

## Experimental procedures

### Flow chart

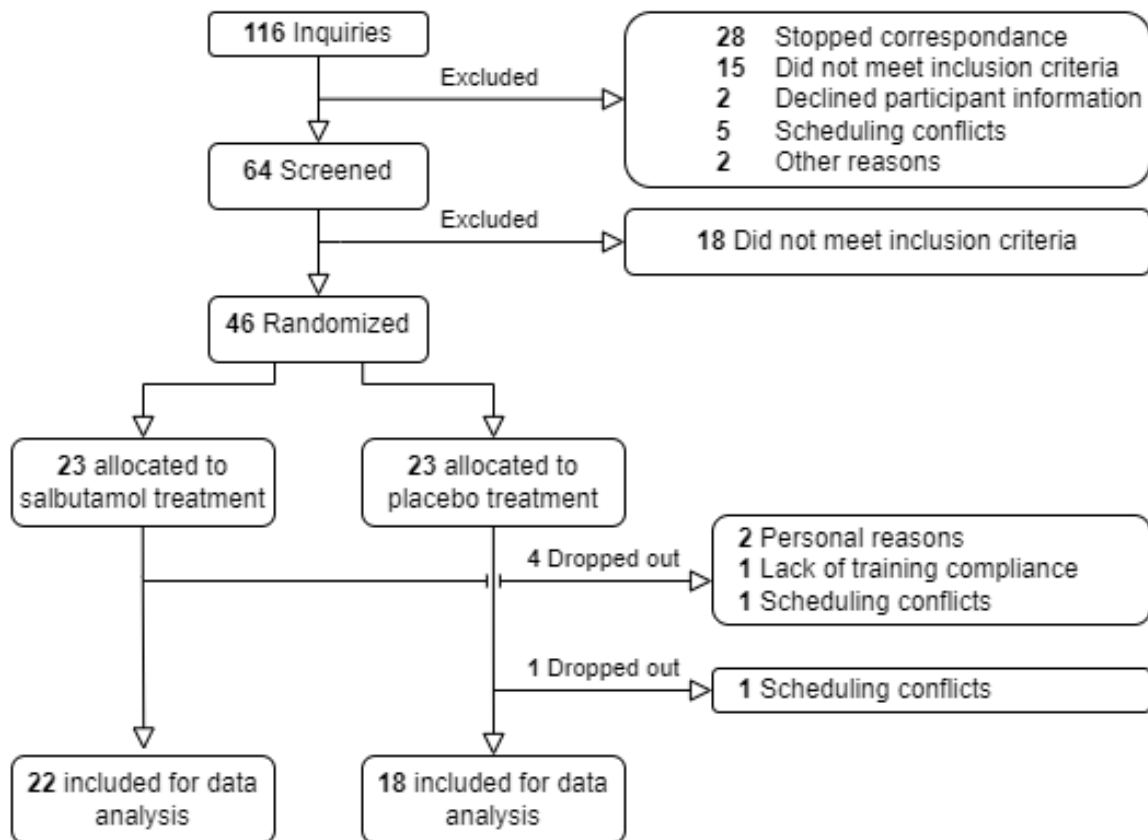


Figure 1. Participant flow diagram.

### Dual-Energy X-ray absorptiometry

DXA scans were performed using a Lunar iDXA (GE Healthcare, Brøndby, Denmark) which was calibrated daily according to manufacturer's instructions. Participants rested on the scanner bed in the supine position for 10 min prior to the scanning to minimize potential effects of body fluid shifts<sup>1</sup>. Each scan was conducted in duplicate to minimize inter-scan variation and the mean of the two scans was used. The scanner was calibrated before each experimental trial day, using daily calibration procedures (Lunar "System Quality Assurance").

### Muscle biopsy

Muscle biopsies were collected under local anaesthesia (20 mg/mL Xylocain without epinephrine; AstraZeneca, Cambridge, UK) through a small incision in the skin over the *vastus lateralis*. Biopsies were collected with a modified Bergström needle with suction<sup>2</sup>. Upon collection, the muscle biopsy piece was rinsed in saline (9 mg/mL, Fresenius Kabi, Sweden), frozen liquid nitrogen, and stored at -80°C<sup>0</sup> until analysis.

### *Immunoblotting and SDS-page*

Protein contents were determined by Western blotting. Approximately 1 mg d.w. muscle tissue was homogenized for 1 min at 30 Hz on a shaking bead-mill (TissueLyser II, Qiagen, Valencia, CA, USA) in ice-cold lysis buffer containing: 10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES (pH 7.5), 1% NP-40, 20 mM  $\beta$ -glycerophosphate, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 10 mM NaF, 2 mM PMSF, 1 mM EDTA (pH 8), 1 mM EGTA (pH 8) 10  $\mu$ g/mL aprotinin, 10  $\mu$ g/mL leupeptin, and 3 mM benzamidine. Samples were rotated end-over-end for 30 min at 4°C and centrifuged (18,320  $\times$  g) for 20 min at 4°C. The protein concentration of each sample was determined in triplicate with a BSA kit (Thermo Fisher Scientific, MA, US) and samples were created in duplicate with 6 $\times$  Laemmli buffer (7 mL 0.5 M Tris-base, 3 mL glycerol, 0.93 g DTT, 1 g SDS, and 1.2 mg bromophenol blue) and ddH<sub>2</sub>O to achieve equal protein concentration. Equal amounts of protein were loaded in wells of pre-cast 4-15% gels (Bio-Rad Laboratories, CA, US) with all samples for each participant loaded on the same gel. Proteins were then separated according to their molecular weight by SDS-PAGE and semi-dry transferred to a PVDF membrane (Millipore A/S, Copenhagen, Denmark). Membranes were blocked for 15 min in either 2% skim milk or 3% BSA in tris-buffered saline (TBS) containing 0.1% Tween-20 (TBST) before an overnight incubation in primary antibody at 4°C and a subsequent incubation in horseradish peroxidase conjugated secondary antibody at room temperature for 1 h. Bands were visualized with ECL (Millipore) and recorded with a digital camera (ChemiDoc MP Imaging System, Bio-Rad Laboratories). Bands were quantified using Image Lab version 6.0 (Bio-Rad Laboratories) and determined as the total band intensity adjusted for background intensity. Primary antibodies used were:  $\beta$ <sub>2</sub>-adrenoceptor: EPR707(N) (#ab182136, Abcam, Cambridge, UK); SERCA1: VE121G9 (#MA3-912, ThermoFisher Scientific, Waltham, MA, USA); SIRT3: D22A3 (#5490, Cell Signaling Technology, Herlev, Denmark); Desmin: D33 (#M076029-2, Dako, Glostrup, Denmark). Secondary antibodies used were:  $\beta$ <sub>2</sub>-adrenoceptor:

Goat Anti-Rabbit (1:5000; #4010-05, SouthernBiotech, Birmingham, AL, USA); SERCA1: Goat Anti-Mouse (1:5000; #P0447, Dako, Glostrup, Denmark); SIRT3: Goat Anti-Rabbit (1:5000; #4010-05, SouthernBiotech, Birmingham, AL, USA); Desmin: Goat Anti-Mouse (1:5000; #P0447, Dako, Glostrup, Denmark).

#### *Fiber type determination with gel electrophoresis*

Muscle fiber type content was determined by myosin heavy chain (MHC) isoform separation. We used the sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) method. The immunoblotting sample preparations were diluted in heavy sample buffer (1:1 100% glycerol to Laemmli buffer) to reach a protein concentration of 0.3 mg/l. Equal amounts of protein were loaded in wells of self-casted gels (separation gel: 30% glycerol, 8% acrylamide, 200 mM Tris-HCl with pH 8.80, 100 mM glycine, 0.4% SDS, 0.1% APS, 0.5% TCE, 0.05% TEMED; stacking gel: 30% glycerol, 4% acrylamide, 70 mM Tris-HCl with pH 6.80, 4 mM EDTA, 0.4% SDS, 0.1% APS, 0.05% TEMED). A lower running buffer (50 mM Tris, 75 mM glycine, 0.05% SDS) and a top running buffer (300 mM Tris, 75 mM glycine, 0.3% SDS) with 1 mM DTT added just before start were used. Electrophoresis ran in ice boxes for 16 h at a constant voltage of 73 V followed by 24 h at a constant current (10 mA). Bands were visualized by 5 min of UV exposure and recorded with a digital camera (ChemiDoc MP Imaging System, Bio-Rad Laboratories). Bands were quantified using Image Lab version 6.0 (Bio-Rad Laboratories) and determined as the total band intensity adjusted for background intensity. Results are expressed as the ratio of MHC type I to MHC type II.

#### *Maximal voluntary contraction*

During measurements of the contractile properties of the quadriceps muscle, participants were positioned on a table with their right leg fixed in a knee joint angle of 90° of flexion. The participants were sitting upright with thighs parallel to the floor and a 90° angle of flexion in the hips. Hands were positioned on handlebars on each side for further tension support. Isometric contraction force was recorded using a strain gauge (Tedeo-Huntleigh) strapped around the right ankle just above the malleoli. Strain gauge signal was fed to an amplifier connected to a computer. Data were recorded at 1 kHz in LabChart 8 (ADInstruments). Before, during and immediately after each MVC, superimposed percutaneous electrical muscle stimulations were delivered to the vastus lateralis muscle and

rectus femoris muscle by two self-adhesive electrodes (PALS Platinum 5 × 9 cm; Alexgaard Manufacturing, Lystrup, Denmark). Electrodes were placed on the skin 25% distal from spina iliaca anterior superior and 25% proximal from patella covering m. vastus lateralis and m. rectus femoris. Muscle stimulations were produced by a constant current stimulator (Stimulator model DS7AH; Digitimer, Hertfordshire, UK) in rectangular pulses of 1 ms. All participants received a progressive familiarization to stimulation intensity at the screening. During the experimental trials an intensity of 999 mA was applied. To determine the degree of voluntary activation (VA) level, a single stimulation was delivered on top of the plateau of each MVC. A single stimulation was delivered 1 s following relaxation of each MVC to determine potentiated peak twitch force ( $T_{Pot}$ ). During MVC measurements, participants received verbal encouragement with no visual feedback.

The following parameters were determined: MVC (N): highest force during an MVC;  $T_{Pot}$  (N): highest force during a potentiated single stimulation 1 s following relaxation from an MVC; half-relaxation time (HRT, ms): time from peak twitch force until force reached half of peak twitch force; and time-to-peak twitch force (TPT, ms): time from single stimulation until peak twitch force was reached.

Degree of VA level was calculated from the single twitches using the following equation <sup>3</sup>:

$$VA = \left[ 1 - \left( \frac{T_S}{T_{Pot}} \right) \right] \times 100$$

where  $T_S$  is the superimposed twitch delivered on top of the plateau of the MVC and  $T_{Pot}$  is the potentiated twitch delivered following relaxation after an MVC. A correction was applied to the equation if the superimposed stimulation was delivered slightly before or after the peak MVC <sup>4</sup>.

## References

- 1 Berg, H. E., Tedner, B. & Tesch, P. A. Changes in lower limb muscle cross-sectional area and tissue fluid volume after transition from standing to supine. *Acta Physiol Scand* **148**, 379-385, doi:10.1111/j.1748-1716.1993.tb09573.x (1993).
- 2 Bergström, J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* **35**, 609-616 (1975).
- 3 Bachasson, D. *et al.* Quadriceps function assessment using an incremental test and magnetic neurostimulation: A reliability study. *Journal of Electromyography and Kinesiology* **23**, 649-658, doi:<https://doi.org/10.1016/j.jelekin.2012.11.011> (2013).

- 4 Strojnik, V. & Komi, P. V. Neuromuscular fatigue after maximal stretch-shortening cycle exercise. *J Appl Physiol* (1985) **84**, 344-350, doi:10.1152/jappl.1998.84.1.344 (1998).