



# Normal to enhanced intrinsic mitochondrial respiration in skeletal muscle of middle- to older-aged women and men with uncomplicated type 1 diabetes

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## Abstract

**Aims/hypothesis** This study interrogated mitochondrial respiratory function and content in skeletal muscle biopsies of healthy adults between 30 and 72 years old with and without uncomplicated type 1 diabetes.

**Methods** Participants (12 women/nine men) with type 1 diabetes ( $48 \pm 11$  years of age), without overt complications, were matched for age, sex, BMI and level of physical activity to participants without diabetes (control participants) ( $49 \pm 12$  years of age). Participants underwent a Bergström biopsy of the *vastus lateralis* to assess mitochondrial respiratory function using high-resolution respirometry and citrate synthase activity. Electron microscopy was used to quantify mitochondrial content and cristae (pixel) density.

**Results** Mean mitochondrial area density was 27% lower ( $p = 0.006$ ) in participants with type 1 diabetes compared with control participants. This was largely due to smaller mitochondrial fragments in women with type 1 diabetes ( $-18\%$ ,  $p = 0.057$ ), as opposed to a decrease in the total number of mitochondrial fragments in men with diabetes ( $-28\%$ ,  $p = 0.130$ ). Mitochondrial respiratory measures, whether estimated per milligram of tissue (i.e. mass-specific) or normalised to area density (i.e. intrinsic mitochondrial function), differed between cohorts, and demonstrated sexual dimorphism. Mass-specific mitochondrial oxidative phosphorylation (OXPHOS) capacity with the substrates for complex I and complex II ( $C_{I+II}$ ) was significantly lower ( $-24\%$ ,  $p = 0.033$ ) in women with type 1 diabetes compared with control participants, whereas mass-specific OXPHOS capacities with substrates for complex I only (pyruvate [ $C_{I\text{ pyr}}$ ] or glutamate [ $C_{I\text{ glu}}$ ]) or complex II only (succinate [ $C_{II\text{ succ}}$ ]) were not different ( $p > 0.404$ ). No statistical differences ( $p > 0.397$ ) were found in mass-specific OXPHOS capacity in men with type 1 diabetes compared with control participants despite a 42% non-significant increase in  $C_{I\text{ glu}}$  OXPHOS capacity ( $p = 0.218$ ). In contrast, intrinsic  $C_{I+II}$  OXPHOS capacity was not different in women with type 1 diabetes ( $+5\%$ ,  $p = 0.378$ ), whereas in men with type 1 diabetes it was 25% higher ( $p = 0.163$ ) compared with control participants. Men with type 1 diabetes also demonstrated higher intrinsic OXPHOS capacity for  $C_{I\text{ pyr}}$  ( $+50\%$ ,  $p = 0.159$ ),  $C_{I\text{ glu}}$  ( $+88\%$ ,  $p = 0.033$ ) and  $C_{II\text{ succ}}$  ( $+28\%$ ,  $p = 0.123$ ), as well as higher intrinsic respiratory rates with low (more physiological) concentrations of either ADP, pyruvate, glutamate or succinate ( $p < 0.012$ ). Women with type 1 diabetes had higher ( $p < 0.003$ ) intrinsic respiratory rates with low concentrations of succinate only. Calculated aerobic fitness (Physical Working Capacity Test [PWC<sub>130</sub>]) showed a strong relationship with mitochondrial respiratory function and content in the type 1 diabetes cohort.

**Conclusions/interpretation** In middle- to older-aged adults with uncomplicated type 1 diabetes, we conclude that skeletal muscle mitochondria differentially adapt to type 1 diabetes and demonstrate sexual dimorphism. Importantly, these cellular alterations were significantly associated with our metric of aerobic fitness (PWC<sub>130</sub>) and preceded notable impairments in skeletal mass and strength.

**Keywords** Aerobic fitness · Mitochondria · Older adults · Oxidative phosphorylation · Skeletal muscle · Type 1 diabetes

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## Research in context

### What is already known about this subject?

- Ageing adults with diabetes are at greater risk of geriatric syndromes, particularly those implicated in skeletal muscle dysfunction
- Mounting evidence indicates that type 1 diabetes may cause deficits in skeletal muscle mitochondrial function early in life (and during disease progression)

### What is the key question?

- Do abnormalities in skeletal muscle mitochondria exist in relatively healthy adults with type 1 diabetes who are beyond young adulthood?

### What are the new findings?

- Middle- to older-aged adults with uncomplicated type 1 diabetes demonstrate alterations to mitochondrial oxidative phosphorylation capacity, substrate sensitivity and ultrastructure, including sexual dimorphism
- Mitochondrial content was lower in the type 1 diabetes cohort despite comparable body composition and calculated aerobic fitness
- Compared with matched counterparts, intrinsic mitochondrial oxidative phosphorylation capacity and respiratory response to low concentrations of ADP and complex I substrates were generally normal in women, but enhanced in men, with type 1 diabetes, and this was positively correlated with our metric of aerobic fitness in both sexes

### How might this impact on clinical practice in the foreseeable future?

- Considering the well-demonstrated importance of mitochondria to muscle health, and the well-established role of muscle in our overall well-being, the findings in this study cohort further stress not only the importance of aerobic fitness in those with type 1 diabetes, but also the need to identify appropriate exercise guidelines that are sex-specific

## Abbreviations

$C_{I+II}$	Complex I and complex II
$C_{I\text{ glu}}$	Complex I-supported respiration stimulated by glutamate
$C_{I\text{ pyr}}$	Complex I substrate respiration stimulated by pyruvate
$C_{II\text{ succ}}$	Complex II substrate respiration stimulated by succinate
CS	Citrate synthase
HR	Heart rate
LBM	Lean body mass
LEAK	State II respiration
MVC	Maximal voluntary contraction
OXPHOS	Oxidative phosphorylation
PGC1 $\alpha$	Peroxisome-proliferator-activated receptor- $\gamma$ coactivator-1 $\alpha$
PmFBs	Permeabilised muscle fibres
PWC <sub>130</sub>	Physical Working Capacity Test at HR of 130 beats/min
STZ	Streptozotocin
TEM	Transmission electron microscopy

## Introduction

For the first time in history, people with type 1 diabetes on intensive insulin therapy are living into the later decades of life [1, 2]. However, ageing adults with diabetes are at higher risk for the occurrence of geriatric syndromes, particularly those associated with deteriorations in skeletal muscle, including frailty [3], falls/fractures [4, 5] and functional disability [6, 7]. These occurrences lead to decreased independence/quality of life [8], higher rates of institutionalisation [9], additional comorbidities [8] and, consequently, premature mortality [9]. The cellular mechanisms underlying deficits to skeletal muscle in type 1 diabetes, particularly in older age, remain largely unknown.

Mitochondrial dysfunction has long been considered to be a primary mediator of age-related muscle decline [10]. In older people without diabetes, declines in *in vivo* mitochondrial oxidative capacity are associated with reduced gait speed [11], muscle strength and quality [12], resting metabolic rate [13] and insulin sensitivity [14]. Though considerable variability in mitochondrial (dys)function exists amongst adults

of the same age (predominantly due to variability in aerobic fitness), the literature generally supports an age-dependent downward trend in skeletal muscle mitochondrial function and content [15, 16].

Evidence exists that mitochondrial dysfunction occurs early in type 1 diabetes, and, hence, at a considerably earlier age in life than in those without type 1 diabetes (reviewed in [17, 18]). We recently reported a decrease in skeletal muscle mitochondrial respiratory function in physically active young adults with type 1 diabetes, despite no change in mitochondrial content [19], suggesting intrinsic mitochondrial deficiencies. However, the deficits in mitochondrial oxidative capacity may not be a universal phenomenon in those with type 1 diabetes as some have reported no differences between control groups and young adults with type 1 diabetes [20–22].

With an ageing population of adults with type 1 diabetes on the horizon, understanding whether deficits in muscle mitochondria exist, persist or worsen beyond young adulthood is imperative. Therefore, we employed a cross-sectional study interrogating mitochondrial respiratory function and ultrastructure in skeletal muscle biopsies of adults between 30 and 72 years of age with uncomplicated type 1 diabetes. We hypothesised that women and men with uncomplicated type 1 diabetes have reduced muscle mitochondrial respiratory function and content compared with matched control groups.

## Methods

### Recruitment and participant characteristics

Female and male participants (hereafter referred to as women and men) with uncomplicated type 1 diabetes between 30 and 72 years old were recruited from the McMaster University campus area and surrounding cities in Southwestern Ontario, Canada. Type 1 diabetes participants were closely matched with participants without diabetes (control group) for age ( $\pm 4$  years), sex (self-identified male or female), BMI ( $\pm 4$  kg/m<sup>2</sup>) and physical activity levels. The latter was assessed by participants' self-reported leisure physical activity type and time. We excluded people diagnosed with type 2 diabetes, statin-induced myopathy or myalgia, heart disease, severe hypertension, stage 3 neuropathy or severe retinopathy; who used assistive walking devices; who used corticosteroids and NSAIDs (non-steroidal anti-inflammatory drugs); and who smoked or used tobacco products. The study was reviewed and approved by the Hamilton Integrated Research Ethics Board (HiREB #5344), in accordance with the Declaration of Helsinki. All participants ( $n = 42$ ) gave verbal and written informed consent.

Participant characteristics are presented in Table 1, including the Physical Working Capacity Test at a heart rate (HR) of 130 beats/min (PWC<sub>130</sub>) and maximal voluntary contraction

(MVC). There were no significant differences in any characteristics except for HbA<sub>1c</sub>, which was significantly higher in individuals with type 1 diabetes compared with control participants. A neurological examination of the peripheral nervous system by a neuromuscular clinician revealed similar scores between groups, except for ~20% of participants with type 1 diabetes, who had evidence of a mild sensory neuropathy (decreased vibration more than mild and/or absent ankle reflexes). No study participants demonstrated severe or even moderate peripheral neuropathy by examination, nor was there any evidence of proximal limb motor deficit.

In both the control and type 1 diabetes groups, ~63% of women were premenopausal ( $n = 7/8$  for control/type 1 diabetes), and ~37% of women were postmenopausal ( $n = 4$  each group). In the control group, 43% reported using hormonal contraceptives (birth control or intrauterine device) compared with 50% in the type 1 diabetes group ( $n = 3$  and 4, respectively). Postmenopausal women ( $n = 5/4$  for control/type 1 diabetes) did not declare use of hormone replacement therapy except for one in the type 1 diabetes group. Menstrual cycle was not controlled for in this study.

Characteristics specific to the type 1 diabetes cohort are presented in Table 2. On average, diabetes duration was 28 ( $\pm 13$ ) years and age of diagnosis was 19 ( $\pm 10$ ) years. Diabetes duration was significantly longer ( $p = 0.027$ ) in men ( $36 \pm 11$  years) than women ( $23 \pm 13$  years). Two participants self-reported symptoms of diabetes-related complications (neuropathy [ $n = 1$ ] and mild retinopathy/neuropathy [ $n = 1$ ], respectively), though neurological examination by a neuromuscular clinician did not confirm these self-reports, as mentioned earlier.

### Study protocol

Participants visited the laboratory twice with 7–9 days between visits. All participants were instructed to consume a standardised meal 1.5–2 h prior to their visits and refrain from caffeine, alcohol and moderate to vigorous exercise for at least 24 h before each visit.

**Visit 1** Body height and weight were recorded to calculate BMI while body composition was assessed via dual-energy x-ray absorptiometry (DEXA; GE Healthcare, Toronto, ON, Canada). Muscle strength was assessed by isometric MVC of the right leg (unless otherwise specified) using an isokinetic dynamometer (Biodex System 3; Biodex Medical Systems, Shirley, NY, USA). Participants were instructed to contract their quadriceps isometrically as hard as they could for 5 s and to rest for 30 s. This was repeated three times with the highest recorded value used. Aerobic fitness was assessed by the PWC<sub>130</sub> cycling test. Participants were provided with a wrist-based HR watch (Polar M430, Polar, USA) and instructed to cycle at a cadence between 50 and 70

**Table 1** Participant characteristics

Characteristic	Combined		Women		Men	
	CON (N=21)	T1D (N=21)	CON (n=12)	T1D (n=12)	CON (n=9)	T1D (n=9)
Age (years)	49 ± 12	48 ± 11	47 ± 10	45 ± 10	51 ± 15	52 ± 13
Weight (kg)	75.8 ± 12.9	78.3 ± 16.3	71.5 ± 14.1	73.2 ± 16.2	81.4 ± 9.0	85.2 ± 14.4
Height (cm)	169 ± 12	170 ± 8	162 ± 10	166 ± 6	179 ± 5	176 ± 7
BMI (kg/m <sup>2</sup> )	26.4 ± 3.3	26.9 ± 4.7	27.1 ± 3.7	26.5 ± 5.6	25.5 ± 2.5	27.5 ± 3.4
Lean mass (kg)	49.2 ± 11.9	49.8 ± 10.4	40.5 ± 6.0	42.8 ± 5.0	60.7 ± 6.5	59.1 ± 8.2
Lean mass (% BW)	64.9 ± 11.3	64.6 ± 10.1	57.9 ± 8.4	60.8 ± 10.6	74.2 ± 7.1	69.7 ± 6.9
Fat mass (kg)	23.7 ± 10.5	25.3 ± 11.6	28.1 ± 10.3	26.9 ± 13.4	17.9 ± 7.9	23.2 ± 8.9
Fat mass (% BW)	64.9 ± 11.3	64.6 ± 10.1	57.9 ± 8.4	60.8 ± 10.6	74.2 ± 7.1	69.7 ± 6.9
HbA <sub>1c</sub> (mmol/mol)	33.8 ± 4.0	56.9 ± 8.3***	34.5 ± 2.9	57.1 ± 7.6***	32.8 ± 5.2	56.8 ± 9.5***
HbA <sub>1c</sub> (%)	5.2 ± 0.4	7.4 ± 0.8***	5.3 ± 0.3	7.4 ± 0.7***	5.2 ± 0.5	7.3 ± 0.9***
PWC <sub>130</sub> (watts)	127.3 ± 62.9	104.3 ± 49.8	94.6 ± 38.3	87.4 ± 41.5	167.2 ± 65.5	133.3 ± 52.4 <sup>a</sup>
PWC <sub>130</sub> (watts/LM)	2.5 ± 0.8	2.2 ± 0.9	2.2 ± 0.7	2.1 ± 1.0	2.7 ± 1.0	2.3 ± 0.8
MVC (N×m)	226 ± 68	212 ± 78	186 ± 48	175 ± 47	279 ± 53	261 ± 85
MVC (N×m/leg LM)	26.1 ± 3.6	24.9 ± 5.5	26.3 ± 3.2	24.1 ± 5.2	25.8 ± 4.3	26.0 ± 5.9

Data are presented as mean ± SD

All variables were measured and/or calculated except for age, which was based on participant self-report

An unpaired, two-tailed Student's *t* test was used to compare variables between the control and type 1 diabetes groups in all the participants (combined) as well as within each respective sex (women and men, respectively)

<sup>a</sup>Methodological issues with HR monitors caused a loss of data from two male participants with type 1 diabetes for this measurement (*n* = 7 for T1D men)

\*\*\**p* < 0.001 CON vs T1D

BW, body weight; CON, control; LM, lean mass; T1D, type 1 diabetes

**Table 2** Characteristics specific to the type 1 diabetes participants

Characteristic	Combined (N=21)	Women (n=12)	Men (n=9)
Disease duration (years)	28±13	23±13	36±11*
Age of diagnosis (years)	19±10	22±11	16±8
Total daily insulin dose (units day <sup>-1</sup> )	47.0±25.4	38.5±16.6	59.2±31.6
Total daily insulin dose (units kg <sup>-1</sup> day <sup>-1</sup> )	0.56±0.22	0.49±0.16	0.67±0.26
Using CSII	11 (52)	6 (50)	5 (56)
Using MDI	10 (48)	6 (50)	4 (44)
Using CGM	14 (67)	8 (67)	6 (67)
Using flash glucose monitoring	7 (33)	4 (33)	3 (33)
Self-reported complications, high BP	3 (14)	1 (8)	2 (22)
Self-reported complications, neuropathy	2 (10)	1 (8)	1 (11)
Self-reported complications, retinopathy	1 (5)	1 (8)	0 (0)

Data are presented as mean ± SD or as *n* (%)

Characteristics above were based on participant self-report

An unpaired, two-tailed Student's *t* test was used to compare variables between women and men with type 1 diabetes

\**p* < 0.050 women vs men

CGM, continuous glucose monitoring; CSII, continuous subcutaneous insulin infusion; MDI, multiple daily injections

rev min<sup>-1</sup> and maintain it for 10 min. Baseline power was set at 25 watts and thereafter adjusted according to the watch HR data. The goal was to carefully adjust workload in order to steadily increase HR to 130 beats/min by 8 min into the cycling test (with cadence being constant). For the remaining 2 min, power was slightly adjusted if HR began to exceed 135 beats/min. Power and HR were recorded every 15 s during the entire fitness test in order to generate a standard curve of power output (x axis) vs HR (y axis). The slope of the standard curve was used to calculate the power output at 130 beats/min and was also normalised to lean body mass (LBM) (Table 1).

**Visit 2** A Bergström needle sample of *vastus lateralis* muscle was obtained under local anaesthetic (2% lidocaine, 0.005 mg/ml adrenaline [epinephrine]) [23]. A portion of the sample muscle (~15–20 mg) was immediately placed in ice-cold BIOPS buffer [19] for subsequent in vitro assessment of mitochondrial respiratory function. Approximately 5 mg of muscle was placed in ice-cold 2% glutaraldehyde (buffered with 0.1 M sodium cacodylate [pH 7.4]) to preserve the muscle ultrastructure for transmission electron microscopy (TEM) analysis. A blood sample was obtained using venepuncture for HbA<sub>1c</sub> analysis at the McMaster Core Facility laboratory.

### Mitochondrial bioenergetics in permeabilised muscle fibres

The freshly obtained muscle sample in ice-cold BIOPS buffer was divided into small muscle bundles (~1.5–5 mg wet weight) for the preparation of permeabilised muscle fibres (PmFBs), as previously described [24]. Following this, the PmFBs were weighed and washed in buffer Z (in mmol/l: 105 K-MES, 30 KCl, 10 KH<sub>2</sub>PO<sub>4</sub>, 5 MgCl<sub>2</sub>·6H<sub>2</sub>O, 1 EGTA and 5 mg ml<sup>-1</sup> BSA [pH 7.1]) until measurements were initiated (<20 min).

Mitochondrial respiratory kinetics were assessed using the Oroboros Oxygraph-2K (Oroboros Instruments, Innsbruck, Austria), similar to our previous study [19]. The chambers were filled with 2 ml of buffer Z supplemented with 20 mmol/l creatine [25] and 5 μmol/l blebbistatin, and experiments were undertaken at 37°C with constant stirring at 750 rev/min and mild hyperoxygenation (250–275 μmol/l O<sub>2</sub>).

ADP-stimulated respiratory kinetics were determined through ADP (A5258; Sigma-Aldrich, USA) titrations in the presence of 5 mmol/l pyruvate (P2256; Sigma-Aldrich), 2 mmol/l malate (M1000; Sigma-Aldrich) and 10 mmol/l succinate (S2378; Sigma-Aldrich) to recruit both complex I and complex II (C<sub>I+II</sub>). Standard pyruvate (C<sub>I pyr</sub>) and glutamate (C<sub>I glu</sub>) (GLU303; BioShop, Burlington, ON, Canada) titrations, including 2 mmol/l malate and 5 mmol/l ADP, were performed to determine complex I kinetics. Complex II kinetics were determined through standard succinate titrations (C<sub>II succ</sub>) in the presence of 10 μmol/l rotenone (R8875; Sigma-

Aldrich) and 5 mmol/l ADP. Mitochondrial LEAK respiration (state II) was measured in the presence of saturating complex I substrates (5 mmol/l pyruvate and 2 mmol/l malate) and in the absence of adenylates (i.e. ADP). In doing so, any change in respiration is largely considered to be the result of increased/decreased coupling of oxidative phosphorylation (OXPHOS) to ATP synthesis due to proton re-entry into the matrix through either uncoupling mechanisms or H<sup>+</sup>-dependent transporters that have been altered [26]. In other words, herein, mitochondrial LEAK respiration refers to the in vitro measure of mitochondrial respiration compensating for proton leak across the inner mitochondrial membrane [27]. Integrity of the mitochondria (outer mitochondrial membrane) was verified by the addition of 10 μmol/l cytochrome *c* (cat no. 192-10; Lee Biosolutions, Maryland Heights, MO, USA). Any experiments that had a > 10% increase in respiration after the addition of cytochrome *c* were excluded.

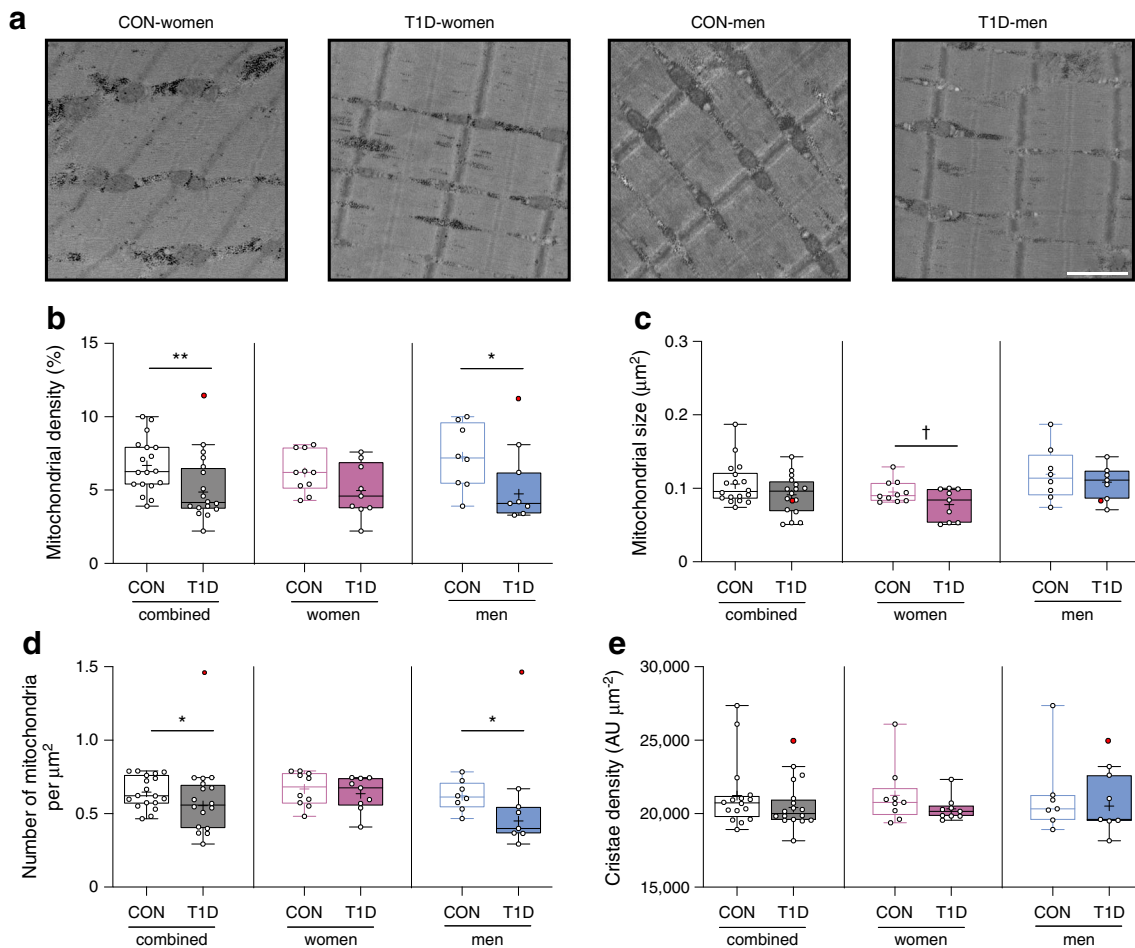
All respirometry data were normalised per milligram wet weight (from here on, referred to as mass-specific mitochondrial respiration); however, this does not discern whether the function per mitochondria has changed or whether mitochondrial content per milligram wet weight has changed. Thus, we further normalised each participant's respiration rates to their own unique mitochondrial content (area density, discussed below). Importantly, since mitochondrial content was determined in a subset of participants (*n* = 18 and 17 for control and type 1 diabetes, respectively), the intrinsic mitochondrial respiration data do not include all participants in the study, only those we could normalise to (from here on, referred to as intrinsic mitochondrial respiration).

### TEM

TEM Facility staff at McMaster University Medical Centre processed the fresh muscle samples fixed in 2% glutaraldehyde, as described previously [28]. Mitochondrial area density, size, number per muscle area and cristae (pixel) density were quantified in a subset of control (*n* = 18) and type 1 diabetes (*n* = 17) participants by Nikon Imaging Software (NIS)-Elements AR (v4.6; Melville, NY, USA) using representative micrographs, as previously described in detail [19].

### Citrate synthase assay

The maximal activities of citrate synthase (CS) were determined as previously described [29]. In brief, muscle homogenate was added to a cuvette containing 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) (D1830; Sigma-Aldrich), acetyl-CoA (A2181; Sigma-Aldrich) and Tris-HCl buffer (pH 8.1) and incubated at 37°C. Oxaloacetate (O4126; Sigma-Aldrich) was then added to initiate the reaction. Absorbance was measured continuously at 412 nm for 2 min at 37°C using a ultraviolet-visible (UV-vis) spectrophotometer (Cary Bio-



**Fig. 1** Quantification of mitochondrial content and characteristics using representative micrographs captured by TEM. Since TEM provides two-dimensional images, mitochondrial content was determined by calculating the percentage mitochondrial area ‘density’ (mean mitochondrial size  $\times$  number of mitochondrial fragments per tissue area, expressed as % area of muscle [45]) in a subset of participants with type 1 diabetes ( $n = 16$ ) and without type 1 diabetes ( $n = 18$ ). Mitochondrial cristae density was also determined by calculating the pixel density of each mitochondrion whereby a lower pixel density is indicative of swollen mitochondria or reduced cristae surface area. **(a)** Representative micrographs. Scale bar, 1  $\mu\text{m}$ . **(b)** Mitochondrial density was significantly lower (\*\* $p = 0.006$ ) in combined type 1 diabetes vs control participants. Compared with control participants, women with type 1 diabetes had observably lower ( $p = 0.110$ ) density while men with type 1 diabetes had significantly lower

(\* $p = 0.033$ ) density. **(c)** Compared with control participants, individual mitochondrion size was observably lower ( $^{\dagger}p = 0.056$ ) in women but not men with type 1 diabetes. **(d)** The number of mitochondrial fragments per unit area was significantly lower in combined participants (\* $p = 0.048$ ) and men (\* $p = 0.014$ ), but not women ( $p = 0.549$ ), with type 1 diabetes. **(e)** Mitochondrial cristae density was not statistically different with type 1 diabetes. The data were analysed using an unpaired Student’s  $t$  test (two-tailed). One male participant with type 1 diabetes was a significant outlier using the ROUT test in GraphPad Prism, depicted as a red circle, and was removed from all statistical analyses in this figure. Boxes represent the interquartile range, whiskers show the maximum and minimum, the horizontal solid line indicates the median and the cross indicates the mean. AU, arbitrary units; CON, control; T1D, type 1 diabetes

300; Varian, Palo Alto, CA, USA), and the slope between 30 s and 90 s was used for calculations. Enzyme activity was measured in duplicate and CS maximal activity (expressed as  $\text{nmol min}^{-1} [\text{mg protein}]^{-1}$ ) was expressed relative to total protein measured with a bicinchoninic acid (BCA) assay kit (Pierce, Rockford, IL, USA).

## Statistics

Statistical analyses were performed using GraphPad Prism (version 8.0) software (San Diego, CA, USA). All data were

initially tested for normal distribution (D’Agostino–Pearson omnibus test) and equal group variance (Brown–Forsythe variance test). The specific analyses used are defined in the figure legends. Statistical significance was established at  $p \leq 0.05$ .

## Results

At study initiation, a design to interrogate sex differences was not considered. However, recent work demonstrating sexual

dimorphism in human skeletal muscle mitochondrial bioenergetics [30–32] made it critical to present control vs type 1 diabetes data as follows: combined (all participants irrespective of sex); women (female participants only); and men (male participants only).

### Skeletal muscle mitochondrial ultrastructure

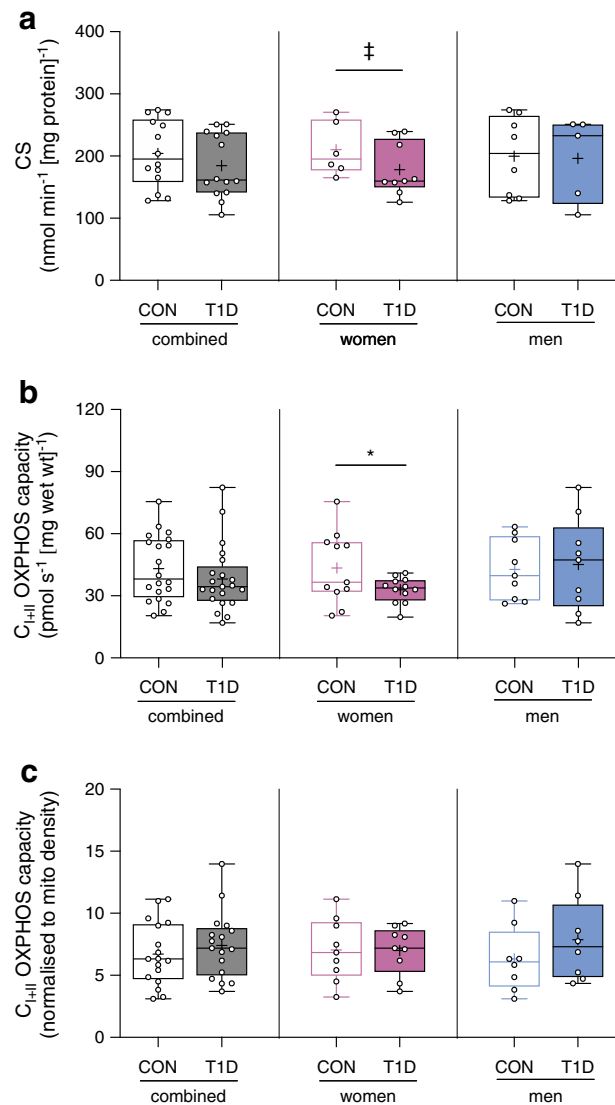
Mean mitochondrial area density was significantly lower ( $p = 0.006$ ) in the type 1 diabetes group ( $-27\%$ ; Fig. 1b) vs control participants. Women with type 1 diabetes demonstrated a  $\sim 20\%$  decrease ( $p = 0.110$ ) in density while men with type 1 diabetes demonstrated a  $\sim 35\%$  decrease ( $p = 0.033$ ) (Fig. 1b). In women, this was largely due to a decrease ( $p = 0.057$ ) in mitochondrial fragment size ( $-18\%$ ; Fig. 1c) and not mitochondrial fragment number per muscle area ( $-9\%$ ,  $p = 0.141$ ) (Fig. 1d). In men, lower mitochondrial density was largely due to decreased ( $p = 0.130$ ) mitochondrial fragment number per muscle area ( $-28\%$ ; Fig. 1d) compared with mitochondrial size ( $-9\%$ ,  $p = 0.380$ ) (Fig. 1c). Mitochondrial cristae density (measured by pixel density) was not different ( $p > 0.270$ ) between cohorts irrespective of sex (Fig. 1e).

### Skeletal muscle mitochondrial OXPHOS capacity

**CS activity** No difference was found in CS activity between cohorts in a subset of participants ( $n = 14$ ) (combined [ $-18\%$ ,  $p = 0.265$ ], women [ $-17\%$ ,  $p = 0.088$ ], men [ $+14\%$ ,  $p > 0.999$ ]) (Fig. 2a).

**$C_{I+II}$ -supported respiration** Mitochondrial  $C_{I+II}$  OXPHOS capacity was comparable between cohorts for both mass-specific ( $-11\%$ ,  $p = 0.165$ ) and intrinsic ( $+11\%$ ,  $p = 0.217$ ) respiration (Fig. 2b,c). Separated by sex, mass-specific  $C_{I+II}$  OXPHOS capacity was significantly lower in women ( $-24\%$ ,  $p = 0.033$ ) but not men with type 1 diabetes ( $+5\%$ ,  $p = 0.397$ ) (Fig. 2b,c). In contrast, intrinsic  $C_{I+II}$  OXPHOS capacity was not different in women with type 1 diabetes ( $+5\%$ ,  $p = 0.378$ ), while in men with type 1 diabetes it was 25% non-significantly higher ( $p = 0.163$ ) compared with control participants (Fig. 2b,c).

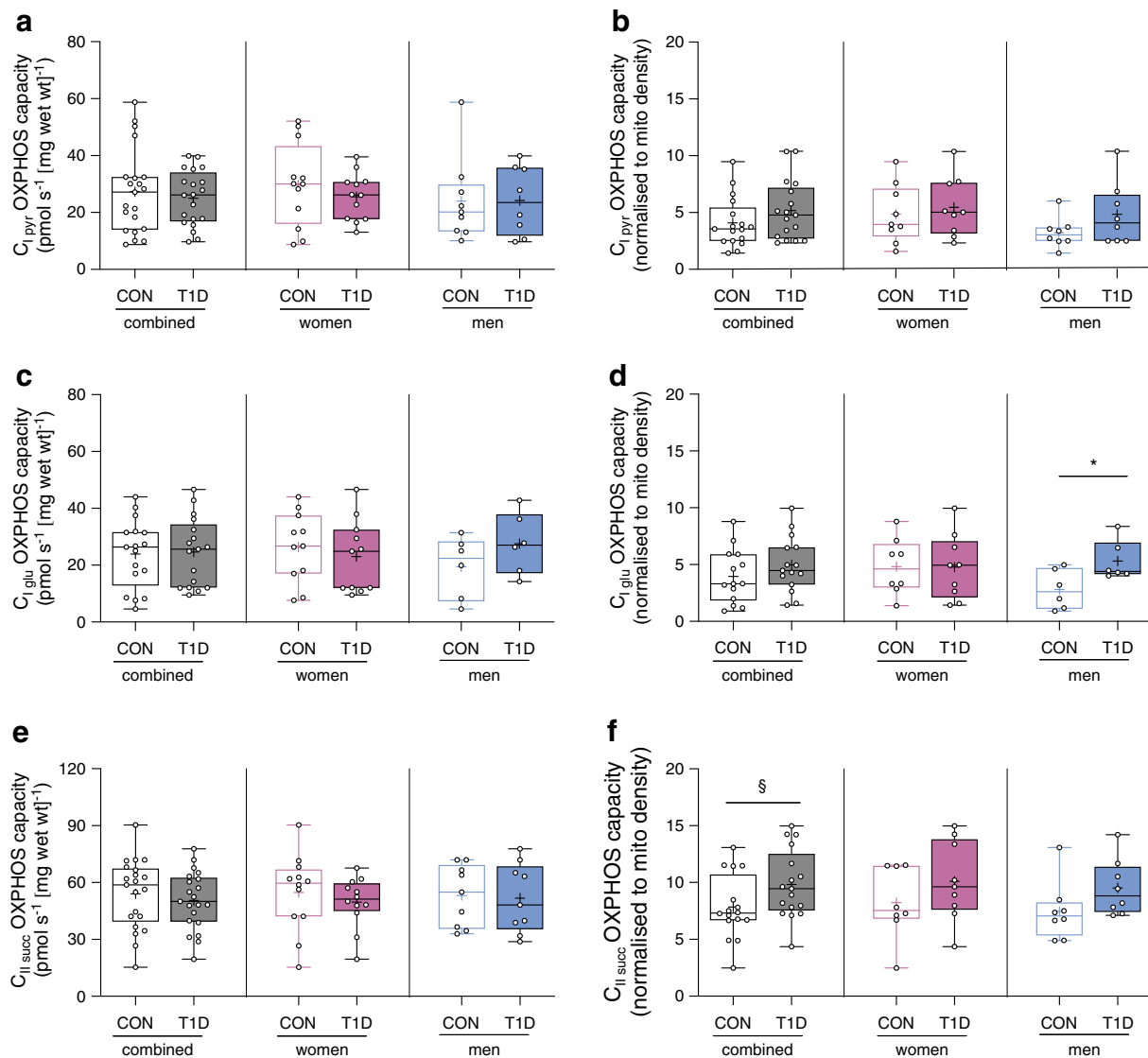
**Complex I-supported respiration** To determine whether alterations exist at the level of complex I or upstream dehydrogenases, specifically pyruvate dehydrogenase (PDH) and glutamate dehydrogenase (GDH),  $C_{I\text{ pyr}}$  and  $C_{I\text{ glu}}$  OXPHOS capacity was also measured as both substrates generate NADH that supports complex I activity. No differences were found between groups with mass-specific  $C_{I\text{ pyr}}$  OXPHOS capacity (combined [ $-8\%$ ;  $p = 0.572$ ], women [ $-14\%$ ;  $p = 0.404$ ], men [ $+1\%$ ;  $p = 0.970$ ]) or  $C_{I\text{ glu}}$  OXPHOS capacity (combined [ $-3\%$ ;  $p = 0.853$ ], women [ $-12\%$ ;  $p = 0.404$ ]), except in men with type 1 diabetes where a 42% non-



**Fig. 2** Skeletal muscle mitochondrial CS activity and OXPHOS capacity. (a) CS activity was non-significantly lower ( $^{\ddagger}p = 0.088$ ) only in women with T1D. Respiration assays were performed in PmFBs, supported by saturating complex I (pyruvate + malate) and complex II (succinate) substrates ( $C_{I+II}$  OXPHOS capacity), and normalised by initial muscle wet weight ( $\text{pmol s}^{-1} [\text{mg wet weight}]^{-1}$ ) and by mitochondrial area density, i.e. intrinsic mitochondrial respiration. (b) Mass-specific  $C_{I+II}$  OXPHOS capacity was significantly lower ( $^*p = 0.042$ ) in women, but not men, with type 1 diabetes compared with control participants. (c) Intrinsic  $C_{I+II}$  OXPHOS capacity was not different between cohorts irrespective of sex. A Student's unpaired  $t$  test (one-tailed) was used to analyse the data. One control female participant was not included in the analyses due to methodological issues. Boxes represent the interquartile range, whiskers show the maximum and minimum, the horizontal solid line indicates the median and the cross indicates the mean. CON, control; mito, mitochondrial; T1D, type 1 diabetes; wt, weight

significant increase was measured compared with control participants ( $p = 0.218$ ) (Fig. 3a,c).

When normalised to mitochondrial content,  $C_{I\text{ pyr}}$  and  $C_{I\text{ glu}}$  OXPHOS capacities were non-significantly higher (both  $+27\%$ ,  $p > 0.201$ ) in participants with type 1 diabetes (Fig. 3b,d). When separated by sex, we observed a non-significant



**Fig. 3** Skeletal muscle OXPHOS capacity of complex I supported by pyruvate ( $C_{I\text{ pyr}}$ ) or glutamate ( $C_{I\text{ glu}}$ ) and complex II supported by succinate ( $C_{II\text{ succ}}$ ) in PmFBs. Respiration assays were normalised by the initial muscle wet weight ( $\text{pmol s}^{-1} [\text{mg wet wt}]^{-1}$ ) (**a**, **c**, **e**) or by mitochondrial area density (i.e. intrinsic mitochondrial function) (**b**, **d**, **f**). Neither (**a**) mass-specific nor (**b**) intrinsic  $C_{I\text{ pyr}}$  OXPHOS capacity was different between cohorts. One male participant with type 1 diabetes was not included in the analyses due to methodological issues. While (**c**) mass-specific  $C_{I\text{ glu}}$  OXPHOS capacity was observably higher in men with type 1 diabetes compared with control men ( $p = 0.220$ ), only (**d**) intrinsic  $C_{I\text{ glu}}$

OXPHOS capacity was significantly higher in men with T1D ( $*p = 0.033$ ). As a result of methodological issues, data were not obtained for  $n = 4$  control (one woman, three men) and  $n = 4$  type 1 diabetic (one woman, three men) participants. Only (**f**) intrinsic  $C_{II\text{ succ}}$  OXPHOS capacity was observably higher ( $§p = 0.057$ ) in the type 1 diabetes cohort. A Student's unpaired  $t$  test (two-tailed) was used to analyse all the data. Boxes represent the interquartile range, whiskers show the maximum and minimum, the horizontal solid line indicates the median and the cross indicates the mean. CON, control; mito, mitochondrial; T1D, type 1 diabetes; wt, weight

50% increase ( $p = 0.159$ ) in intrinsic  $C_{I\text{ pyr}}$  OXPHOS capacity and a significant 88% increase ( $p = 0.033$ ) in intrinsic  $C_{I\text{ glu}}$  OXPHOS capacity in men with type 1 diabetes only (Fig. 3b,d).

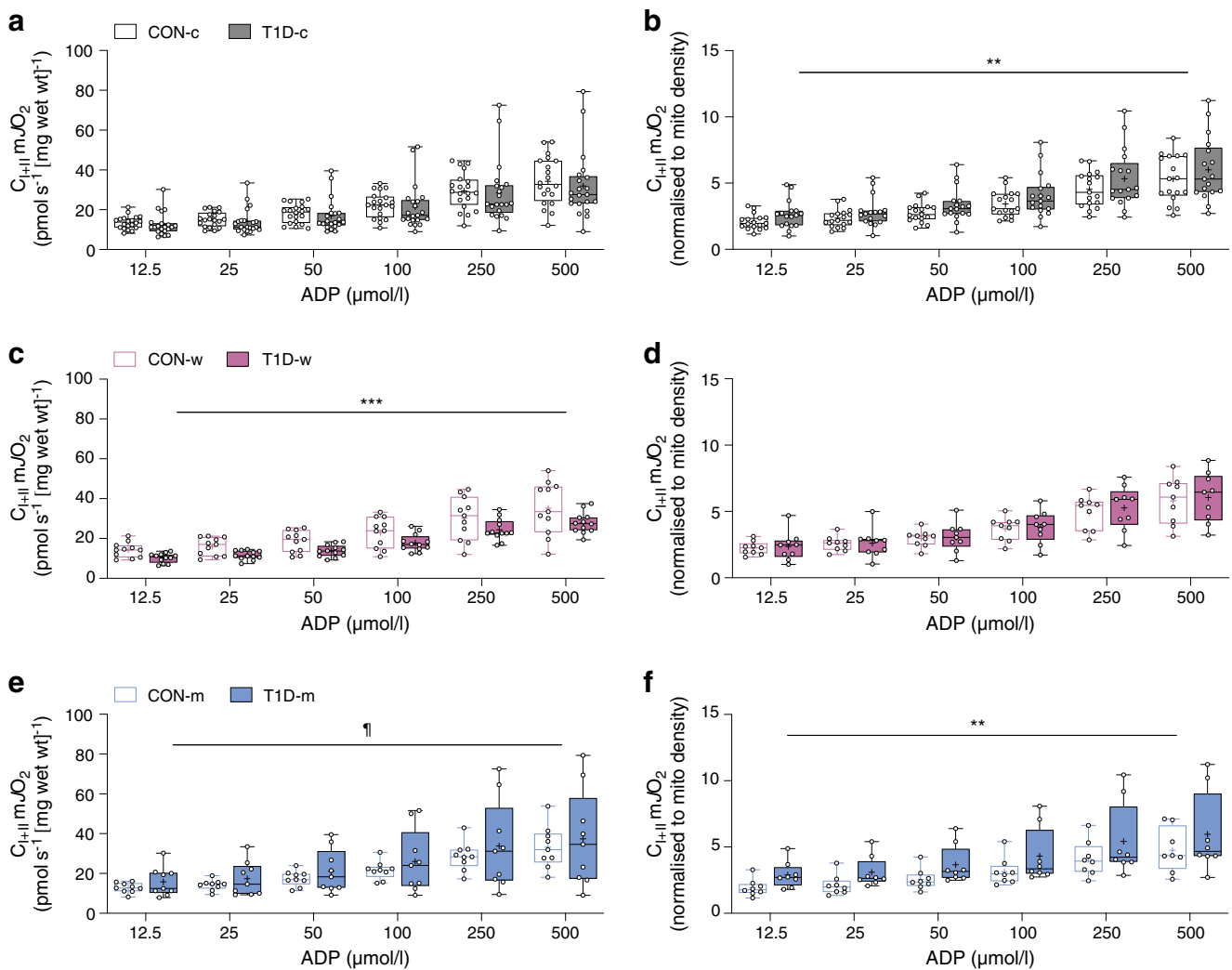
**Complex II-supported respiration** No differences were found in mass-specific  $C_{II\text{ succ}}$  OXPHOS capacity between groups (combined [ $-6\%$ ;  $p = 0.502$ ], women [ $-9\%$ ;  $p = 0.475$ ], men [ $-3\%$ ;  $p = 0.868$ ]) (Fig. 3e). Normalised to mitochondrial content,  $C_{II\text{ succ}}$  OXPHOS capacity was 25% higher in the

type 1 diabetes cohort ( $p = 0.058$ ) (Fig. 3f). However, women and men with type 1 diabetes demonstrated non-significant 23% ( $p = 0.268$ ) and 28% ( $p = 0.123$ ) increases, respectively, in intrinsic  $C_{II\text{ succ}}$  OXPHOS capacity (Fig. 3f).

### Skeletal muscle mitochondrial respiratory response to low substrate concentrations

**ADP** The ability of mitochondria to respond to changing energy demands (i.e. range of subsaturating ADP concentrations)





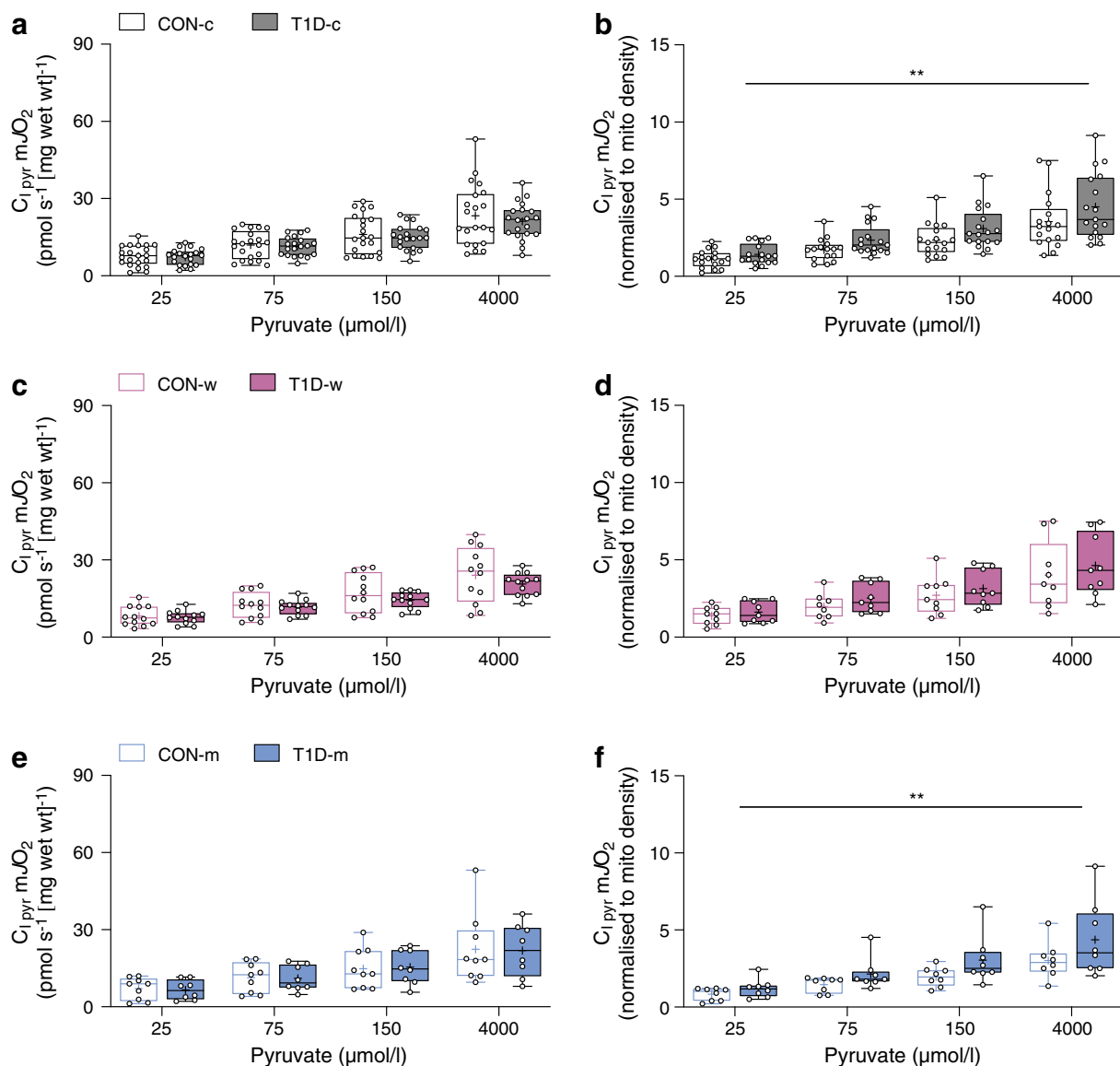
**Fig. 4**  $C_{I+II}$ -supported mitochondrial respiration stimulated by subsaturating ADP concentrations ( $C_{I\text{ pyr}} + C_{II\text{ succ}}$   $\text{mJO}_2$ ) in PmFBs. Respiration assays were normalised by initial muscle wet weight ( $\text{pmol s}^{-1} [\text{mg wet weight}]^{-1}$ ) (a, c, e) and by mitochondrial area density (i.e. intrinsic mitochondrial function) (b, d, f). Compared with control participants, mass-specific ADP-stimulated respiration showed a main effect for lower rates ( $**p < 0.001$ ) in type 1 diabetes for women (c) and observably higher rates ( $^{\#}p = 0.095$ ) in men with type 1 diabetes (e). However, intrinsic ADP-stimulated respiration showed similar rates in type 1 diabetes for women (d) compared with control participants,

was interrogated. ADP was titrated at increasing physiological concentrations considered to be representative of conditions of sedentary/low physical activity to moderate/high physical activity and beyond. Collectively, mass-specific submaximal ADP-stimulated respiration was similar ( $p = 0.230$ ) between cohorts (Fig. 4a). When separated by sex, women with type 1 diabetes demonstrated a reduction in ADP-stimulated respiration (main effect  $p < 0.001$ ), while in men it was non-significantly higher (main effect  $p = 0.095$ ) (Fig. 4c,e). In contrast, intrinsic submaximal ADP-stimulated respiration was significantly higher (main effect  $p = 0.002$ ) in individuals with type 1 diabetes (Fig. 4b); however, no statistical difference was found in women (main

whereas a main effect for higher respiration rates was found in type 1 diabetes for combined participants ( $**p = 0.002$ ) and men ( $**p = 0.001$ ) (b and f, respectively). A two-way ANOVA was performed to determine a main effect of disease state. One control female participant with type 1 diabetes was not included in the analyses due to methodological issues. Boxes represent the interquartile range, whiskers show the maximum and minimum, the horizontal solid line indicates the median and the cross indicates the mean. c, combined; CON, control; m, men; mito, mitochondrial;  $\text{mJO}_2$ , mitochondrial oxygen flux; T1D, type 1 diabetes; w, women; wt, weight

effect  $p = 0.474$ ), whereas a significant increase was found in men (main effect  $p = 0.001$ ) (Fig. 4d,f).

**Pyruvate and glutamate** We also interrogated the response of complex I and/or upstream dehydrogenases to low (i.e. subsaturating) concentrations of pyruvate or glutamate. No differences were found between groups for mass-specific submaximal pyruvate-supported respiration (combined  $[-8\%; p = 0.258]$ , women  $[-12\%; p = 0.545]$ , men  $[-2\%; p = 0.894]$ ) (Fig. 5a,c,e) or submaximal glutamate-supported respiration (combined  $[+9\%; p = 0.232]$ , women  $[-0.7\%; p = 0.956]$ , men  $[+30\%; p = 0.197]$ ) (Fig. 6a,c,e). In contrast, intrinsic pyruvate



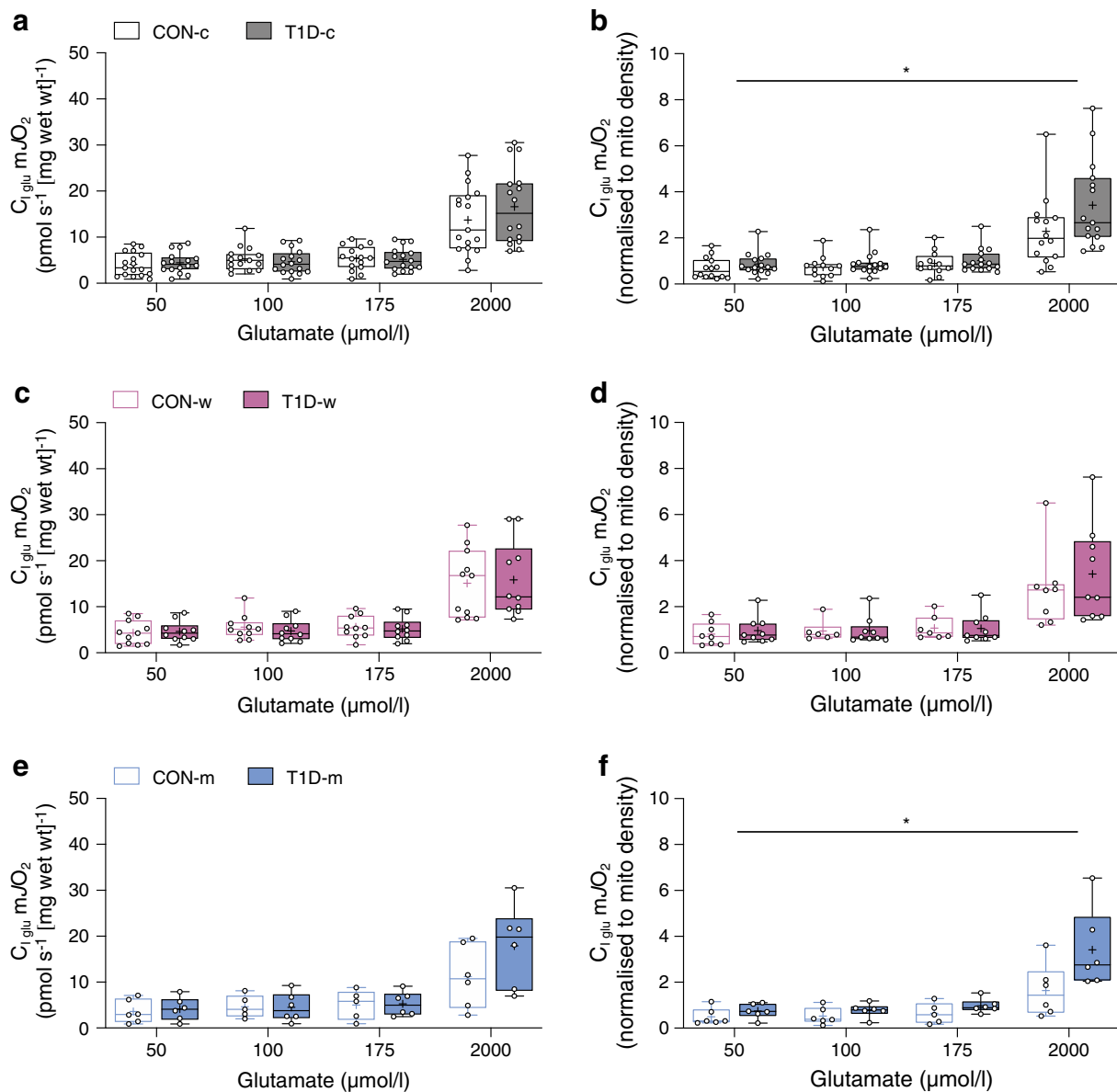
**Fig. 5** Mitochondrial respiration stimulated by subsaturating pyruvate concentrations ( $C_{1\text{pyr}} m\text{JO}_2$ ) in PmFBs. Respiration assays were normalised by initial muscle wet weight ( $\text{pmol s}^{-1} [\text{mg wet weight}]^{-1}$ ) (**a**, **c**, **e**) and by mitochondrial area density (i.e. intrinsic mitochondrial function) (**b**, **d**, **f**). No statistical differences were found ( $p > 0.050$ ) in mass-specific mitochondrial respiration between cohorts (**a**, **c** and **e**). Intrinsic pyruvate-stimulated respiration showed a main effect for higher rates in type 1 diabetes for combined participants (\*\* $p = 0.003$ ) and men (\*\* $p = 0.005$ )

but not women ( $p = 0.118$ ) (**b**, **f** and **d**, respectively). A two-way ANOVA was performed to determine a main effect of disease state. One control female participant and one male participant with type 1 diabetes were not included in the analyses due to methodological issues. Boxes represent the interquartile range, whiskers show the maximum and minimum, the horizontal solid line indicates the median and the cross indicates the mean. c, combined; CON, control; m, men; mito, mitochondrial;  $m\text{JO}_2$ , mitochondrial oxygen flux; T1D, type 1 diabetes; w, women; wt, weight

and glutamate submaximal respiration were both significantly increased (main effect  $p = 0.003$  and  $p = 0.030$ , respectively) in the type 1 diabetes cohort (Figs 5b, 6b). Only men with type 1 diabetes showed a significant increase in both pyruvate- (main effect  $p = 0.005$ ) and glutamate-supported (main effect  $p = 0.012$ ) respiration (Figs 5f, 6f).

**Succinate** The response of complex II to low concentrations of succinate was also interrogated. No significant differences

were found in mass-specific submaximal succinate-supported respiration between groups (combined  $[-6\%$ ;  $p = 0.335$ ], women  $[-2\%$ ;  $p = 0.813$ ], men  $[+17\%$ ;  $p = 0.149$ ] (Fig. 7a,c,e). In contrast, intrinsic submaximal succinate-supported respiration was significantly increased in the type 1 diabetes group (main effect  $p < 0.001$ ), and this was consistent in both women and men (main effect  $p = 0.003$  and  $p < 0.001$ , respectively) (Fig. 7b,d,f).



**Fig. 6** Mitochondrial respiration stimulated by subsaturating glutamate concentrations ( $C_{1\text{glu}} \text{ mJO}_2$ ) in PmFBs. Respiration assays were normalised by initial muscle wet weight ( $\text{pmol s}^{-1} [\text{mg wet weight}]^{-1}$ ) (a, c, e) and by mitochondrial area density (i.e. intrinsic mitochondrial function) (b, d, f). No statistical differences ( $p > 0.050$ ) were found in mass-specific mitochondrial respiration between cohorts (a, c and e). Compared with control participants, intrinsic glutamate-stimulated respiration showed a main effect for higher rates in type 1 diabetes for combined participants ( $*p = 0.030$ ) and men ( $*p = 0.012$ ) but not women ( $p = 0.438$ ) (b, f and d,

respectively). A two-way ANOVA was performed to determine a main effect of disease state. As a result of methodological issues, data were not obtained in four control participants (one woman, three men) and four participants with type 1 diabetes (one woman, three men). Boxes represent the interquartile range, whiskers show the maximum and minimum, the horizontal solid line indicates the median and the cross indicates the mean. c, combined; CON, control; m, men; mito, mitochondrial;  $\text{mJO}_2$ , mitochondrial oxygen flux; T1D, type 1 diabetes; w, women; wt, weight

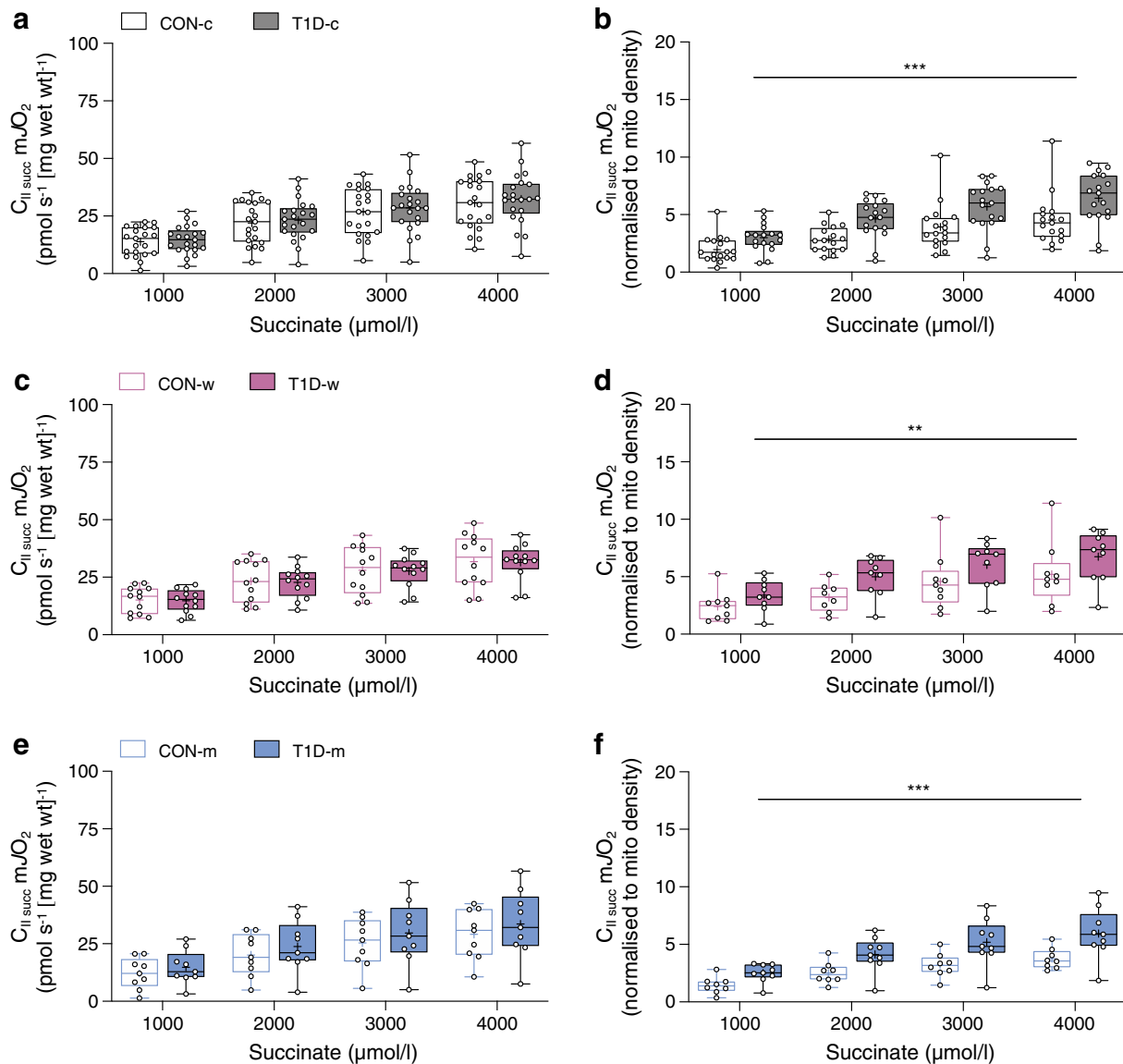
### Skeletal muscle mitochondrial coupling

Collectively, mitochondrial LEAK respiration was 40% higher ( $p = 0.007$ ) in participants with type 1 diabetes compared with control participants (Fig. 8a). When separated by sex, LEAK respiration was not different in women with type 1 diabetes (+8%;  $p = 0.608$ ), while in men with type 1

diabetes LEAK respiration was 82% greater ( $p = 0.002$ ) (Fig. 8b,c), compared with control participants.

### Correlation analyses

Figure 9 contains a customised heat map summarising the correlational analyses performed in the type 1 diabetes cohort, separated into women (Fig. 9a) and men



**Fig. 7** Mitochondrial respiration stimulated by subsaturating succinate concentrations ( $C_{II\text{ succ}} mJO_2$ ) in PmFBs. Respiration assays were normalised by initial muscle wet weight ( $\text{pmol s}^{-1} [\text{mg wet weight}]^{-1}$ ) (**a**, **c**, **e**) and by mitochondrial area density (i.e. intrinsic mitochondrial function) (**b**, **d**, **f**). No statistical differences ( $p > 0.050$ ) were found in mass-specific mitochondrial respiration between cohorts (**a**, **c** and **e**). Compared with control participants, intrinsic succinate-stimulated respiration showed a main effect for higher rates in type 1 diabetes for

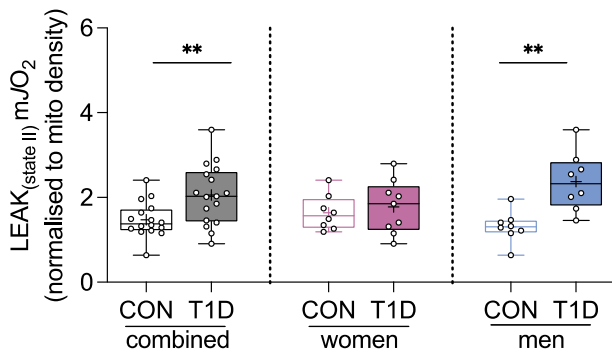
combined participants ( $***p < 0.001$ ), women ( $**p = 0.003$ ) and men ( $***p < 0.001$ ) (**b**, **d**, and **f**). A two-way ANOVA was performed to determine a main effect of disease state. Boxes represent the interquartile range, whiskers show the maximum and minimum, the horizontal solid line indicates the median and the cross indicates the mean. c, combined; CON, control; m, men; mito, mitochondrial;  $mJO_2$ , mitochondrial oxygen flux; T1D, type 1 diabetes; w, women; wt, weight

(Fig. 9b), between metrics that we considered clinically relevant and the mitochondrial respiratory measures undertaken.

## Discussion

This is the first study to interrogate mitochondrial respiratory function and content in muscle biopsies of adults with uncomplicated type 1 diabetes with a wide age span (30–72 years

old) in comparison with strictly matched control participants (age, sex, BMI and self-reported physical activity levels). The success in our matching strategy was supported by the comparable LBM, muscle strength and calculated aerobic fitness between cohorts. Despite the similarities in these clinically relevant metrics, mitochondrial OXPHOS capacity, mitochondrial respiratory response to low (‘physiological’) substrate concentrations and ultrastructure were altered with type 1 diabetes and demonstrated sexual dimorphism. Specifically, at the whole-muscle level, we found



**Fig. 8** Intrinsic mitochondrial LEAK (state II) respiration. Mitochondrial respiration was measured in the absence of ADP but presence of saturating complex I substrates (pyruvate + malate) to drive (maximise) proton leak-dependent respiration (i.e. absence of adenylates). Compared with control participants, LEAK respiration (i.e. state II) was significantly higher with type 1 diabetes for combined participants (\*\* $p = 0.007$ ) and men (\*\* $p = 0.002$ ) but not women ( $p = 0.408$ ). A Student’s unpaired  $t$  test (two-tailed) was used to analyse the data. One control female participant was not included in the analyses due to methodological issues. Boxes represent the interquartile range, whiskers show the maximum and minimum, the horizontal solid line indicates the median and the cross indicates the mean. CON, control; T1D, type 1 diabetes; wt, weight

mitochondrial OXPHOS capacity and submaximal ADP respiration were significantly lower in women, but not men, with type 1 diabetes compared with control groups. We also found mitochondrial density to be significantly lower in women and men with type 1 diabetes, with decreased mitochondrial fragment size in women and reduced number of mitochondrial fragments in men being the primary drivers of the decreased density. When respiration rates were normalised to mitochondrial density, the mitochondrial respiratory deficiencies in women with type 1 diabetes disappeared, whereas in men with type 1 diabetes, we found significant increases in mitochondrial OXPHOS capacity and mitochondrial respiratory rates in response to low concentrations of ADP, pyruvate, glutamate or succinate, indicating normal and enhanced intrinsic mitochondrial respiratory function between sexes, respectively. Altogether, these novel findings reveal differential adaptations of muscle mitochondria to type 1 diabetes in middle- to older-aged women and men without overt diabetes complications.



**Fig. 9** Heat map of correlations between mitochondrial measures performed and metrics of clinical relevance in women (a) and men (b) with type 1 diabetes. The heat map shows the computed Pearson  $r$  correlations using GraphPad Prism. Positive correlations are given in shades of blue/green, and negative correlations in shades of red, with intensity

indicating the magnitude of the correlation. \* $p \leq 0.050$  and • $p \leq 0.120$ . Aerobic fitness, muscle mass, muscle strength and fat mass refer to PWC<sub>130</sub>, % LBM, MVC and % flash monitoring, respectively. Glu, glutamate; mito, mitochondrial; Pyr, pyruvate; Succ, succinate; T1D, type 1 diabetes

Previous works from our group and others have reported decrements in muscle mitochondrial respiration in young people with type 1 diabetes. These include attenuations in *in vivo* and *in vitro* muscle oxidative capacity [19, 30, 33–35], decreased submaximal ADP-stimulated respiration [19, 30], decreased complex II submaximal and maximal respiration [19, 30] and decreased complex IV activity [22]. However, these findings are not consistent across all studies, as some groups did not observe differences in mitochondrial OXPHOS capacity [20, 22] and complex II function [22], with only deficiencies in complex IV function reported [22]. These *in vitro* studies also measured markers of mitochondrial content and reported no differences in mitochondrial area density [19], protein expression of complexes I to V [19, 30] or CS activity [22], indicating intrinsic mitochondrial respiratory deficiencies occur early in the progression of type 1 diabetes in human muscle. While informative, the bulk of studies to date have studied adolescents and young adults, making it unclear if older adults with type 1 diabetes exhibit similar or worsened muscle mitochondria dysfunction. In this study of older adults with type 1 diabetes who are relatively healthy, we found normal CS activity and normal or enhanced intrinsic mitochondrial respiratory function, which was also observably different between sexes. Women with type 1 diabetes had normal intrinsic mitochondrial  $C_{I+II}$  OXPHOS capacity, submaximal ADP respiration, and  $C_{I\text{ pyr}}$  and  $C_{I\text{ glu}}$  submaximal respiration, whereas intrinsic  $C_{II\text{ succ}}$  submaximal respiration was significantly higher. In contrast, men with type 1 diabetes had a non-significant increase ( $p = 0.163$ ) in intrinsic mitochondrial  $C_{I+II}$  OXPHOS capacity and significantly greater  $C_{I\text{ glu}}$  OXPHOS capacity, mitochondrial ADP submaximal respiration and  $C_{I\text{ pyr}}$ ,  $C_{I\text{ glu}}$  and  $C_{II\text{ succ}}$  submaximal respiration, respectively. While these novel findings contradicted our working hypothesis [17] and the current literature available in young adults with type 1 diabetes, they provide for the first time invaluable insight and evidence that muscle health, as it pertains to mitochondrial function, can generally be preserved in adults with type 1 diabetes with increasing age and disease duration.

Enhanced intrinsic mitochondrial respiratory function has been previously reported in type 2 diabetes muscle in spite of many studies suggesting normal or dysfunctional mitochondria in adults with type 2 diabetes. Specifically, Larsen and colleagues found higher intrinsic mitochondrial substrate sensitivity to glutamate and succinate in biopsies from middle-aged adults with type 2 diabetes compared with age- and BMI-matched control groups [36]. While the mechanisms remain to be fully delineated, some studies suggest this is a compensatory effect and/or adaptation during the progression of diabetes [36, 37]. Interestingly, in the streptozotocin (STZ)-induced type 1 diabetes rodent model, Larsen and colleagues also found intrinsic mitochondrial OXPHOS capacity to be

elevated in soleus, and to a varying degree in plantaris, and this occurred in parallel with reduced mitochondrial content [38], similar to our present work. Since insulin resistance is also a feature of type 1 diabetes, the enhanced intrinsic mitochondrial response to physiological substrate concentrations and/or capacity can be a compensatory mechanism for decreased glucose flux into the cell because of insulin resistance. However, this is strictly speculative at this time. We did measure the protein expression of mitochondrial pyruvate carrier 1 and 2 (MPC1, MPC2) in a subset of participants but found no statistical difference between groups (see Electronic supplementary material [ESM] Fig. 1).

Another potential explanation for the sexual dimorphism and altered mitochondrial respiratory function in type 1 diabetes is fibre type differences. While muscle fibre type was not measured, we have preliminary data that (compared with control groups) women with type 1 diabetes display a greater proportion of type IIX and type I fibres, while men exhibit more type IIX and reduced type I fibres. Both men and women with type 1 diabetes have less type IIA fibres. As type II fibres have greater substrate sensitivity than type I fibres, it may be that the differential fibre type expression between men and women with type 1 diabetes explains, in part, the sex differences in mitochondrial respiratory function observed herein.

Interestingly, we did not find significant differences in mitochondrial respiratory function and ultrastructure between pre- and postmenopausal women irrespective of type 1 diabetes (ESM Fig. 2). Altogether, if we compare these study findings with our previous work in younger cohorts with type 1 diabetes [19], we propose that in otherwise healthy women and men with uncomplicated type 1 diabetes entering their fourth decade of life and beyond, intrinsic mitochondrial respiratory function does not worsen, but, rather, differentially adapts to the type 1 diabetes environment to preserve mitochondrial health and, ultimately, muscle metabolic health. Indeed, this is a significant stray from our initial hypothesis of accelerated muscle ageing in those with type 1 diabetes [17], but if we consider the complexity and heterogeneity of type 1 diabetes, what remains clear is that there is significant research needed to be done in the area, beginning with larger cohort studies in both sexes.

In the present study, there was the collective ~27% decrease in mitochondrial content (i.e. area density) in the type 1 diabetes cohort. This is in contrast to previous work and is intriguing considering that our participants were strictly matched, had comparable physical activity levels and scored similarly on the aerobic fitness test. Indeed, it is important to recognise that  $PWC_{130}$  is not a direct measure of maximal aerobic capacity ( $\dot{V}O_{2\text{max}}$ ), but rather submaximal aerobic capacity. Thus, we cannot negate the possibility that our type 1 diabetes cohort had a lower maximal aerobic capacity compared with matched control participants, and a lower mitochondrial content as a

result. On the other hand, a similar aerobic capacity between those with/without type 1 diabetes, as is suggested by our data, would indicate deficits in the mechanisms regulating skeletal muscle adaptations to increased physical activity/exercise in type 1 diabetes (i.e. mitochondrial biogenesis/degradation/quality control). In STZ rodent models, but not type 2 or insulin-resistant models, a decrease in peroxisome-proliferator-activated receptor- $\gamma$  coactivator-1 alpha (PGC1a) expression, and consequently reduced transcription of mitochondrial genes, has been reported [39]. Insulin deprivation in adults with type 1 diabetes (and thus hyperglycaemia) reduces rates of mitochondrial ATP production and the expression of OXPHOS genes [40]. A recent study in STZ-induced diabetes also found reductions in OXPHOS capacity (but not intrinsic OXPHOS capacity) and reduced protein expression of PGC1a and proteins of the ETC and substrate transport (voltage-dependent anion channel [VDAC], adenine nucleotide translocase [ANT]) with acute insulin deprivation [41]. Interestingly, we did not find differences in adenine nucleotide translocase 1 (ANT1) or adenine nucleotide translocase 2 (ANT2) protein expression between sexes in a subset of participants with type 1 diabetes (ESM Fig. 1). Thus, while speculative, perhaps after more than two decades of exogenous insulin treatment concurrent with muscle insulin resistance, mitochondria undergo numerous adaptations to ensure adequate redox balance and ATP supply, similar to what has been previously suggested in type 2 diabetes [36, 37].

## Perspectives and conclusions

Advances for type 1 diabetes care, while not a cure, are allowing those with this chronic disease to live consistently longer than ever before. However, the literature indicates that people with diabetes are more likely to suffer from fatigue, weakness and frailty with increasing age compared with control groups [4, 9]. Given that deficits in muscle mitochondria are well known to trigger catabolic signalling cascades and promote atrophy and loss of muscle strength [42], the present findings provide, for the first time, evidence that alterations to mitochondrial respiratory function and ultrastructure are occurring in middle- to older-aged adults with type 1 diabetes. Our findings also indicate that the mitochondrial changes may be an adaptation/compensation to the pathological consequences of type 1 diabetes (i.e. dysglycaemia and hyper/hypoinsulinaemia) and/or the loss of mitochondrial content. It is important to note that the participants recruited were active (recreationally to very active), mostly resided in a university-dwelling community, had at least a post-secondary education and, if they had type 1 diabetes, were well controlled and knowledgeable on the importance of a healthy lifestyle for managing their disease. Thus, the results of this study should be restricted to these demographics and support the dire need for further research into other demographics with type 1 diabetes, including those who have poor glycaemic

management (~83% of youth and ~79% of adults with type 1 diabetes [43]), are sedentary and/or have unhealthy body weight (~60% of adults with type 1 diabetes are overweight/obese [44]).

Taken together, as we continue to uncover how muscle adapts to the type 1 diabetes environment in humans, what remains clear is that women and men are differentially impacted at the cellular level, and future studies must not only consider sex in the treatment and management of type 1 diabetes, but must also consider the importance of regular exercise/physical activity to maintain skeletal muscle metabolic health across the lifespan.

**Supplementary Information** The online version of this article (<https://doi.org/10.1007/s00125-021-05540-1>) contains peer-reviewed but unedited supplementary material.

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**Data availability** All relevant data are included in this article.

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**Authors' relationships and activities** The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

**Contribution statement** CMFM, AGD and TJH conceived and designed the experiments. CGRP, VL and MAT contributed to the conception of experiments. CMFM wrote the manuscript. CMFM performed all experiments with assistance from IAR and JPN. CMFM, AGD, MN, IAR, JPN and LVT analysed data. CMFM, CGRP and TJH interpreted data. MAT performed muscle biopsies and venous blood draw. CMFM, AGD and LVT assisted with muscle and blood sample collection and processing. All authors edited the manuscript and provided final approval of the version to be published. TJH is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of data and the accuracy of the data analysis.

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