Methods

Patient recruitment and muscle biopsy collection

Two groups of patients, with and without type 2 diabetes, and meeting all study criteria were selected for study. The length of time between the diagnosis of type 2 diabetes and the muscle sampling was variable and ranged from 241 to 2178 days. The average duration of this period of time was 1072 ± 211 days. It is however important to note that, given the challenges in dating the exact onset of type 2 diabetes, it is possible and likely that the actual onset of diabetes predated the clinical diagnosis.

Rectus abdominis samples were obtained in the operating room from the incision sites of laparoscopic equipment while patients were under general anesthesia. Skeletal muscle sample collection was limited to regular working hours and varied based on operating room schedules. The rectus abdominis was chosen because of ease of access to the site during the surgery, which permitted functional and biochemical characterizations of this stabilizer muscle.

High-resolution Respirometry

Briefly, rectus abdominis samples were placed in ice-cold relaxation medium (BIOPS) [1] immediately after harvesting. Individual muscle fibers were mechanically separated and saponin permeabilized (50µg/ml) on ice for 30 minutes. Fibers were rinsed in mitochondrial respiration medium (MiR05)[1], and weighed amounts were placed in the respirometry chambers. Two separate protocols were used and runs were performed at
37°C. The concentration of oxygen was kept between 200 and 400 nmol/mL for the duration of the measurements. The first protocol involved progressive additions of 2mM malate, 5mM pyruvate (L\textsubscript{N} - adenylate free leak respiration), 10mM glutamate, 5mM ADP (P\textsubscript{CI} – Complex I-supported respiration), 10mM succinate (P\textsubscript{CI+CII} – Complex I- and II-supported respiration), 0.25\mu M titrations of carbonyl cyanide p-trifluoromethoxyphenyl hydrazine (FCCP) (U\textsubscript{C} – Maximal uncoupled respiration), 2.5\mu M antimycin A (AA) and 2mM N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride (TMPD) with 2mM ascorbate (Tm – Cytochrome C Oxidase (COX) activity). This last measurement has been used as an indicator of mitochondrial content in various works and is arguably better used to normalize high-resolution respirometry data as the measurement is performed on the same sample as the rest of the protocol [1]. The second protocol included consecutive additions of 2mM malate, 200\mu M octanoyl carnitine, 5mM ADP (P\textsubscript{ETF} – fatty acid-supported respiration), 5mM pyruvate, 10mM glutamate, 10mM succinate, 2.5\mu M oligomycin (L\textsubscript{Omy} – Leak respiration). All runs were performed in duplicate and values are corrected to non-mitochondrial oxygen consumption (AA).

**Mitochondrial Supercomplex and Total Protein Determinations**

ATP synthase assembly and ETC supercomplex assembly were analyzed by blue native polyacrylamide gel electrophoresis (BN-PAGE) [2, 3]. Rectus abdominis tissue was flash frozen and subsequent steps were performed on ice. Samples were homogenized in sucrose buffer (250mM sucrose, 20mM imidazole/HCl, pH 7.0). Mitochondria were pelleted at 10,000 x g for 10 minutes and resuspended in 50mM imidazole/HCl pH 7.0,
50mM NaCl, 5mM 6-aminohexanoic acid, 1mM EDTA with 1% digitonin (final digitonin to tissue ratio of 1:12 w/w) for 30 minutes. Samples were cleared by centrifugation for 30 minutes at 14,000 x g. Protein was loaded with 5% glycerol and a 1:10 dye:digitonin ratio of Coomassie Blue G-250 onto 3-13% large gradient gels. Gels were run in high Coomassie Blue cathode buffer for 2 hours at 150V and switched to low Coomassie cathode buffer overnight at 200V. Gels were transferred to nitrocellulose membrane at 500mA for 2.5 hours and membranes were blotted as described below.

For determinations of total mitochondrial protein amounts, samples were subjected to standard Western blotting procedures (SDS-PAGE).

**Immunoblotting**

The following primary antibodies were used for BN-PAGE blots: Complex I [NDUFA9] (459100, Invitrogen – 1:2,000), Complex II [Fp] (459200, Invitrogen – 1:10,000), Complex III [UQCRC2] (Ab14745 MitoSciences – 1:2,000), Complex IV [subunit I] (459600, Invitrogen – 1:1,000), Complex V [ATP5A] (Ab14748, MitoSciences – 1:2,000). For SDS-PAGE, antibodies for Complex III and IV were substituted with: Complex III [subunit core I] (459140/D2035, Invitrogen – 1:3,000), Complex IV [subunit I] (MS404/E0594 MitoSciences – 1:2,000). Membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies and visualized using enhanced chemiluminescence. Bands were quantified using ImageJ and data was normalized to protein content as assessed by Ponceau S staining of membranes.
References