistance. The greater insulin resistance (particularly in those with advanced T2D), may be associated with greater vascular constriction due to increased expression of endothelin-1 and reduced nitric oxide phosphorylation (38), resulting in reduced skeletal muscle blood flow during exercise and more perturbed metabolic milieu during exercise in people with

T2D compared to controls. For example, there is evidence of impaired potassium handling and calcium regulation (20), and increased lactate concentrations (19, 33) in people with T1D and T2D after exercise compared with controls. It is therefore probable that people with T2D and diabetic polyneuropathy or other complications of advanced T2D may have even more severe fatigability of lower limb muscles than evidenced among our cohort, and these associations warrant further investigation.

No Sex Differences in Fatigability with T2D

A unique finding of this study was there were no sex-related differences in fatigability of the knee extensor muscles in a middle-to-older aged cohort of healthy controls or people with T2D for a fast velocity dynamic fatiguing task. Typically, there are sex differences in fatigability for isometric and slow-to-moderate velocity fatiguing tasks, particularly in the upper limb in young healthy and older adults (24, 25). However, the magnitude of the sex differences in fatigability of young and old adults was diminished for fast-velocity fatiguing contraction tasks with both the elbow flexor and knee extensor muscles (42), and we found this to be the case in the middle-to-older aged adults in this study. We also observed no sex difference in the reduction in the MVIC measured immediately after the dynamic tasks and during recovery. However, in several other studies, men showed greater reductions than women in the MVIC immediately after the dynamic fatiguing contraction (40-42). The mechanism for the sex difference in the slower recovery of the men than the women in that study was due to contractile mechanisms with no sex difference in reductions in voluntary activation (40). The sex difference in fatigability can diminish for older adults for both isometric tasks and dynamic tasks (24, 25, 45). The lack of sex difference in fatigability between our current cohorts could be due to the age of our participants, whose average age was 60 years, which is older compared to previous reports demonstrating sex differences (40, 41). The lack of sex differences in fatigability within our

cohort could be secondary to our *a priori* participant matching criteria, including similar estimated maximal aerobic capacity (VO_2 peak). Women are expected to have a lower maximal aerobic capacity than men due to a number of physiological factors including smaller hearts, less haemoglobin, greater body fat (24, 27); thus, the women in our cohort could be relatively more fit than the men.

Conclusion

Men and women with T2D who exhibited no clinical signs of diabetic polyneuropathy, were more fatigable during and in recovery from a fast-velocity dynamic fatiguing task with the knee extensors muscles than controls without diabetes who were matched for age, body mass index, and physical activity. This difference in fatigability occurred when measured as a loss of power and the reduction of MVIC torque. Furthermore, there was no sex-based differences in fatigability for the people with T2D and controls. The greater fatigability was associated with glycemic control and contractile mechanisms, with no observed impairments in neuromuscular transmission. Although neural mechanisms of fatigability contributed to reductions in knee extensor power, the lower neural drive was moderate relative to the larger contribution of contractile mechanisms that explained the greater fatigability of the lower limb in the men and women with T2D.

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Author Contributions

J.S., A.R.H., and S.K.H. conceived and designed research; J.S., A.H. and A.R.H. performed experiments; J.S. and A.R.H. analyzed data; J.S., S.B.M., A.H., A.R.H., and S.K.H. interpreted results of experiments; J.S., A.R.H., and S.K.H. drafted manuscript; J.S., S.B.M., A.H., A.R.H., and S.K.H. edited and revised manuscript; J.S., S.B.M., A.H., A.R.H., and S.K.H. approved final version of manuscript; J.S. and S.K.H. prepared figures.

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	Units	Type 2 Diabetes *(n=17; 10 men)*	Control *(n = 21, 11 men)*
Age	years	59.6 ± 9.0	59.5 ± 9.6
BMI	$kg \cdot m^{-2}$	29.4 ± 7.0	27.2 ± 4.3
Body Fat	%	36.2 ± 13.8	32.4 ± 7.2
Duration of Diabetes	years	6.83 ± 6.45	0 *
HbA1c	%	7.04 ± 1.11	5.41 ± 0.23 *
Fasting Plasma Glucose	$mg \cdot dL^{-1}$	126.1 ± 32.1	87.1 ± 6.6 *
Fasting Plasma Insulin	pMol	59.1 ± 25.7	35.1 ± 17.5 *
HOMA-IR	AU	3.08 ± 1.71	1.28 ± 0.63 *
Estimated VO_2 Peak	mL/kg/min	27.9 ± 8.3	30.1 ± 7.8
Leg Muscle Mass	kg	8.22 ± 1.75	8.52 ± 2.36
Daily Step Count	n	8334 ± 3446	8295 ± 3218
Questionnaires			
PSQI	AU	4.19 ± 2.61	4.90 ± 2.41
FIS total	AU	24.18 ± 29.65	7.19 ± 14.91
FIS Cognitive	AU	6.38 ± 7.43	3.62 ± 4.42
FIS Physical	AU	7.63 ± 8.02	4.62 ± 5.84
FIS Psychological	AU	11.69 ± 15.53	6.67 ± 11.03
GDS	AU	2.00 ± 0	1.95 ± 0.22

Table 1: Participant characteristics and questionnaire scores. Values are displayed as mean ± SD. BMI, body mass index; HbA_{1c}, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; AU, arbitrary unit; PSQI, Pittsburgh Sleep Quality Index; FIS, Fatigue Impact Scale; GDS, Geriatric Depression Scale. (* denotes group difference between controls and T2D, $P < 0.05$)

		Type 2 Diabetes (*n = 17)*			Control (*n = 21*)		
		Baseline	Task End	Δ (%)	Baseline	Task End	Δ (%)
MVCC Power	Watts	291 ± 139	163 ± 96	-42.8 ± 24.2†	261 ± 85	199 ± 90	-26.4 ± 15.0†*
MVCC Velocity	$deg \cdot s^{-1}$	330 ± 62	209 ± 74	-36.2 ± 4.5†	333 ± 37	273 ± 61	-18.9 ± 4.6†*
Duty Cycle	%	13.7 ± 1.2	18.0 ± 2.0	+29.5 ± 20.5†	14.5 ± 2.1	18.1 ± 2.8	+25.8 ± 19.7†
MVIC Torque	Nm	176 ± 80	105 ± 46	-37.6 ± 18.2†	160 ± 59	115 ± 45	-26.4 ± 12.1†*
Twitch Amplitude	Nm	41.1 ± 16.8	21.2 ± 10.1	-44.0 ± 20.4†	43.4 ± 20.1	31.1 ± 12.0	-35.4 ± 20.0†*
Contraction Time	ms	83.1 ± 7.0	80.5 ± 14.0	NS	83.7 ± 10.0	82.4 ± 11.5	NS
Half-relaxation time	ms	70.2 ± 20.7	87.8 ± 30.0	+28.0 ± 12.7†	64.2 ± 18.4	74.1 ± 31.2	+27.4 ± 9.5†
VA (ES)	%	93.4 ± 3.4	84.2 ± 9.3	-12.1 ± 2.6†	92.8 ± 5.0	86.4 ± 7.3	-12.4 ± 4.4†
SIT	% MVIC	2.71 ± 1.72	4.76 ± 2.79	+55.8 ± 41.1†	3.19 ± 1.72	5.04 ± 5.73	+47.7 ± 47.2†
PAP	%	63.2 ± 51.8			74.1 ± 47.3		
M_{max} Amplitude							
RF	mV	4.88 ± 1.35	5.13 ± 1.32	NS	4.67 ± 0.78	4.47 ± 0.63	NS
VL	mV	6.40 ± 2.40	5.59 ± 1.27	NS	5.88 ± 2.30	5.70 ± 2.18	NS
VM	mV	7.16 ± 3.40	7.52 ± 3.86	NS	6.82 ± 2.20	6.77 ± 1.20	NS
MEP Amplitude							
RF	$\%M_{max}$	39.5 ± 24.0	54.0 ± 33.2	+50.0 ± 71.0†	49.3 ± 38.6	70.9 ± 44.5	+36.5 ± 59.6†
VL	$\%M_{max}$	38.2 ± 24.1	42.8 ± 24.7	+11.3 ± 34.3†	29.0 ± 12.6	40.9 ± 24.5	+27.5 ± 37.1†
VM	$\%M_{max}$	47.1 ± 28.9	53.6 ± 37.1	NS	47.7 ± 29.8	52.2 ± 37.4	NS
EMG Silent Period							
RF	ms	149 ± 116	233 ± 167	+59.5 ± 71.2†	134 ± 41	203 ± 96	+61.0 ± 91.4†
VL	ms	150 ± 112	252 ± 169	+70.5 ± 74.7†	138 ± 58	189 ± 94	+47.3 ± 77.0†
VM	ms	151 ± 112	249 ± 169	+66.5 ± 62.3†	134 ± 36	166 ± 94	+47.1 ± 67.3†

Table 2: Baseline Muscle Function Before and After the Dynamic Fatiguing Contraction in People with T2D and Age-and Physical Activity-Matched Healthy Controls without T2D. Values are displayed as mean ± SD. The relative reduction (%) shown is from baseline to immediately after the fatiguing tasks (Task End). People with T2D demonstrated greater reductions in MVCC power, MVIC torque, and potentiated twitch amplitude compared with healthy controls. (* denotes group difference between controls and T2D, $P < 0.05$; † denotes difference between baseline and task end, $P < 0.05$).

Abbreviations: MVCC, maximal voluntary concentric contraction; MVIC, maximal voluntary isometric contraction; VA, voluntary activation; ES, electrical stimulation; SIT, superimposed twitch; PAP, post-activation potentiation; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; M_{max}, maximal compound muscle action potential; NS, not statistically significant.

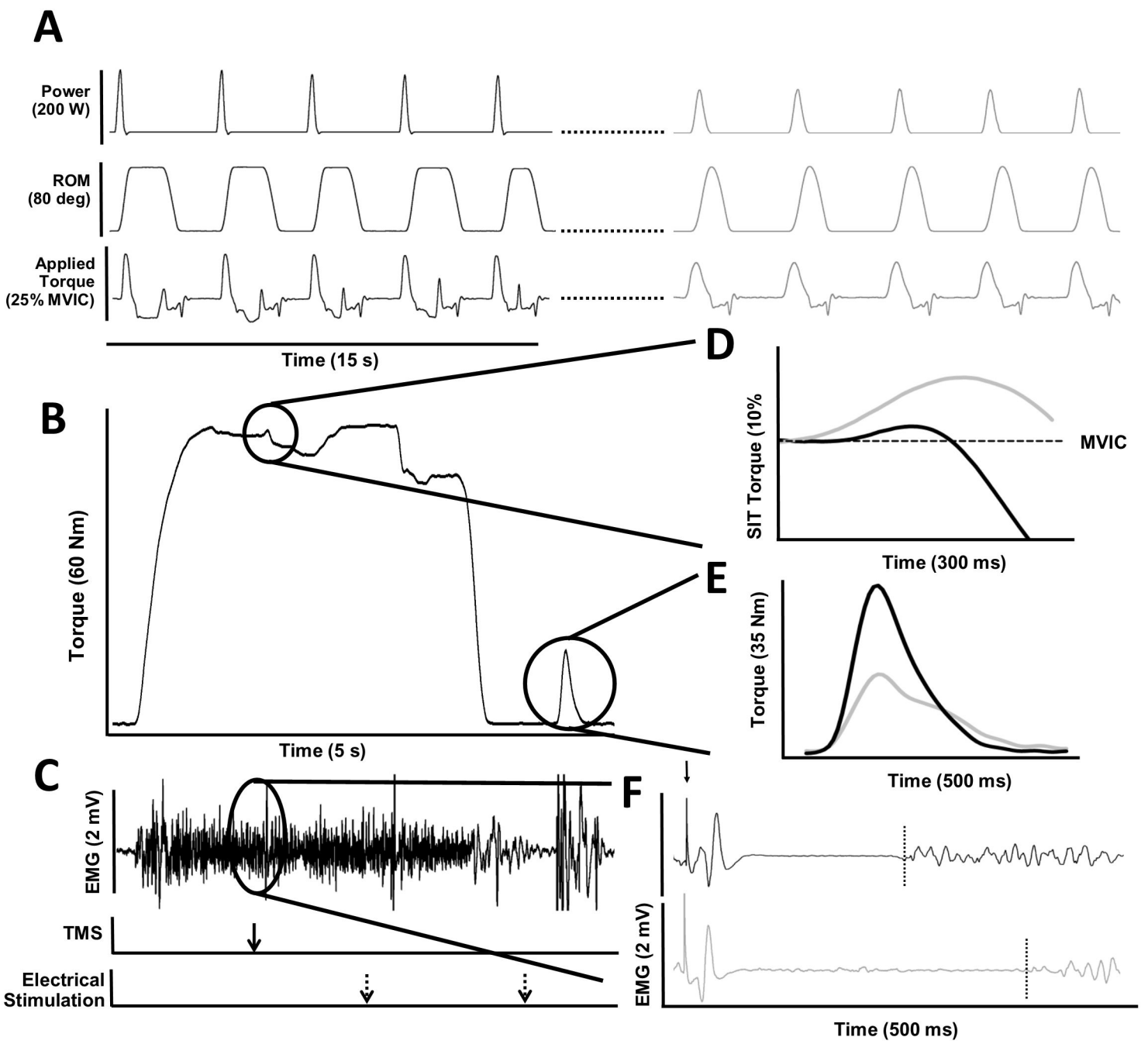
FIGURE LEGENDS

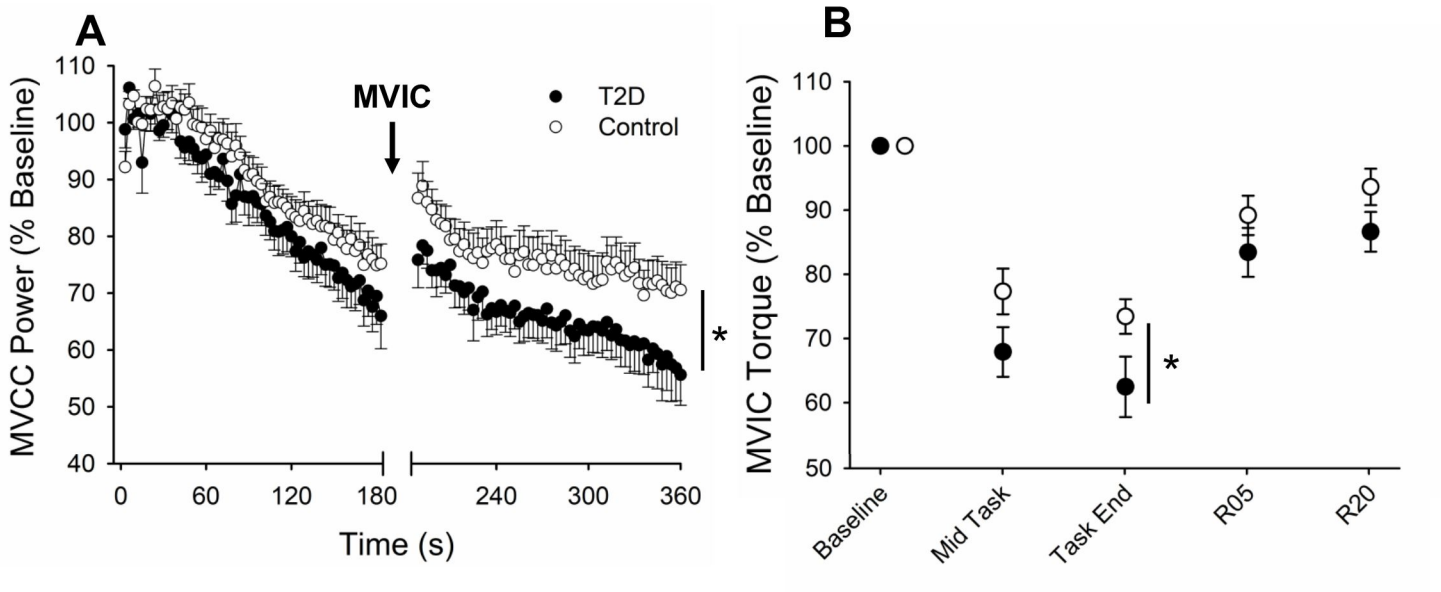
Figure 1: Representative data for maximal voluntary concentric contraction (MVCC) power, range of motion and applied torque, maximal voluntary isometric contraction (MVIC) torque, superimposed twitch (SIT) torque, potentiated twitch, motor evoked potential (MEP) and EMG silent period. *A.* Calculated power (applied torque × half-wave rectified velocity), range of motion and applied torque signals of a 62-year old control woman performing five MVCCs at the start (black lines) and end (grey lines) of the fatiguing task. The torque (***B***) and EMG (***C***) signal of the woman performing an MVIC with TMS-elicited SIT during the MVIC and electrical stimulation evoked twitches during the MVIC and at rest. The TMS-elicited SIT (***D***), electrically-evoked potentiated twitch (***E***) torque, and vastus lateralis EMG (***F***) signal displaying the MEP and EMG silent period from before (black line) and after the fatiguing task (grey line).

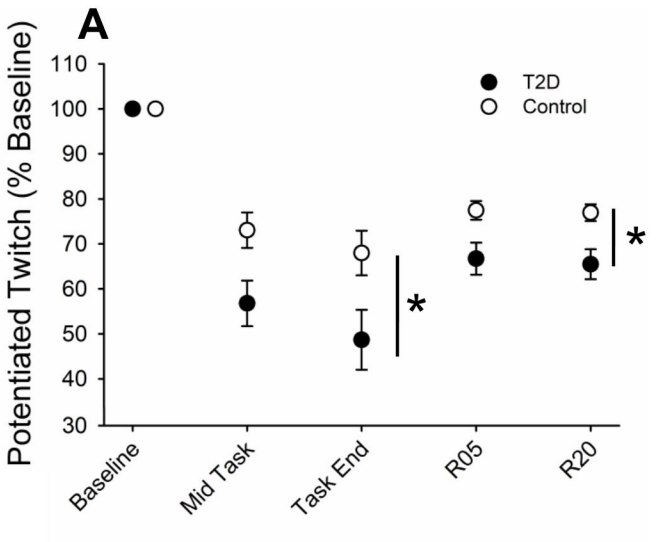
Figure 2: Fatigability of the maximal voluntary concentric contraction (MVCC) power (% baseline) (*A*) and maximal voluntary isometric contraction (MVIC) torque (% baseline) (*B*) in response to a dynamic fatiguing task. Values are displayed as mean ± SEM. ***A.*** The T2D group had greater reductions in the mean MVCC power (% baseline power of the mean of first 5 contractions) than controls by the last five contractions of the dynamic fatiguing task. Recovery of power during the MVCCs at 5 min (R05) and 20 mins (R20) was less for the T2D than control group. ***B.*** MVIC torque (% baseline) declined more for the T2D than the control group by the end of the dynamic fatiguing task (Task End). Recovery of MVIC was similar between people with T2D and controls for MVIC torque up to 20 mins after the fatiguing task (R20). (* group differences at $P < 0.05$).

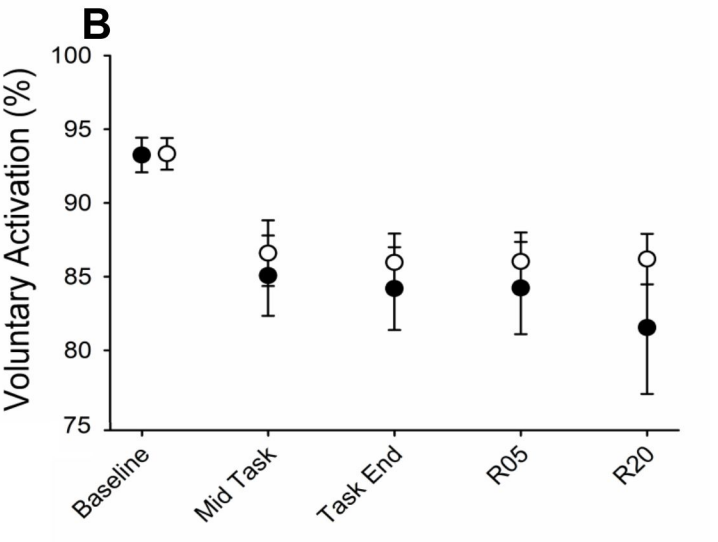
Figure 3: Electrically-evoked potentiated twitch amplitude (A) and voluntary activation (B) during and after the dynamic fatiguing task. Values are displayed as mean ± SEM. ***A.*** The electrically-evoked potentiated twitch amplitude (% baseline) was reduced more for the T2D group than controls and remained depressed during the 20 mins recovery ($P < 0.05$). ***B.*** *V*oluntary activation (assessed with electrical stimulation) declined in both people with T2D and controls ($P < 0.05$) but did not differ between groups ($P > 0.05$).

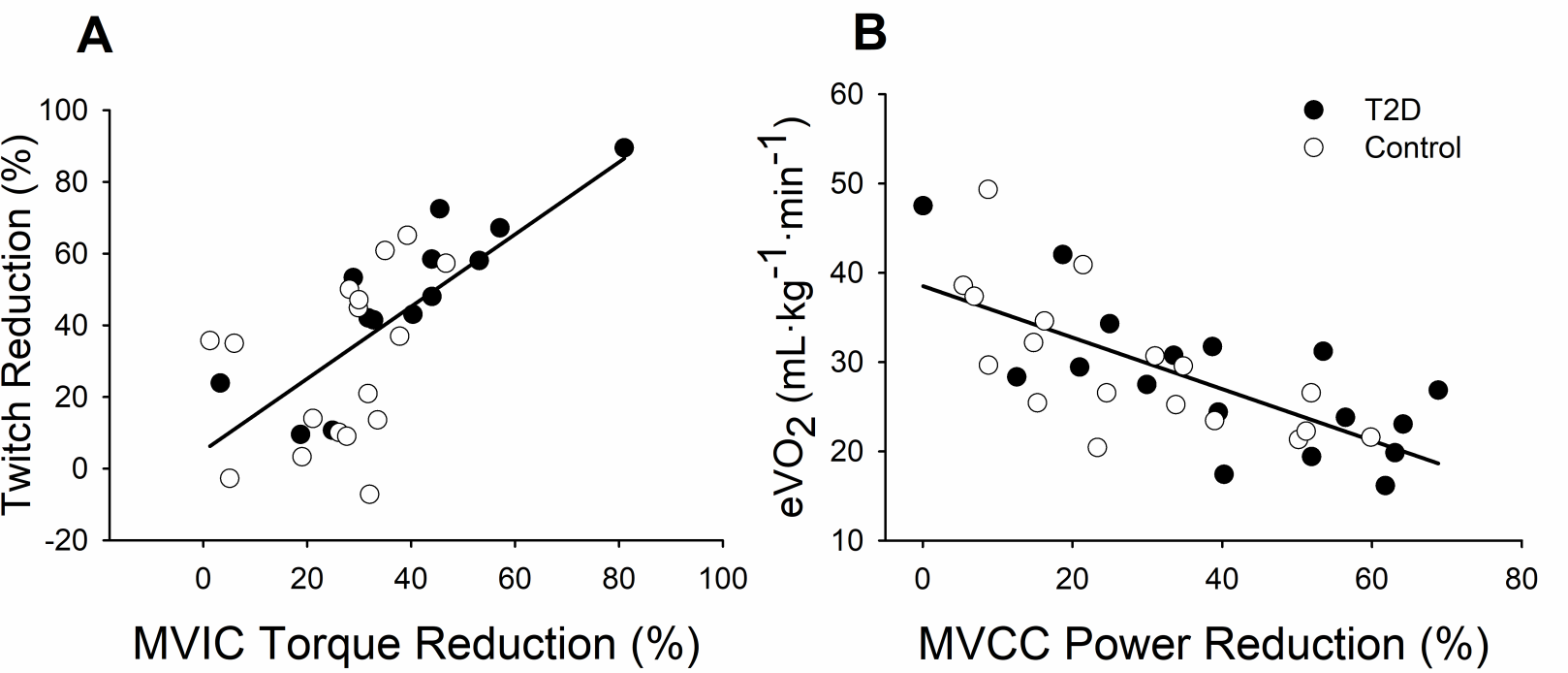
Figure 4: Associations with fatigability. *A.* The reduction in MVIC torque (%) was associated with the reduction in potentiated twitch amplitude (%) (***A***; $r = 0.603$, $r^2 = 0.364$, $P = 0.002$). ***B***. The reduction in MVCC power (%) was associated with estimated peak aerobic capacity (eVO_2) (***B***; $r = -0.703$, $r^2 = 0.494$, $P < 0.001$).


A
Power (200 W)
ROM (80 deg)
Applied Torque (25% MVIC)
Time (15 s)
B
Torque (60 Nm)
Time (5 s)
C
EMG (2 mV)
TMS
Electrical Stimulation
D
SIT Torque (10%
MVIC
Time (300 ms)
E
Torque (35 Nm)
Time (500 ms)
F
EMG (2 mV)
Time (500 ms)


A
MVCC Power (% Baseline)
110
100
90
80
70
60
50
40
MVIC
T2D
Control
*
0
60
120
180
240
300
360
Time (s)
B
MVIC Torque (% Baseline)
110
100
90
80
70
60
50
*
Baseline
Mid Task
Task End
R05
R20


A
Potentiated Twitch (% Baseline)
110
100
90
80
70
60
50
40
30
T2D
Control
*
*
Baseline
Mid Task
Task End
R05
R20


B
Voluntary Activation (%)
100
95
90
85
80
75
Baseline
Mid Task
Task End
R05
R20


A
B
Twitch Reduction (%)
MVIC Torque Reduction (%)
eVO2 (mL·kg-1·min-1)
MVCC Power Reduction (%)
T2D
Control
100
80
60
40
20
0
-20
0
20
40
60
80
100
60
50
40
30
20
10
0
20
40
60
80