Translational studies of chronic supplementation with a mitochondria-targeted antioxidant to improve physical function with ageing

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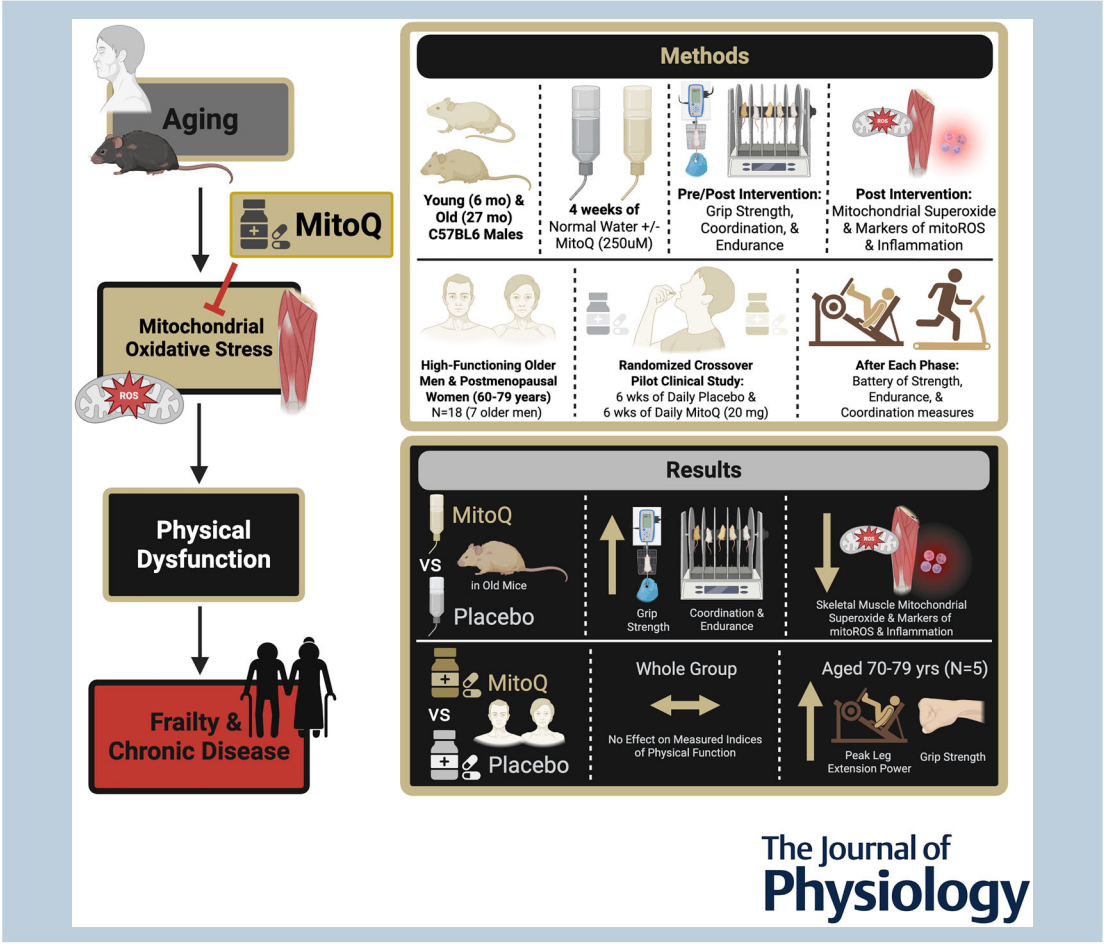

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Abstract figure legend Chronic oral supplementation with the mitochondria-targeted antioxidant MitoQ (4 weeks; 250 μM) mitigates age-related physical dysfunction in old mice, which is associated with reductions in skeletal muscle mitochondria-specific superoxide and markers of mitochondrial reactive oxygen species (mitoROS)-related oxidative stress and inflammation. The effects of MitoQ in old mice did not directly translate to humans as there were no convincing effects of MitoQ on measures of motor function in a randomized, placebo-controlled, cross-over design clinical trial of 6 weeks of 20 mg day^{-1} MitoQ *vs*. placebo. However, in participants aged 70 years or older ($N = 5$), we observed possible evidence of efficacy of MitoQ supplementation for improving select measures of strength.

Abstract Declines in physical function with advancing age increase the risk of functional limitations and chronic disease. Excess mitochondrial reactive oxygen species (mitoROS)-related oxidative stress is linked to physical dysfunction with ageing, but the effects of therapies targeting excess mitoROS on age-associated physical dysfunction are unclear. Here, we determined the efficacy of the mitochondria-targeted antioxidant MitoQ for improving multiple domains of physical function, first in old mice and then in high-functioning older adults in a randomized, placebo-controlled, cross-over design clinical trial. In old male C57BL6/N mice ($N = 22$–26; 27 months), we found that 4 weeks of treatment with MitoQ (250 μM in the drinking water) attenuated the age-related decline in grip strength, co-ordination, and endurance without effects in young mice ($N = 18$–20; 6 months). The effects of MitoQ in old mice were accompanied by lower levels of skeletal muscle mitochondria-specific superoxide production and markers of mitoROS-related oxidative stress (i.e. phosphorylated SHC adaptor protein 1, isoform p66) and inflammation (i.e. interleukin-6, tumour necrosis factor-alpha, interferon-gamma). In the clinical trial, we did not observe convincing effects of 6 weeks of MitoQ (20 mg day^{-1}) treatment on physical function in healthy older adults ($N = 18$; aged 60–79 years). However, exploratory subgroup analyses suggest possible effects of MitoQ on peak leg extension power and grip strength in participants ≥70 years of age. Our findings provide preclinical, proof-of-concept evidence for targeting excess mitoROS with MitoQ to reverse physical dysfunction with ageing. Although the effects of MitoQ did not directly translate to high functioning older adults, our initial observations suggest MitoQ may have greater efficacy in older, more physically frail individuals.

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Key points

- Excess mitochondrial reactive oxygen species (mitoROS)-related oxidative stress is linked to physical dysfunction with ageing, but the effects of therapies targeting excess mitoROS on age-associated physical dysfunction are unclear.
- In old mice, chronic supplementation with the mitochondria-targeted antioxidant MitoQ improves measures of physical function, which was accompanied by reductions in mitochondria-specific superoxide production in skeletal muscle.
- The effects of MitoQ in old mice did not directly translate to humans as there were no convincing effects on measures of motor function in a randomized, placebo-controlled, cross-over design clinical trial of 6 weeks of 20 mg day^{-1} MitoQ.
- However, in participants ≥70 years of age, we observed possible evidence of efficacy of MitoQ supplementation for improving select measures of strength.
- Future clinical trials with MitoQ and possibly other mitochondria-targeted antioxidant approaches for enhancing physical function with ageing should focus on older adults of more advanced age or more frail clinical populations.

Introduction

Advancing age is associated with reductions in multiple domains of physical function, including skeletal muscle strength (Celis-Morales et al., 2018), co-ordination (Tieland et al., 2018) and endurance (Bemben, 1998). Declines in physical function increase the risk of functional limitations, disabilities, loss of independence and other chronic diseases of ageing (Guralnik et al., 1995; James et al., 2024; Kennedy et al., 2014; Rantanen et al., 1999). One mechanism that contributes to age-associated physical dysfunction is increased skeletal muscle reactive oxygen species (ROS)-related oxidative stress (Chen et al., 2022; Sonjak et al., 2019; Szentesi et al., 2019), a key source of which is excess ROS production by mitochondria (mitoROS). Establishing interventions that decrease mitoROS-associated oxidative stress holds promise for improving physical function with ageing to reduce the risk of future disability.

Healthy mitochondria are necessary for maintaining skeletal muscle function, including through the production of physiological levels of mitoROS, which exert key cellular signalling functions (Ahn et al., 2019; Grevendonk et al., 2021; Hood et al., 2019). Excess production of mitoROS, however, can impair skeletal muscle function in part by activating/phosphorylating SHC adaptor protein 1, isoform p66 (p66 SHC) (Barbieri & Sestili, 2012; Granatiero et al., 2017; Potes et al., 2024), a marker and regulator of mitoROS-related oxidative stress that promotes mitoROS in a feedforward manner (Barbieri & Sestili, 2012; Granatiero et al., 2017; Potes et al., 2024). Excess mitoROS also stimulates pro-inflammatory cytokines, driving skeletal muscle inflammation (Chen et al., 2022; Peake et al., 2010; Wang et al., 2017). MitoROS-related oxidative stress increases in skeletal muscle with ageing (Chistiakov et al., 2014; Dai et al., 2014; Grevendonk et al., 2021; Mansouri et al., 2006; Sakellariou, Pearson, Lightfoot, Nye, Wells, Giakoumaki, Vasilaki et al., 2016; Seo et al., 2010, 2016; Sonjak et al., 2019) and experimentally augmenting mitochondrial antioxidant capacity can improve skeletal muscle function in old mice (Umanskaya et al., 2014). However, effective pharmacological treatments that target excess mitoROS to ameliorate physical dysfunction with ageing remain to be established.

Mitochondria-targeted antioxidants represent an innovative approach for reducing mitoROS to improve physiological function in states of excess mitoROS-related oxidative stress. MitoQ is a mitochondria-targeted antioxidant consisting of the naturally occurring antioxidant ubiquinol conjugated to a lipophilic cation; the lipophilicity and positive charge of this biochemical modification enable MitoQ to cross cell membranes and drive the compound to the inner mitochondrial membrane where it is optimally positioned to decrease mitoROS-related oxidative stress (Murphy & Smith, 2007; Smith & Murphy, 2010). We recently showed that supplementation with MitoQ for 4 weeks completely ameliorated age-related vascular dysfunction in old mice (Gioscia-Ryan et al., 2014, 2018). We then translated these findings in a randomized, placebo-controlled, cross-over design pilot clinical trial in older men and postmenopausal women and demonstrated that older men and postmenopausal women had improved vascular function after 6 weeks of MitoQ supplementation compared to placebo (Rossman et al., 2018). However, the efficacy of chronic supplementation with MitoQ for improving physical function with ageing is unknown.

In this translational investigation, we first tested the hypothesis that targeting excess mitoROS with chronic oral MitoQ supplementation in old mice would attenuate age-related declines in multiple domains of physical function and that the improvements in physical function would be associated with lower levels of skeletal muscle mitochondria-specific superoxide production and protein markers of mitochondrial oxidative stress [i.e. phosphorylated (p)-p66 SHC] and inflammation [i.e. interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ)], as well as higher protein levels of a marker of mitochondria-specific antioxidant capacity (i.e. superoxide dismutase 2, mitochondrial; SOD2). We then sought to gain initial insight into the potential effects of chronic oral MitoQ supplementation on physical function in older men and postmenopausal women in a randomized, double-blind, placebo-controlled, cross-over design clinical trial.

Kevin O. Murray has completed his postdoctoral fellowship in Dr Douglas Seals' Integrative Physiology of Aging laboratory at the University of Colorado Boulder. His training focused on determining the role of and targeting mitochondrial oxidative stress for vascular and physical dysfunction with ageing. He has accepted a position to continue in this area of research as faculty at Colorado State University in the Department of Food Science and Human Nutrition. **Rachel Gioscia-Ryan** earned her PhD from the University of Colorado, studying mitochondria-targeted strategies to mitigate age-related physiological dysfunction. She completed medical school and residency in anaesthesiology at the University of Michigan, followed by fellowship training in critical care and (currently) adult cardiothoracic anaesthesiology at Brigham and Women's Hospital. She aspires to work as a physician-scientist with research focused on identifying pragmatic interventions for clinical dilemmas in perioperative and critical care settings.

Methods

Preclinical study

Ethical approval for animals. All studies were approved by the Institutional Animal Care and Use Committee at the University of Colorado Boulder (Ethics Committee Reference # 2539) and conformed to the Guide for the Care and Use of Laboratory Animals (2016).

Animals. Male C57BL6/N mice, an established model of age-related physical dysfunction (Clayton et al., 2022; Justice, Carter et al., 2014; Justice, Gioscia-Ryan et al. 2015; Murray et al., 2024), were purchased from the ageing colony at the National Institute of Aging (National Institutes of Health, Bethesda, MD, USA) at ∼4 and ∼25 months of age and allowed to acclimate to our facilities for 2 weeks before beginning treatment. Mice were housed in standard cages under a 12:12 h light/dark photocycle and were provided with normal rodent chow (Harlan 7917; Harlan, Indianapolis, IN, USA) and water *ad libitum*. Body mass and water intake were monitored daily throughout the study. At the time of terminal tissue collection, mice were killed using an approach consistent with the American Veterinary Medical Association guidelines (Leary et al., 2020), as previously described (Casso et al., 2022). Mice were anaesthetized with 2% inhaled isoflurane (open-drop delivery in anaesthesia box). The depth of anaesthesia was confirmed by the absence of pedal withdrawal reflex. Mice were then killed by exsanguination via cardiac puncture.

MitoQ treatment. Mice were randomly assigned to treatment with MitoQ [250 μM; in the form of Mitoquinone mesylate adsorbed to β-cyclodextrin (22% MitoQ by weight) from Antipodean Pharmaceuticals, San Francisco, CA, USA]. We have shown that this dose and duration of treatment is effective for reducing mitoROS and improving vascular function in old mice (Gioscia-Ryan et al., 2014, 2018; Rodriguez-Cuenca et al., 2010; Smith & Murphy, 2010). Young MitoQ-treated (YMQ, ∼6 months, $n = 18$) and old MitoQ-treated (OMQ, ∼27 months, $n = 22$) mice were compared with mice provided normal drinking water [young control (YC, ∼6 months, $n = 20$) and old control (OC, ∼27 months, $n = 26$)] for 4 weeks. MitoQ was prepared fresh from powder (the preparation is water-soluble) and administered in light-protected water bottles changed every 3 days.

Experimental measurements

Physical function. Physical function testing was administered over a 3 day period. Forelimb grip strength (day 1) and accelerating (day 2) and endurance (day 3) rota-rod run ability were assessed, as previously described by our laboratory (Clayton et al., 2022; Justice, Carter et al., 2014; Justice, Gioscia-Ryan et al. 2015; Murray et al., 2024).

Grip strength of the forelimbs was assessed using a customized grip strength device which contained a force transducer (0.5 kg; PS Series; Imada Inc., Northbrook IL, USA) attached to a trapeze grip of ∼1.5 in mm diameter. Briefly, the mouse was grasped by the tail, suspended just above the trapeze bar, and lowered until it successfully grasped the bar with both forepaws. A gradual horizontal tug was then applied until the mouse released its grip. Five trials were taken with 30 s between trials. Trials in which the mouse forcefully displaced the bar rather than simply releasing its grasp were included (Clayton et al., 2022; Justice, Carter et al., 2014; Justice, Gioscia-Ryan et al. 2015; Murray et al., 2024, 2025).

Co-ordination was measured as latency to fall from an accelerating rota-rod (Ugo Basile, Comerio, Italy) over three trials. Mice were subjected to three trials separated by an inter-trial interval of ∼1 h. The rota-rod speed was increased during each trial from 4 to 40 rpm over a 5 min period and a cut-off time was set at 6 min. On the day prior to testing, each mouse was introduced to the test by placing it on the rota-rod until it was able to maintain its balance while the rod accelerated for 90 s (Clayton et al., 2022; Justice, Carter et al., 2014; Justice, Gioscia-Ryan et al. 2015; Murray et al., 2024).

Endurance tests were conducted 24–48 h after the accelerating rota-rod test. The maximal speed each mouse could sustain on the three accelerating rota-rod trials was used to set the standardized submaximal speed for the rota-rod run. Mice with similar maximal speeds ran at the same time on the five-panel rota-rod. The test comprised four consecutive phases: refresh, warm-up, endurance 1 and endurance 2. In the refresh period, the rota-rod was accelerated to 25% of maximal baseline speed and maintained for 2 min. Next, the mice performed a warm-up run during which the rota-rod was accelerated to 50% of maximum for 5 min. Mice that fell during either the refresh or warm-up phases were immediately placed back on the rota-rod to continue running. Subsequently, the rota-rod was accelerated to 75% of maximum speed for the endurance 1 phase and the time to fall from the rota-rod was recorded. If a mouse did not fall after 10 min, the rota-rod was further accelerated to 100% of maximum speed for the endurance 2 phase for up to 30 min or until a fall. The speed of each phase was recorded and used to determine the total distance ran across all phases (Clayton et al., 2022; Justice, Carter et al., 2014; Justice, Gioscia-Ryan et al. 2015; Murray et al., 2024).

Skeletal muscle mitochondria-specific superoxide production. Measurement of mitochondria-specific superoxide production in the soleus skeletal muscle was

performed using electron paramagnetic resonance (EPR) spectroscopy (Abdel-Rahman et al., 2016; Griendling et al., 2016; Jackson, 2016; Jackson et al., 1985). This was assessed in a similar manner to prior studies completed by our laboratory measuring mitochondria-specific superoxide production in segments of the thoracic aorta (Clayton et al., 2020; Gioscia-Ryan et al., 2014, 2016). Briefly, the soleus skeletal muscle was removed and dissected from the hind leg of the old mice. Segments of the soleus were incubated for 1 h at 37°C in Krebs-Hepes buffer with the mitochondria superoxide-specific spin probe MitoTEMPO-H (0.5 mM; Enzo Life Sciences, Inc., Long Island, NY, USA) for detection of mitochondria-specific superoxide production (Clayton et al., 2020; Gioscia-Ryan et al., 2014, 2016). The signal amplitude was analysed using an MS300 X-band EPR spectrometer (Magnettech GmbH, Berlin, Germany) with the following settings: centerfield, 3350 G; sweep 80 G; microwave modulation, 3000 mG; and microwave attenuation, 7 dB (Clayton et al., 2020; Gioscia-Ryan et al., 2014, 2016).

Skeletal muscle markers of mitochondrial oxidative stress and antioxidant capacity: western blot. Skeletal muscle abundance of proteins related to mitochondrial oxidative stress was determined in quadriceps skeletal muscle homogenate by a standard western blot, as previously reported (de Picciotto et al., 2016; Fleenor et al., 2012; Gioscia-Ryan et al., 2018; Justice, Gioscia-Ryan et al. 2015; LaRocca et al., 2014).

Briefly, quadriceps skeletal muscles were homogenized in ice-cold radioimmunoprecipitation assay 1x lysis buffer (10x RIPA buffer base, 100 mM Na_3VO_4, 0.1 M phenylmethylsulfonyl fluoride, 250 mM sodium fluoride and 100 mM sodium pyrophosphate) containing protease and phosphatase inhibitors [Protease Inhibitor Cocktail Tablet (Roche, Indianapolis, IN, USA) and 0.01% phosphatase inhibitor cocktail (Sigma, St Louis, MO, USA)]. Protein concentration was determined using the Pierce BCA assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Lysates were mixed 1:4 with 2x Laemmli Buffer (BioRad, Hercules, CA, USA). Samples were then denatured at 95°C for 10 min prior to loading. Equal amounts of protein (15 μg) were loaded into each lane of 4–12% stain-free TGX gels (Bio-Rad). After loading the protein, electrophoresis was performed at 150 V for 60 min with the chamber surrounded by ice (Criterion System; Bio-Rad). Proteins were transferred to a nitrocellulose membrane with a Trans-Blot Turbo transfer pack (BioRad). Membranes were incubated (overnight at 4°C) with primary antibodies: phosphorylated-p66 SHC (dilution 1:500, p-p66 SHC, anti-mouse, ab54518; Abcam, Cambridge, UK), superoxide dismutase isoform 2, mitochondrial (dilution 1:1500, SOD2, catalog. no. ADI-SOD-111-D; Enzo Life Sciences, Inc.), and glyceraldehyde 3-phosphate dehydrogenase (dilution 1:1,000, normalizer, GAPDH, catalog. no. 2118; Cell Signaling, Danvers, MA, USA). After primary antibody incubation, the membranes were washed in Tris-buffered saline-Tween 20 (TBS-T) (3 × 10 min), incubated in secondary antibody (Jackson ImmunoResearch, West Grove, PA, USA) for 60 min at room temperature, washed in TBS-T (3 × 10 min), and then rinsed in 1× TBS prior to imaging. Proteins were visualized on a digital acquisition system (ChemiDoc-It; UVP, Upland, CA, USA) in ECL substrate (Pierce, Rockford, IL, USA). Relative intensity was quantified using ImageJ (NIH, Bethesda, MD, USA) and normalized to GAPDH intensity (obtained from the same blots after antibody stripping) and then expressed as a ratio of the mean intensity of the young control group.

Skeletal muscle inflammatory cytokines: enzyme-linked immunosorbent assay (ELISA). Briefly, quadriceps skeletal muscles were homogenized in ice-cold radioimmunoprecipitation assay 1x lysis buffer (10x RIPA buffer base, 100 mM Na_3VO_4, 0.1 M phenylmethylsulfonyl fluoride, 250 mM sodium fluoride and 100 mM sodium pyrophosphate) containing protease and phosphatase inhibitors [Protease Inhibitor Cocktail Tablet (Roche) and 0.01% phosphatase inhibitor cocktail (Sigma)]. Protein concentration was determined using the Pierce BCA assay kit (Thermo Fisher Scientific). Equal amounts of protein (15 μg) were used to determine abundance of the pro-inflammatory cytokines IL-6, TNF-α and IFN-γ by multiplex ELISA (Mouse Inflammatory Cytokine Kit; Aushon Biosystems; Billerica, MA, USA) in accordance with the the manufacturer's instructions, as described previously (Clayton et al., 2022; Gioscia-Ryan et al., 2018).

Pilot clinical study

We assessed measures of physical function as exploratory outcomes during our previously completed clinical trial (registered on ClinicalTrials.gov, NCT02597023), in which we reported the effects of MitoQ on vascular function (Rossman et al., 2018), with the goal of informing the design of a potential future clinical trial focused on physical function. Measures of physical function were obtained in 18 of the 20 participants who completed the pilot clinical study assessing the effect of MitoQ on vascular function in older adults (Rossman et al., 2018). The remaining two participants opted out of completing these measures.

Ethical approval for humans. The study conformed with the *Declaration of Helsinki*. All procedures were reviewed and approved by the institutional review board (Ethics Committee Reference # 15-0402) at the University of Colorado at Boulder. The nature, benefits and risks of all

study procedures as well as the ability to withdraw from the study at any time were explained to the volunteers, and their written informed consent was obtained before their participation in the study.

Participants. As previously reported in our study describing the effects of MitoQ on vascular function in healthy older adults (Rossman et al., 2018), high-functioning older men and postmenopausal women aged 60–79 years from Boulder County, Colorado, and the surrounding areas were studied. All participants were non-smokers and free of overt clinical diseases, including peripheral artery disease (ankle-brachial index >0.90) and advanced cardiovascular disease as determined by medical history, physical examination, blood chemistries, and blood pressure and electrocardiogram at rest and during incremental treadmill exercise. Potential participants were excluded if they had abnormal blood chemistries, alcohol dependence, uncontrolled thyroid disease, severe obesity (body mass index >40 kg m^{-2}) or were not weight stable (defined as >2.5 kg change in body mass) for at least 3 months before enrolling in the study.

Study design, randomization and intervention. As previously described (Rossman et al., 2018), the pilot clinical trial used a 2 × 6-week randomized, double-blind, placebo-controlled, cross-over design. Participants consumed MitoQ (MitoQ Limited, Auckland, New Zealand) at a dose of 20 mg day^{-1} or identical placebo capsules for 6 weeks before crossing over to the other treatment arm in a randomly determined order. Randomization was performed by a member of the study team not involved in the assessment of outcomes, and a block randomization scheme stratified for age and sex was used. MitoQ or placebo capsules were taken once each morning with breakfast. Every 2 weeks during the active treatment and placebo phases, in-person check-in visits were performed to exchange intervention capsules (a precise number of capsules were allocated until the participant's next visit) and to assess participant adherence by survey and capsule count.

Safety and tolerability have been previously reported (Rossman et al., 2018). Briefly, no treatment-related serious adverse events occurred during the pilot clinical study. In total, four treatment-emergent adverse events were reported by four of the 24 participants enrolled in the study. All self-reported adverse events were mild in severity and included gastrointestinal discomfort ($n = 1$) during the MitoQ condition and gastrointestinal discomfort ($n = 2$) and diarrhoea ($n = 1$) under the placebo condition. None of the enrolled participants dropped out of the study because of side effects (Rossman et al., 2018).

Measurements. All measurements were performed after a 12 h fast from food (water allowed) and caffeine; a 24 h abstention from alcohol, physical activity, prescription medications and the study compound (MitoQ and placebo); and a 48 h abstention from over-the-counter medications and supplements. The study team was blinded to treatment condition during physical function data acquisition and analysis (Rossman et al., 2018).

Participant characteristics. Body mass index was determined by anthropometry (Lohman et al., 1992) and arterial systolic and diastolic blood pressures were assessed in triplicate over the brachial artery at rest with a semi-automated device (Dinamap XL; Johnson & Johnson, New Brunswick, NJ, USA) at the end of each intervention phase and at check-in visits. Leisure-time physical activity was determined by the Modifiable Activity Questionnaire at the end of each intervention phase (Pereira et al., 1997).

Blood samples were drawn from an intravenous catheter placed in an antecubital vein at screening and after each treatment phase. The Colorado Clinical and Translational Sciences Institute Clinical Translational Research Center Core Laboratory and Boulder Community Hospital Clinical Laboratory performed the following blood assays, as previously described (DeVan et al., 2016; Rossman et al., 2018). Fasting serum lipids were determined with standard assays. Fasting plasma glucose was measured by reflective spectrophotometry (Ortho Clinical Diagnostics, Raritan, NJ, USA). Plasma oxidized low-density lipoprotein and serum IL-6 were assessed by ELISA (Mercodia, Uppsala, Sweden). Serum high-sensitivity C-reactive protein (CRP) was measured by immunoturbidimetry (Beckman Coulter, Brea, CA, USA) (Rossman et al., 2018). In addition, EDTA-treated plasma collected 24 h after capsule ingestion following each 6 week intervention period was obtained to determine the effects of chronic MitoQ supplementation on circulating MitoQ levels in plasma. Plasma samples were analysed by reversed-phase liquid chromatography using gradient elution with acetonitrile, water and formic acid, and the deuterated compound (d_{15}-MitoQ) was used as an internal standard, as previously described (Li et al., 2007; Rossman et al., 2018).

Physical function. Physical function was assessed with select measures of the NIH Toolbox Motor Battery, as previously described (Craighead et al., 2024; Reuben et al., 2013), including domains of strength (grip strength test), dexterity (9-hole pegboard dexterity test and mobility (4 m walk gait speed test). Grip strength was determined as the maximum force produced on a hand dynamometer over three attempts with the dominant hand. The results from the pegboard test are presented as the average from

two attempts with the dominant hand. The 4 m walk gait speed test is reported as the fastest of three attempts.

In addition to the NIH Toolbox, strength (leg extensor strength and peak power with leg press), mobility (stair ascent test), endurance [6 min walk test and treadmill endurance (during a modified Balke treadmill protocol)], cardiorespiratory fitness ($\dot{V}_{O_2max}$ determined using a modified Balke treadmill protocol), balance (rapid step test time and number of errors) and fatigability (heel-rise test; fatigue severity scale questionnaire) were also evaluated with other established measures as described previously by our laboratory and others (Cho et al., 2004; Craighead et al., 2024; Evans et al., 1995; Justice, Mani et al., 2014; Justice, Johnson et al., 2015; Kirn et al., 2016; Laboratories, 2002; Lunsford & Perry, 1995; Santos-Parker et al., 2017; Vestergaard et al., 2009). Briefly, leg extensor strength was defined as the highest force produced from a maximum of eight trials, whereas peak lower body power was determined as the maximal power produced during five leg press attempts with resistance set at 40% and 70% of an individual's maximal lower body leg strength. The stair ascent test was calculated as the average of three trials. The 6 min walk test was quantified from a single attempt. The rapid step test time and number of errors were quantified as the average of three tests, whereas the heel-rise test was a single attempt.

For $\dot{V}_{O_2max}$, treadmill speed was set to reach ∼75% of each participant's age-predicted maximal heart rate. Treadmill grade was initially 0% and then increased by 2% every 2 min until volitional exhaustion, which was determined as the participant-expressed inability to continue treadmill exercise, despite strong encouragement to continue by the study team. Breath-by-breath expired gas volume and composition were collected and analysed using the Ultima CPX metabolic stress testing system (MGC Diagnostics, Saint Paul, MN, USA). Exercise tolerance was assessed as total treadmill exercise time during the modified Balke $\dot{V}_{O_2max}$ test.

Statistical analysis

Statistical analyses were performed with G*Power 3.1 (http://www.gpower.hhu.de) and Prism, version 10 (GraphPad Software Inc., San Diego, CA, USA). Prior to the analysis of the study outcomes, normality of each variable was assessed with the Shapiro–Wilk test. One outlier was identified in each the old control and old MitoQ groups for the p-p66 SHC western blot (Grubbs test, $\alpha < 0.05$). For preclinical studies, changes in indices of physical function and bodyweight were analysed with three-way repeated measures mixed model ANOVAs with Tukey's multiple comparisons test. Effects of time (pre- *vs.* post-intervention; within group), age (young *vs.* old; between groups), treatment (placebo *vs.* MitoQ; between groups) and the interaction (time × age × treatment) were assessed. Levels of MitoQ intake through the drinking water, mitochondria-specific superoxide production in soleus skeletal muscle and protein levels of markers of mitoROS-related oxidative stress and inflammation in quadriceps skeletal muscle were analysed using an unpaired *t* test. Mouse characteristics measured only post-intervention period were analysed using an ordinary two-way ANOVA with Tukey's multiple comparisons test. Clinical physical function outcomes were analysed with paired *t* tests. For preclinical outcomes, data are presented as the mean ± SD. For clinical outcomes, clinical characteristics are presented as the the mean ± SD and physical function data are presented as the mean ± 95% confidence interval. Statistical significance was set *a priori* at $\boldsymbol{\alpha} < 0.05$.

Results

Preclinical study

Mouse characteristics. MitoQ consumption across the 4 week treatment period was similar to our previous studies (Gioscia-Ryan et al., 2014, 2018) and not different between young (2.45 ± 0.68 mL water day^{-1}; 0.61 ± 0.17 mmol MitoQ day^{-1}) and old mice (2.36 ± 1.22 mL water day^{-1}; 0.59 ± 0.31 mmol MitoQ day^{-1}) ($P = 0.828$). Select morphological characteristics are shown in Table 1. Although there were differences in body ($P < 0.001$) (see Appendix, Table A1), heart ($P < 0.001$) (see Appendix, Table A2) and quadriceps ($P = 0.0080$) (see Appendix, Table A2) masses with ageing, we did not observe an effect of receiving MitoQ *vs.* normal drinking water in young or old mice on these morphological indices, consistent with our previous studies (see Appendix, Tables A1 and A2) (Gioscia-Ryan et al., 2014, 2018).

MitoQ treatment improves grip strength in old mice. At baseline, absolute grip strength was 20% lower ($P = 0.0070$) (Fig. 1*A* and *B*; see also Appendix, Table A3) and grip strength normalized to body mass (Fig. 1*C* and *D*; see also Appendix, Table A4) was 30% lower ($P < 0.001$) in old control (OC) mice compared to young control (YC) mice. Absolute grip strength was not changed ($P = 0.076$) but grip strength normalized to body mass was lower ($P = 0.0420$) over 4 weeks in OC mice (Fig. 1*A*–*D*; see also Appendix, Tables A3 and A4). Absolute grip strength (Fig. 1*A* and *B*; see also Appendix, Table A3) and grip strength normalized to body mass (Fig. 1*C* and *D*; see also Appendix, Table A4) were improved with MitoQ in old (OMQ) mice by 19% ($P < 0.001$) and 25% ($P < 0.001$) to overcome the decrease in grip strength normalized to body mass in OC mice over the 4 week intervention period and to mitigate the age-related reduction in absolute grip strength and grip strength normalized to body mass by

49% and 45%, respectively. There were no effects of MitoQ on absolute ($P = 0.995$) or normalized grip strength ($P = 0.998$) in young MitoQ (YMQ) mice (Fig. 1*A*–*D*; see also Appendix, Tables A3 and A4).

MitoQ supplementation improves measures of co-ordination and endurance in old mice. Co-ordination was measured as latency to fall from a constantly accelerating rota-rod (Fig. 2). At baseline, latency to fall was 42% lower ($P < 0.001$) in OC mice compared to YC mice (Fig. 2*A* and *B*; see also Appendix, Table A5). Latency to fall did not change during the intervention period in OC mice ($P = 0.0780$) (Fig. 2*A* and *B*; see also Appendix, Table A5). Latency to fall improved in OMQ mice by 44% ($P < 0.001$) to ameliorate the age-related reduction in co-ordination (Fig. 2*A* and *B*; see also Appendix, Table A5). There were no effects of MitoQ in YMQ mice ($P > 0.999$) (Fig. 2*A* and *B*; see also Appendix, Table A5).

The results of the latency to fall test were also used to determine the speeds utilized during the endurance run test (i.e. longer latency to fall results in faster set speed during endurance run test) (Fig. 3). As a result, OC and YC mice had similar endurance run times ($P = 0.997$) (Fig. 3*A* and *B*; see also Appendix, Table A6), but old mice had 43% shorter endurance run distances ($P = 0.0020$) (Fig. 3*C* and *D*; see also Appendix, Table A7). Endurance run time ($P = 0.889$) and endurance run distance ($P > 0.999$) did not change over 4 weeks in OC mice (Fig. 3*A*–*D*; see also Appendix, Tables A6 and A7). Endurance run time improved by 95% ($P < 0.001$) and endurance run distance improved by 71% ($P < 0.001$) (Fig. 3*A*–*D*; see also Appendix, Tables A6 and A7) in OMQ mice to reverse the ageing-related reduction in endurance run distance (Fig. 3*A*–*D*; see also Appendix, Tables A6 and A7). There were no effects of MitoQ on endurance run time ($P > 0.999$) or distance in YMQ mice ($P > 0.999$) (Fig. 3*A*–*D*; see also Appendix, Tables A6 and A7).

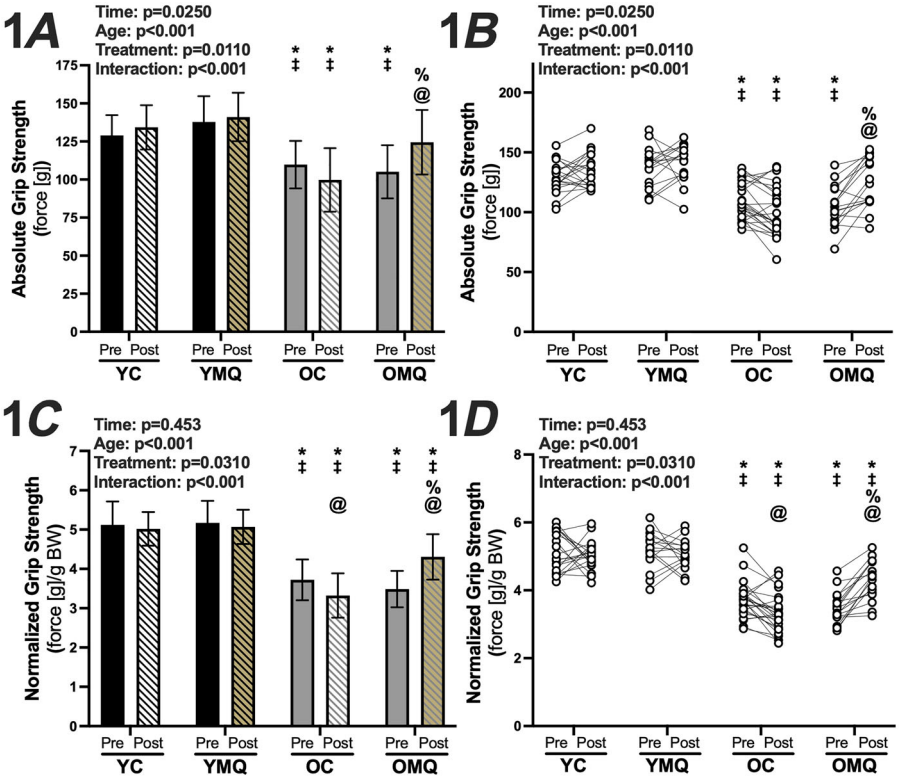

Figure 1. Grip strength and grip strength normalized to body mass before and after the 4-week intervention period in young and old mice
Absolute grip strength (*A* and *B*). Grip strength normalized to body mass (*C* and *D*). $P < 0.05$ with respect to *young control (YC) pre- or post-intervention; ‡young MitoQ (YMQ) pre- or post-intervention; %old control (OC) post-intervention; and @pre-intervention within group. Three-way repeated measures mixed model ANOVA with Tukey's multiple comparisons *post hoc* test corrected for multiple comparisons. Exact *P* values are reported in the Appendix (Tables A3 and A4). OMQ, old MitoQ. $N = 16$–25 per group, YC = 20; YMQ = 16; OC = 25; OMQ = 17. Data presented as mean ± SD.

Table 1. Mouse characteristics

Group	Young control		Young MitoQ		Old control		Old MitoQ		
Number, males	20		18		26		22		
Time point	Pre	Post	Pre	Post	Pre	Post	Pre	Post	P value
Body mass (g)	25.5 ± 3.6	26.3 ± 2.7	25.9 ± 3.3	27.1 ± 3.0	29.7 ± 3.4*‡	30.0 ± 3.3*	30.1 ± 2.2*‡	28.9 ± 2.8	0.169
Heart mass (mg)		137 ± 16		131 ± 14		183 ± 32*‡		174 ± 30*‡	0.833
Liver mass (g)		1.5 ± 0.26		1.4 ± 0.12		1.6 ± 0.3		1.4 ± 0.4	0.504
Quadriceps mass (mg)		170 ± 25		189 ± 29		140 ± 22*‡		142 ± 23*‡	0.213
Visceral fat mass (mg)		284 ± 98		233 ± 57		268 ± 101		224 ± 74	0.893

Body mass was measured pre and post the 4 week intervention period. All other measures were assessed only post the 4 week intervention period. Young control/MitoQ mice (~6 months of age); old control/MitoQ mice (~27 months of age). $P < 0.05$ with respect to *young control pre- or post-intervention and ‡young MitoQ pre- or post-intervention. Body mass analysed via three-way repeated measures mixed model ANOVA and organ masses analysed with an ordinary two-way ANOVA. Each corrected with Tukey's multiple comparisons test. The *P* value for each respective interaction is reported in the final column. All *P* values are reported in the Appendix (Tables A1 and A2). Data are presented as the mean ± SD.

Mitochondria-specific superoxide production and protein markers of mitoROS-associated oxidative stress and inflammation were lower in skeletal muscle of old mice following MitoQ treatment. To investigate possible underlying mechanisms contributing to the effects of MitoQ on physical function in old mice, we assessed levels of mitochondria-specific superoxide production via electron paramagnetic resonance spectroscopy in the soleus skeletal muscle and protein abundance of key markers of mitoROS-related oxidative stress, mitochondria-specific antioxidant capacity, and inflammation in quadriceps skeletal muscle from old mice.

Levels of mitochondria-specific superoxide production were lower in OMQ mice compared to OC mice ($P = 0.0331$) (Fig. 4*A*). Additionally, activated (phosphorylated) p66 SHC (Fi. 4*B*; see also Appendix, Fig. A1), a marker and regulator of mitoROS-induced oxidative stress, was lower ($P = 0.00270$), whereas the mitochondria-specific superoxide scavenger (superoxide dismutase 2, mitochondrial; SOD2) (Fig. 4*C*; see also Appendix, Fig. A1) was unchanged ($P = 0.375$) in OMQ mice compared to OC mice. Lastly, pro-inflammatory cytokines IL-6 ($P = 0.0414$), TNF-α ($P = 0.00580$) and IFN-γ ($P = 0.0748$) (Fig. 4*D*–*F*) were generally found to be lower in OMQ mice compared to OC mice.

Pilot clinical trial

After observing evidence of efficacy of chronic MitoQ supplementation for improving physical function in old mice, we sought to obtain initial insight into the effects of MitoQ on physical function in older adults. We included a comprehensive battery of physical function measures

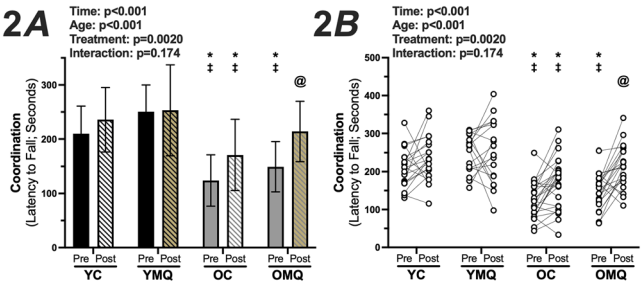

Figure 2. Co-ordination before and after the 4 week intervention period in young and old mice
$P < 0.05$ with respect to *young control (YC) pre- or post-intervention; ‡young MitoQ (YMQ) pre- or post-intervention; and @pre-intervention within group. Three-way repeated measures mixed model ANOVA with Tukey's multiple comparisons *post hoc* test corrected for multiple comparisons. Exact *P* values are reported in the Appendix (Table A5). OC, old control; PMQ, old MitoQ. $N = 18$–26 per group, YC = 20; YMQ = 18; OC = 26; OMQ = 22. Data presented as the mean ± SD.

as ancillary outcomes in our previously completed pilot, randomized, placebo-controlled cross-over clinical trial focused on vascular function in older men and post-menopausal women (Rossman et al., 2018).

Participants. Participant characteristics, exclusion, withdrawal, adherence, safety and vascular outcomes for this study have been previously reported (Murray et al., 2023; Rossman et al., 2018). Overall, measures of physical function were obtained in 18 (seven older men and 11 postmenopausal women) of the 20 participants who completed the pilot clinical study assessing the effect of MitoQ on vascular function in older adults (Rossman et al., 2018). The remaining two participants opted out of completing these measures.

Participant characteristics. In previous studies, we showed that older adults had higher vascular endothelial function, higher plasma levels of MitoQ (Murray et al., 2023; Rossman et al., 2018) and lower plasma levels of oxidized low-density lipoprotein after 6 weeks of oral MitoQ supplementation vs. placebo (Murray et al., 2023; Rossman et al., 2018). We also found that aortic stiffness was lower after MitoQ in a subset of participants who exhibited age-associated aortic stiffening under placebo conditions (Rossman et al., 2018). All other participant characteristics were not different between conditions (Table 2).

Effects of MitoQ supplementation on physical function in older adults. The measures of physical function assessed were not different between placebo and MitoQ conditions in the whole group ($N = 18$) (Table 3) or sex-based analyses [postmenopausal women ($N = 11$) (see Appendix, Table A8) and older men ($N = 7$) (see Appendix, Table A9)].

To assess whether age might influence responsiveness to the intervention, we performed exploratory subgroup analyses in participants aged 60–69 ($N = 13$) years *vs.* 70–79 years ($N = 5$). We observed initial evidence of differences in two of our measured indices: peak leg extension power ($P = 0.0113$) (Fig, 5*A*; see also Appendix,

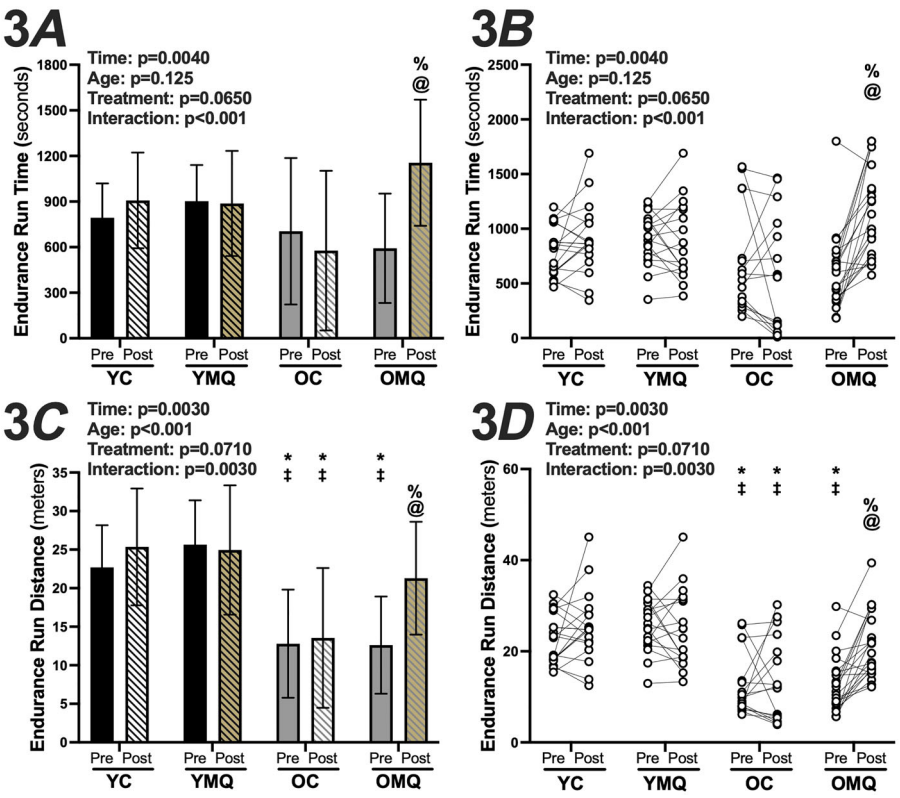

Figure 3. Endurance measures before and after the 4 week intervention period in young and old mice
Endurance run time (*A* and *B*) and endurance run distance (*C* and *D*). $P < 0.05$ with respect to *young control (YC) pre- or post-intervention; ‡young MitoQ (YMQ) pre- or post-intervention; %old control (OC) post-intervention; and @pre-intervention within group. Three-way repeated measures mixed model ANOVA with Tukey's multiple comparisons *post hoc* test corrected for multiple comparisons. Exact *P* values are reported in the Appendix (Tables A6 and A 7). OMQ, old MitoQ. $N = 18$–21 per group, YC = 19; YMQ = 18; OC = 16; OMQ = 21. Data presented as the mean ± SD.

Table 2. Participant characteristics

Participant characteristics			
N (men/women)	18 (7/11)		
Age	67.2 ± 4.7		
Treatment	Placebo	MitoQ	*P* value
Body mass index (kg m^{-2})	23.3 ± 3.8	23.4 ± 3.7	0.190
Systolic blood pressure (mmHg)	112 ± 12	114 ± 17	0.489
Diastolic blood pressure (mmHg)	67 ± 6	66 ± 7	0.461
Resting heart rate (beats min^{-1})	64 ± 10	62 ± 10	0.124
Fasting glucose (mg dL^{-1})	84 ± 7	82 ± 6	0.207
Total cholesterol (mg dL^{-1})	196 ± 38	196 ± 40	>0.999
High-density lipoprotein (mg dL^{-1})	53 ± 14	54 ± 14	0.976
Low-density lipoprotein (mg dL^{-1})	124 ± 32	121 ± 34	0.683
Triglycerides (mg dL^{-1})	105 ± 83	109 ± 55	0.686
Physical activity energy expenditure (kcal week^{-1})	5223 ± 2263	5279 ± 3307	0.934

Paired *t* tests. Data presented as the mean ± SD. *N* = 18 per group.

Fig. A2*A* and *B*) and grip strength ($P = 0.0424$) (Fig. 5*B*; see also Appendix, Fig. A2*C* and *D*) were higher in older adults 70–79 years of age after 6 weeks of treatment with MitoQ compared to after 6 weeks of treatment with placebo. We did not observe an effect of MitoQ supplementation on peak leg extension power ($P = 0.264$) or grip strength ($P = 0.607$) in the whole group, nor in older men ($P = 0.530$; $P = 0.569$) or postmenopausal women ($P = 0.383$; $P = 0.568$), separately (Fig. 5*A* and *B*).

Discussion

In the present study, we showed that 4 weeks of supplementation with the mitochondria-targeted antioxidant MitoQ improved measures of strength, endurance and co-ordination in old mice and the improvements were associated with reduced levels of mitochondria-specific superoxide production and abundance of markers of mitochondrial oxidative stress and inflammation in skeletal muscle. By contrast to our observations in old

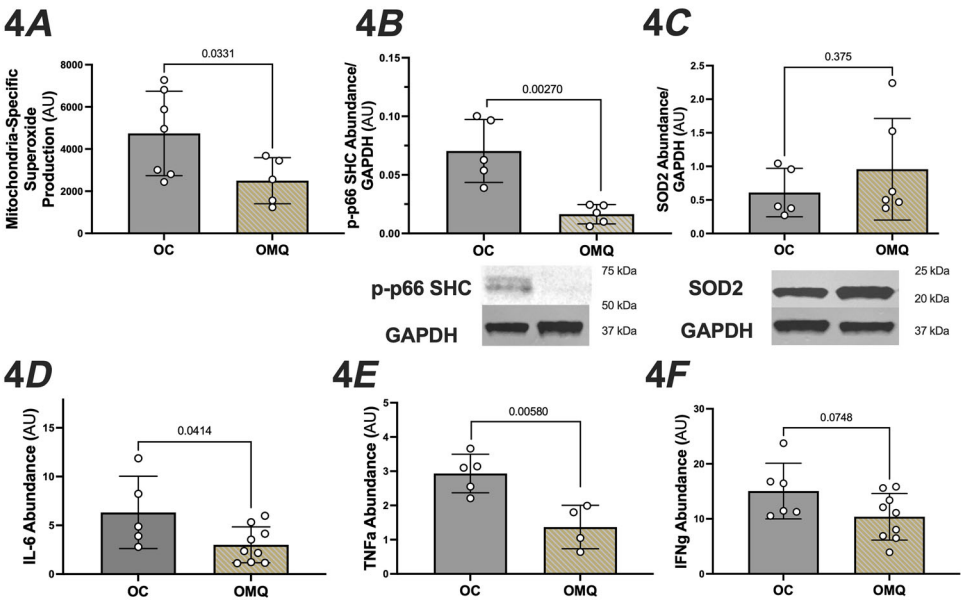

Figure 4. Mitochondria-specific superoxide production (soleus) and protein markers of mitoROS-derived oxidative stress, mitochondria-specific antioxidant capacity and inflammation (quadriceps) after the 4 week intervention period in skeletal muscle from old mice
Mitochondria-specific superoxide production (A) and protein markers of mitoROS-derived oxidative stress (B and C) and inflammation (D-F). N = 4–9 per group. YC, young control; YMQ, young MitoQ; OQ, old control; OMQ, old MitoQ. Data analysed via an unpaired *t* test. Data presented as the mean ± SD.

Table 3. Domains of physical function measured in older adults after 6 weeks of supplementation with placebo and after 6 weeks of supplementation with MitoQ (*N* = 10–18 per group)

Domain	Assessment	Placebo	MitoQ	Mean differences	*P* value
Skeletal muscle strength					
	Chair stand (s)	9.6 (8.4 to 10.8)	10.0 (9.1 to 10.9)	0.4 (–0.4 to 1.1)	0.290
	Knee flexion torque (J)	793.9 (694.5 to 893.3)	807.0 (718.3 to 895.7)	13.1 (–33.1 to 59.4)	0.556
	Knee extension torque (J)	837.4 (697.0 to 977.8)	808.4 (647.2 to 969.6)	−29.0 (–147.0 to 88.9)	0.609
Manual dexterity					
	9-hole pegboard (s)	20.9 (19.8 to 22.0)	21.2 (20.0 to 22.3)	0.3 (–0.9 to 1.4)	0.622
Mobility					
	4 m walk gait speed (m s^{-1})	2.9 (2.7 to 3.1)	3.1 (2.9 to 3.4)	0.2 (–0.07 to 0.4)	0.145
	Stair ascent (s)	3.7 (3.4 to 4.1)	3.7 (3.4 to 4.0)	−0.03 (–0.3 to 0.2)	0.799
Endurance and cardiorespiratory fitness					
	6 min walk (m)	581.9 (532.4 to 631.4)	581.9 (524.4 to 639.5)	0.02 (–20.6 to 20.6)	0.998
	Treadmill endurance (s)	556.5 (493.2 to 619.8)	562.3 (497.7 to 627.0)	5.8 (–29.5 to 41.2)	0.732
	$\dot{V}_{O_2max}$ (mL kg^{-1} m^{-1})	31.9 (28.2 to 35.5)	31.9 (27.6 to 36.2)	−0.01 (–1.0 to 1.0)	0.982
Balance					
	Rapid step test (s)	44.1 (41.2 to 47.1)	44.1 (40.9 to 47.3)	−0.04 (–1.8 to 1.8)	0.960
	Rapid step test error	2.8 (1.2 to 4.4)	2.3 (1.4 to 3.3)	−0.5 (–1.9 to 0.9)	0.479
Fatigability					
	Heel rise (s)	87.3 (56.7 to 117.9)	88.5 (63.4 to 113.6)	1.2 (–19.6 to 21.9)	0.900
	Fatigue severity scale	20.7 (15.4 to 26.0)	19.8 (15.2 to 24.4)	−0.6 (–4.1 to 3.1)	0.759

Paired *t* tests. Data presented as mean ± 95% confidence intervals. RM = repetition maximum.

mice, we did not observe convincing effects of chronic MitoQ supplementation on physical function in a small cohort of healthy, high-functioning older men and post-menopausal women. However, our preliminary subgroup analyses suggest possible effects of MitoQ on peak leg extension power and grip strength in participants ≥70 years of age, consistent with the idea of more pronounced effects in older and more frail individuals. Taken together, we provide preclinical evidence for the efficacy of MitoQ to improve select domains of physical function in old mice. Translation of these findings to healthy older adults was limited, but our preliminary observations support a need for future trials with MitoQ on physical function in older and/or more physically frail populations.

A decline in physical function with advancing age is a major independent risk factor for age-associated functional limitations, disabilities, loss of independence and other chronic diseases of ageing (Bemben, 1998; Celis-Morales et al., 2018; Guralnik et al., 1995; James et al., 2024; Kennedy et al., 2014; Rantanen et al., 1999; Tieland et al., 2018). There is accumulating evidence that mitoROS increases with ageing in skeletal muscle (Chistiakov et al., 2014; Dai et al., 2014; Grevendonk et al., 2021; Sakellariou, Pearson, Lightfoot, Nye, Wells, Giakoumaki, Vasilaki et al., 2016; Seo et al., 2010, 2016; Sonjak et al., 2019) and genetically enhancing mitochondrial antioxidant capacity can improve skeletal muscle function in old mice (Umanskaya et al., 2014). However, effective treatments targeting excess mitoROS for ameliorating age-related declines in physical function are lacking. Here, we show that 4 weeks of supplementation with the mitochondria-targeted antioxidant MitoQ mitigates the age-related decline in strength and reverses the decrease in co-ordination and endurance with ageing. The effects of MitoQ on physical function were specific to old age because we did not observe any effects in young animals. Importantly, the subdomains of physical function improved with MitoQ supplementation reflect important subdomains of physical function in humans (Bemben, 1998; Celis-Morales et al., 2018; Tieland et al., 2018).

A previous study did not observe any effects of MitoQ on isolated myofibres or skeletal muscle mitoROS in old mice (Sakellariou, Pearson, Lightfoot, Nye, Wells, Giakoumaki, Griffiths et al., 2016). However, the study used a lower dose of MitoQ (Sakellariou, Pearson, Lightfoot, Nye, Wells, Giakoumaki, Griffiths et al., 2016) and did not assess physical function *in vivo*. The absence of an effect of MitoQ in this prior study was probably related to an insufficient dose because excess mitoROS and its related oxidative stress (Fitts, 2008; Lamb & Westerblad, 2011; Powers & Schrager, 2022; Xu et al., 2025) and pro-inflammatory cytokines (Peake et al., 2010; Supinski & Callahan, 2007; Tuttle et al., 2020) have been shown to negatively affect myofibrillar force production and calcium handling/sensitivity. It is also possible that our observed improvements in physical function occurred in the absence of effects at the level of the contractile

apparatus (Enoka, 1988). Indeed, the observed effects on physical function may have been secondary to effects of MitoQ on skeletal muscle-associated cells involved in the regulation of skeletal muscle function, such as resident immune cells (Fortner et al., 2020), myoblasts/satellite cells (Pin et al., 2022) and/or fibroblasts (Goh et al., 2019), or potentially effects on neuromuscular co-ordination (Enoka, 1988). The latter idea is consistent with the effects of MitoQ supplementation on co-ordination in the present study. Lasty, the effects of MitoQ on physical function may have been related to cardiovascular adaptations (Dare et al., 2015; Gioscia-Ryan et al., 2014, 2018; Kim et al., 2020; Kirkman et al., 2023; Park et al., 2020; Ribeiro Junior et al., 2018).

Although the complete mechanisms are unknown, the antioxidant effects of MitoQ are considered to be related to the interaction of the active (reduced) ubiquinol form of MitoQ with mitoROS, which oxidizes ubiquinol to ubiquinone. Ubiquinone, in turn, is subsequently reduced to the active ubiquinol via reaction with mitochondrial respiratory chain complex II (Murphy & Smith, 2007; Smith & Murphy, 2010). MitoQ may also modulate mitochondrial superoxide production by reverse electron transport at mitochondrial respiratory chain complex I (Robb et al., 2018). In the present study, we found evidence that four weeks of treatment with MitoQ in the drinking water reduced levels of mitochondria-specific superoxide production and associated markers of mitoROS-related

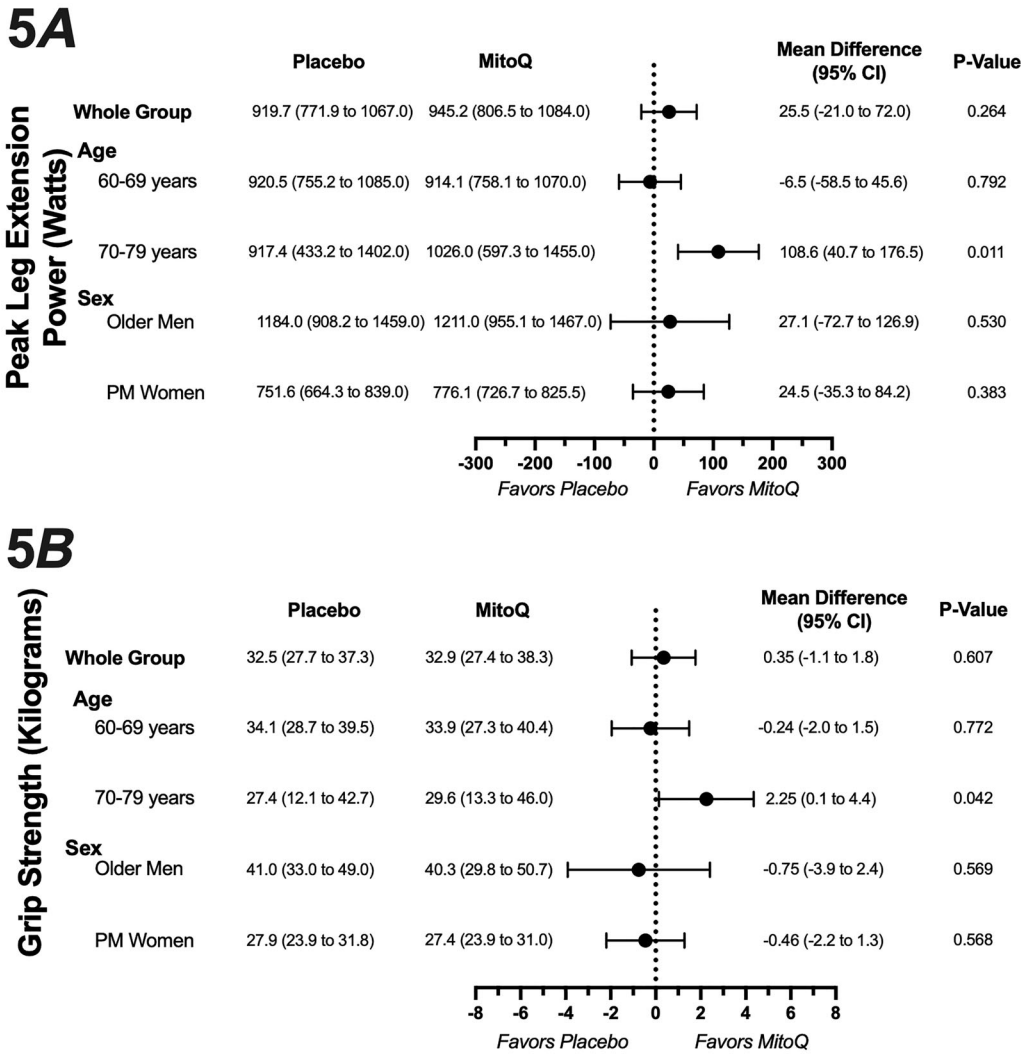

Figure 5. Forest plots for select assessments of physical function measured in older adults after 6 weeks of placebo supplementation and after 6 weeks of MitoQ supplementation
Peak leg extension power (*A*) and grip strength (*B*) are shown. Analyses performed in the whole group ($N = 18$) and subgroups stratified for age (60–69 years: $N = 13$; 70–79 years: $N = 4$–5) and sex [older men: $N = 7$; postmenopausal (PM) women: $N = 11$]. Paired t tests. Data presented as the mean ± 95% confidence interval.

oxidative stress (i.e. p-p66 SHC) in the absence of differences in mitochondria-specific antioxidant capacity (i.e. SOD2) in skeletal muscle. Taken together, these data are consistent with the idea that MitoQ supplementation reduces skeletal muscle mitoROS-related oxidative stress. These changes were accompanied by evidence of lower pro-inflammatory signalling in skeletal muscle, which supports the idea of reduced mitoROS-linked inflammation after MitoQ treatment. As such, our observations suggest reduced mitoROS-related oxidative stress and associated pro-inflammatory signalling are key mechanisms that may contribute to improvements in physical function with MitoQ in old mice.

Our findings extend observations with pharmaceutical-based interventions targeting mitochondria, such as the mitochondrial-targeted peptide elamipretide (SS-31), which has been shown to improve select indices of skeletal muscle function (Campbell et al., 2019; Pharaoh et al., 2023; Sahu et al., 2018; Siegel et al., 2013) and treadmill endurance (Campbell et al., 2019) in old mice. However, the mechanisms of action of SS-31 are considered to be distinct from MitoQ, as well as primarily related to charged interactions of the compound with phosphate and fatty acyl chains in cardiolipin (Birk et al., 2013; Mitchell et al., 2020; Szeto & Liu, 2018*a*) and associated effects on mitochondrial cristae density (Russo et al., 2022, 2024; Szeto & Liu, 2018*b*) and mitochondrial peroxidase activity (Birk et al., 2013).

We have shown that chronic oral supplementation with MitoQ improves vascular function in old mice (Gioscia-Ryan et al., 2014, 2018). More recently, we demonstrated that the effects of MitoQ on vascular function appear to translate to humans, as we showed in a randomized pilot clinical trial that 6 weeks of MitoQ supplementation improves vascular function in older adults (Murray et al., 2023; Rossman et al., 2018). Thus, as ancillary measures collected during this pilot clinical trial, we sought to determine whether the effects of MitoQ on physical function in old mice translate to humans. By contrast to our observations in old mice, we did not observe robust effects of MitoQ supplementation in our overall cohort of healthy older men and postmenopausal women. However, it should be noted that the participants in our trial were all healthy and largely high functioning because overall group means for $\dot{V}_{O_2max}$ (Kaminsky et al., 2022) and grip strength (Tomkinson et al., 2025) tests place these individuals above the median for their age, which probably limited our ability to observe general improvements. Indeed, the 27-month-old mice studied in our preclinical investigation are considered to be the corollary of ∼75 human years of age (Flurkey et al., 2007) and exhibit more severe age-related reductions in physical function (Liu et al., 2013).

To explore the possibility that the high functional status of our participants may have limited our ability to detect an effect of MitoQ and that individuals of more advanced age and/or greater dysfunction at baseline may stand to benefit more from intervention, we performed subgroup analyses restricted to the participants ≥70 years of age. Although our subgroup analyses were conducted in a small cohort, peak leg extension power was ∼12% higher after MitoQ compared to placebo. This finding is consistent with the notion that leg power is associated with quadriceps mitochondrial bioenergetic function in adults aged 70 and older (Mau et al., 2023). Given that a 9–10% improvement in leg extension power is considered clinically relevant and is in the range of that observed following a long-term resistance training physical therapy intervention (Kirn et al., 2016), the effect of MitoQ on leg extension power, if confirmed in a larger trial, has the potential to be clinically meaningful (Kirn et al., 2016). We also observed 2.2 kg higher grip strength after MitoQ *vs.* placebo in the ≥70 years of age subgroup. This effect also could be clinically impactful because grip strength represents an important biomarker of physical function and frailty that is linearly related with risk of all-cause mortality (Bohannon, 2019; Celis-Morales et al., 2018; Rantanen et al., 1999). In combination, our preliminary analyses support the idea that older and/or more frail populations may benefit from mitochondria-targeted antioxidant therapy, although these initial observations would need to be confirmed in a larger, properly powered clinical trial.

There are several limitations of the present study that should be acknowledged. First, the precise mechanism of action of MitoQ remains to be determined. We showed that MitoQ reduced several well-accepted markers of mitoROS and inflammation. However, given that the primary goal of our study was to assess effects of MitoQ on physical function, the sample size for some of our mechanistic measures was small and more studies are needed to determine how MitoQ modulates mitoROS in skeletal muscle. Second, because our clinical trial was a pilot study and the measures of physical function were ancillary outcomes, we did not assess whether the 20 mg day^{-1} dose of MitoQ leads to an accumulation of MitoQ in skeletal muscle or reduced mitoROS-related oxidative stress. However, this dose of MitoQ has been shown to lower maximal skeletal muscle mitochondrial H_2O_2 production (Broome et al., 2025), mildly suppress skeletal muscle mitochondrial respiration-associated H_2O_2 production (Pham et al., 2020) and augment the aerobic exercise training-induced increase in skeletal muscle gene expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (Broome et al., 2022). Furthermore, administration of a single, higher dose of MitoQ (160 mg) reduced skeletal muscle mitochondrial oxidative burden (lower PRDX3 dimerization) and improved skeletal muscle mitochondrial bioenergetics in

overweight and prediabetic middle-aged men (Fiorenza et al., 2024). This latter study also documented the ability of oral administration of MitoQ to increase levels of MitoQ in skeletal muscle (Fiorenza et al., 2024), providing some evidence that oral MitoQ can be taken up in skeletal muscle in humans as has also been shown in mice (Miquel et al., 2014).

Conclusions

Here, we have demonstrated preclinical proof-of-concept support for targeting excess mitoROS with the mitochondria-targeted antioxidant MitoQ with respect to improving physical function with ageing. By contrast to our observations in old mice, we observed limited effects of MitoQ on a variety of assessments of physical function in healthy, high-functioning older adults. However, we provide preliminary support for the idea that MitoQ may have greater efficacy in older and/or more physically frail individuals. Future trials with MitoQ and possibly other mitochondria-targeted antioxidant approaches for enhancing physical function with ageing should focus on older adults of more advanced age or more frail clinical populations. In addition, future studies should consider longer treatment durations and/or higher doses of MitoQ to overcome potential maladaptive skeletal muscle tissue remodelling because of chronic elevations in mitoROS with ageing.

Appendix A

Table A1. Body mass

Three-way ANOVA	*P* value
Time	0.0750
Age	<0.001
Treatment	0.982
Comparison	Adjusted *P* value
Pre:YC *vs.* Pre:YMQ	>0.999
Pre:YC *vs.* Pre:OC	<0.001
Pre:YC *vs.* Pre:OMQ	<0.001
Pre:YC *vs.* Post:YC	0.0850
Pre:YC *vs.* Post:YMQ	0.785
Pre:YC *vs.* Post:OC	<0.001
Pre:YC *vs.* Post:OMQ	0.0270
Pre:YMQ *vs.* Pre:OC	0.0140
Pre:YMQ *vs.* Pre:OMQ	0.01
Pre:YMQ *vs.* Post:YC	0.988
Pre:YMQ *vs.* Post:YMQ	0.367
Pre:YMQ *vs.* Post:OC	0.0050
Pre:YMQ *vs.* Post:OMQ	0.169
Pre:OC *vs.* Pre:OMQ	>0.999
Pre:OC *vs.* Post:YC	0.070
Pre:OC *vs.* Post:YMQ	0.308
Pre:OC *vs.* Post:OC	0.995
Pre:OC *vs.* Post:OMQ	0.994
Pre:OMQ *vs.* Post:YC	0.0490
Pre:OMQ *vs.* Post:YMQ	0.211
Pre:OMQ *vs.* Post:OC	>0.999
Pre:OMQ *vs.* Post:OMQ	0.322
Post:YC *vs.* Post:YMQ	>0.999
Post:YC *vs.* Post:OC	0.0270
Post:YC *vs.* Post:OMQ	0.524
Post:YMQ *vs.* Post:OC	0.169
Post:YMQ *vs.* Post:OMQ	0.827
Post:OC *vs.* Post:OMQ	0.955

All *P* values for all comparisons using Tukey's multiple comparisons test following a three-way repeated measures ANOVA for Table 1.

Table A2. Organ mass characteristics

Liver	
Two-way ANOVA	*P* value
Age	0.380
Treatment	0.020
Comparison	Adjusted *P* value
YC *vs.* YMQ	0.597
YC *vs.* OC	0.631
YC *vs.* OMQ	0.730
YMQ *vs.* OC	0.098
YMQ *vs.* OMQ	>0.999
OC *vs.* OMQ	0.171
Heart	
Two-way ANOVA	*P* value
Age	<0.001
Treatment	0.216
Interaction	0.833
Comparison	Adjusted *P* value
YC *vs.* YMQ	0.878
YC *vs.* OC	<0.001
YC *vs.* OMQ	<0.001
YMQ *vs.* OC	<0.001
YMQ *vs.* OMQ	<0.001
OC *vs.* OMQ	0.741
Quadriceps	
Two-way ANOVA	*P* value
Age	<0.001
Treatment	0.126
Comparison	Adjusted *P* value
YC *vs.* YMQ	0.190
YC *vs.* OC	0.0080
YC *vs.* OMQ	0.0350

Table A2. (Continued)

Liver	
YMQ *vs*. OC	<0.001
YMQ *vs*. OMQ	<0.001
OC *vs*. OMQ	0.997
Visceral fat mass	
Two-way ANOVA	*P* value
Age	0.596
Treatment	0.0490
Interaction	0.893
Comparison	Adjusted *P* value
YC *vs*. YMQ	0.332
YC *vs*. OC	0.960
YC *vs*. OMQ	0.336
YMQ *vs*. OC	0.673
YMQ *vs*. OMQ	0.993
OC *vs*. OMQ	0.619

All *P* values for all comparisons using Tukey's multiple comparisons test following an ordinary two-way ANOVA for Table 1.

Table A3. Absolute grip strength

Comparison	Adjusted *P* value
Pre:YC *vs*. Pre:YMQ	0.788
Pre:YC *vs*. Pre:OC	0.0070
Pre:YC *vs*. Pre:OMQ	0.0010
Pre:YC *vs*. Post:YC	0.856
Pre:YC *vs*. Post:YMQ	0.424
Pre:YC *vs*. Post:OC	<0.001
Pre:YC *vs*. Post:OMQ	0.993
Pre:YMQ *vs*. Pre:OC	<0.001
Pre:YMQ *vs*. Pre:OMQ	<0.001
Pre:YMQ *vs*. Post:YC	0.999
Pre:YMQ *vs*. Post:YMQ	0.995
Pre:YMQ *vs*. Post:OC	<0.001
Pre:YMQ *vs*. Post:OMQ	0.339
Pre:OC *vs*. Pre:OMQ	0.989
Pre:OC *vs*. Post:YC	<0.001
Pre:OC *vs*. Post:YMQ	<0.001
Pre:OC *vs*. Post:OC	0.0760
Pre:OC *vs*. Post:OMQ	0.127
Pre:OMQ *vs*. Post:YC	<0.001
Pre:OMQ *vs*. Post:YMQ	<0.001
Pre:OMQ *vs*. Post:OC	0.975
Pre:OMQ *vs*. Post:OMQ	<0.001
Post:YC *vs*. Post:YMQ	0.938
Post:YC *vs*. Post:OC	<0.001
Post:YC *vs*. Post:OMQ	0.666
Post:YMQ *vs*. Post:OC	<0.001
Post:YMQ *vs*. Post:OMQ	0.110
Post:OC *vs*. Post:OMQ	<0.001

All *P* values for all comparisons using Tukey's multiple comparisons test following a three-way repeated measures mixed model ANOVA for Fig. 1*A* and *B*.

Table A4. Normalized grip strength

Comparison	Adjusted *P* value
Pre:YC *vs*. Pre:YMQ	>0.999
Pre:YC *vs*. Pre:OC	<0.001
Pre:YC *vs*. Pre:OMQ	<0.001
Pre:YC *vs*. Post:YC	0.996
Pre:YC *vs*. Post:YMQ	>0.999
Pre:YC *vs*. Post:OC	<0.001
Pre:YC *vs*. Post:OMQ	<0.001
Pre:YMQ *vs*. Pre:OC	<0.001
Pre:YMQ *vs*. Pre:OMQ	<0.001
Pre:YMQ *vs*. Post:YC	0.990
Pre:YMQ *vs*. Post:YMQ	0.998
Pre:YMQ *vs*. Post:OC	<0.001
Pre:YMQ *vs*. Post:OMQ	<0.001
Pre:OC *vs*. Pre:OMQ	0.839
Pre:OC *vs*. Post:YC	<0.001
Pre:OC *vs*. Post:YMQ	<0.001
Pre:OC *vs*. Post:OC	0.0420
Pre:OC *vs*. Post:OMQ	0.0120
Pre:OMQ *vs*. Post:YC	<0.001
Pre:OMQ *vs*. Post:YMQ	<0.001
Pre:OMQ *vs*. Post:OC	0.974
Pre:OMQ *vs*. Post:OMQ	<0.001
Post:YC *vs*. Post:YMQ	>0.999
Post:YC *vs*. Post:OC	<0.001
Post:YC *vs*. Post:OMQ	0.0020
Post:YMQ *vs*. Post:OC	<0.001
Post:YMQ *vs*. Post:OMQ	0.001
Post:OC *vs*. Post:OMQ	<0.001

All *P* values for all comparisons using Tukey's multiple comparisons test following a three-way repeated measures mixed model ANOVA for Fig. 1*C* and *D*.

Table A5. Co-ordination

Comparison	Adjusted *P* value
Pre:YC *vs*. Pre:YMQ	0.380
Pre:YC *vs*. Pre:OC	<0.001
Pre:YC *vs*. Pre:OMQ	0.0190
Pre:YC *vs*. Post:YC	0.693
Pre:YC *vs*. Post:YMQ	0.294
Pre:YC *vs*. Post:OC	0.327
Pre:YC *vs*. Post:OMQ	>0.999
Pre:YMQ *vs*. Pre:OC	<0.001
Pre:YMQ *vs*. Pre:OMQ	<0.001
Pre:YMQ *vs*. Post:YC	0.994
Pre:YMQ *vs*. Post:YMQ	>0.999
Pre:YMQ *vs*. Post:OC	<0.001
Pre:YMQ *vs*. Post:OMQ	0.504
Pre:OC *vs*. Pre:OMQ	0.803
Pre:OC *vs*. Post:YC	<0.001
Pre:OC *vs*. Post:YMQ	<0.001
Pre:OC *vs*. Post:OC	0.0780

(*Continued*)

Table A5. (Continued)

Pre:OC *vs*. Post:OMQ	<0.001
Pre:OMQ *vs*. Post:YC	<0.001
Pre:OMQ *vs*. Post:YMQ	<0.001
Pre:OMQ *vs*. Post:OC	0.896
Pre:OMQ *vs*. Post:OMQ	<0.001
Post:YC *vs*. Post:YMQ	0.984
Post:YC *vs*. Post:OC	0.0050
Post:YC *vs*. Post:OMQ	0.926
Post:YMQ *vs*. Post:OC	<0.001
Post:YMQ *vs*. Post:OMQ	0.404
Post:OC *vs*. Post:OMQ	0.173

All *P* values for all comparisons using Tukey's multiple comparisons test following a three-way repeated measures mixed model ANOVA for Fig. 2.

Table A6. Endurance run time

Comparison	Adjusted *P* value
Pre:YC *vs*. Pre:YMQ	0.986
Pre:YC *vs*. Pre:OC	0.997
Pre:YC *vs*. Pre:OMQ	0.684
Pre:YC *vs*. Post:YC	0.899
Pre:YC *vs*. Post:YMQ	0.994
Pre:YC *vs*. Post:OC	0.681
Pre:YC *vs*. Post:OMQ	0.050
Pre:YMQ *vs*. Pre:OC	0.778
Pre:YMQ *vs*. Pre:OMQ	0.164
Pre:YMQ *vs*. Post:YC	>0.999
Pre:YMQ *vs*. Post:YMQ	>0.999
Pre:YMQ *vs*. Post:OC	0.184
Pre:YMQ *vs*. Post:OMQ	0.409
Pre:OC *vs*. Pre:OMQ	0.985
Pre:OC *vs*. Post:YC	0.745
Pre:OC *vs*. Post:YMQ	0.842
Pre:OC *vs*. Post:OC	0.889
Pre:OC *vs*. Post:OMQ	0.0080
Pre:OMQ *vs*. Post:YC	0.138
Pre:OMQ *vs*. Post:YMQ	0.217
Pre:OMQ *vs*. Post:OC	>0.999
Pre:OMQ *vs*. Post:OMQ	<0.001
Post:YC *vs*. Post:YMQ	>0.999
Post:YC *vs*. Post:OC	0.159
Post:YC *vs*. Post:OMQ	0.414
Post:YMQ *vs*. Post:OC	0.238
Post:YMQ *vs*. Post:OMQ	0.330
Post:OC *vs*. Post:OMQ	<0.001

All *P* values for all comparisons using Tukey's multiple comparisons test following a three-way repeated measures mixed model ANOVA for Fig. 3*A* and *B*.

Table A7. Endurance run distance

Comparison	Adjusted *P* value
Pre:YC *vs*. Pre:YMQ	0.912
Pre:YC *vs*. Pre:OC	0.0020
Pre:YC *vs*. Pre:OMQ	<0.001
Pre:YC *vs*. Post:YC	0.824
Pre:YC *vs*. Post:YMQ	0.979
Pre:YC *vs*. Post:OC	0.0050
Pre:YC *vs*. Post:OMQ	0.999
Pre:YMQ *vs*. Pre:OC	<0.001
Pre:YMQ *vs*. Pre:OMQ	<0.001
Pre:YMQ *vs*. Post:YC	>0.999
Pre:YMQ *vs*. Post:YMQ	>0.999
Pre:YMQ *vs*. Post:OC	<0.001
Pre:YMQ *vs*. Post:OMQ	0.555
Pre:OC *vs*. Pre:OMQ	>0.999
Pre:OC *vs*. Post:YC	<0.001
Pre:OC *vs*. Post:YMQ	<0.001
Pre:OC *vs*. Post:OC	>0.999
Pre:OC *vs*. Post:OMQ	0.0090
Pre:OMQ *vs*. Post:YC	<0.001
Pre:OMQ *vs*. Post:YMQ	<0.001
Pre:OMQ *vs*. Post:OC	>0.999
Pre:OMQ *vs*. Post:OMQ	<0.001
Post:YC *vs*. Post:YMQ	>0.999
Post:YC *vs*. Post:OC	<0.001
Post:YC *vs*. Post:OMQ	0.626
Post:YMQ *vs*. Post:OC	<0.001
Post:YMQ *vs*. Post:OMQ	0.755
Post:OC *vs*. Post:OMQ	0.0250

All *P* values for all comparisons using Tukey's multiple comparisons test following a three-way repeated measures mixed model ANOVA for Fig. 3*C* and *D*.

Table A8. Domains of physical function measured in postmenopausal women after 6 weeks of supplementation with placebo and after 6 weeks of supplementation with MitoQ (*N* = 5–11 per group)

Domain	Assessment	Placebo	MitoQ	Mean differences	*P* value
Skeletal muscle strength					
	Chair stand (s)	10.6 (9.0–12.1)	10.8 (9.6–12.0)	0.2 (−0.8 to 1.3)	0.641
	Leg extension 1RM (kg)	129.2 (114.5–143.9)	136.2 (112.9–159.6)	−7.0 (−25.9 to 11.6)	0.426
Manual dexterity					
	9-hole pegboard dexterity (s)	20.6 (19.0–22.3)	21.2 (19.6–22.8)	0.6 (−1.2 to 2.3)	0.499
Mobility					
	4 m walk gait speed (m/s)	3.0 (2.7–3.3)	3.1 (2.8–3.5)	0.1 (−0.2 to 0.4)	0.441
	Stair ascent (s)	3.9 (3.5–4.4)	3.9 (3.5–4.2)	−0.09 (−0.5 to 0.3)	0.593
Endurance and cardiorespiratory fitness					
	6 min walk (m)	535.6 (471.7–599.5)	527.6 (466.7–588.6)	−7.96 (−26.7 to 210.77)	0.366
	Treadmill endurance (s)	527.6 (454.4–600.9)	522.7 (429.3–616.2)	−4.9 (−53.3 to 43.5)	0.826
	$\dot{V}_{O_2max}$ (mL kg^{-1} m^{-1})	27.8 (23.9–31.6)	27.2 (22.4–32.0)	−0.6 (−1.9 to 0.8)	0.384
Balance					
	Rapid step test (s)	44.7 (40.2–49.3)	43.6 (39.0–48.2)	−1.1 (−3.0 to 0.7)	0.210
	Rapid step test error	2.2 (1.0–3.5)	2.2 (0.9–3.5)	−0.03 (−1.6 to 1.6)	0.967
Fatigability					
	Heel rise (s)	69.2 (34.4–103.9)	79.6 (29.3–130.9)	10.5 (−6.5 to 27.4)	0.144
	Fatigue severity scale	24.9 (16.3–33.5)	22.8 (15.6–30.0)	−2.1 (−8.1 to 3.9)	0.447

Paired *t* tests. Data presented as the mean ± 95% confidence intervals. RM = repetition maximum.

Table A9. Domains of physical function measured in older men after 6 weeks of supplementation with placebo and after 6 weeks of supplementation with MitoQ (*N* = 5–7 per group)

Domain	Assessment	Placebo	MitoQ	Mean dfferences	*P* value
Skeletal muscle strength					
	Chair stand (s)	8.1 (6.6–9.7)	8.8 (7.9–9.7)	0.7 (–0.8 to 2.1)	0.306
	Leg extension 1RM (kg)	225.3 (173.6–269.0)	221.3 (180.8–269.8)	4.0 (–10.1 to 18.2)	0.512
Manual dexterity					
	9-hole pegboard dexterity (s)	21.4 (19.6–23.2)	21.1 (19.0–23.3)	−0.3 (–1.5 to 0.9)	0.609
Mobility					
	4 m walk gait speed (m/s)	2.8 (2.4–3.3)	3.1 (2.5–3.7)	0.3 (−0.2 to 0.8)	0.221
	Stair ascent (s)	3.4 (2.8–3.9)	3.4(2.8–4.0)	0.07 (−0.2 to 0.3)	0.515
Endurance and cardiorespiratory fitness					
	6 min walk (m)	654.4 (605.3–704.1)	667.2 (573.2–761.2)	12.6 (−40.2 to 65.4)	0.582
	Treadmill endurance (s)	601.9 (464.2–739.5)	624.6 (540.6–708.5)	22.7 (−43.3 to 88.7)	0.432
	$\dot{V}_{O_2max}$ (mL kg^{-1} m^{-1})	38.3 (34.3–42.4)	39.2 (34.6–43.8)	0.8 (−1.0 to 2.7)	0.302
Balance					
	Rapid step test (s)	43.2 (38.9–47.6)	44.9 (39.1–50.7	1.7 (−2.3 to 5.7)	0.349
	Rapid step test error	3.7 (−0.8–8.1)	2.5 (–4.2 to 1.9)	−1.1 (−4.2 to 1.9)	0.389
Fatigability					
	Heel rise (s)	101.8 (43.3–160.4)	95.6 (52.3–138.9)	−6.3 (−49.6 to 37.1)	0.709
	Fatigue severity scale	13.9 (10.1–17.6)	15.6 (10.7–20.5)	1.7 (−1.9 to 5.4)	0.294

Paired *t tests*. Data presented as the mean ± 95% confidence intervals. RM = repetition maximum.

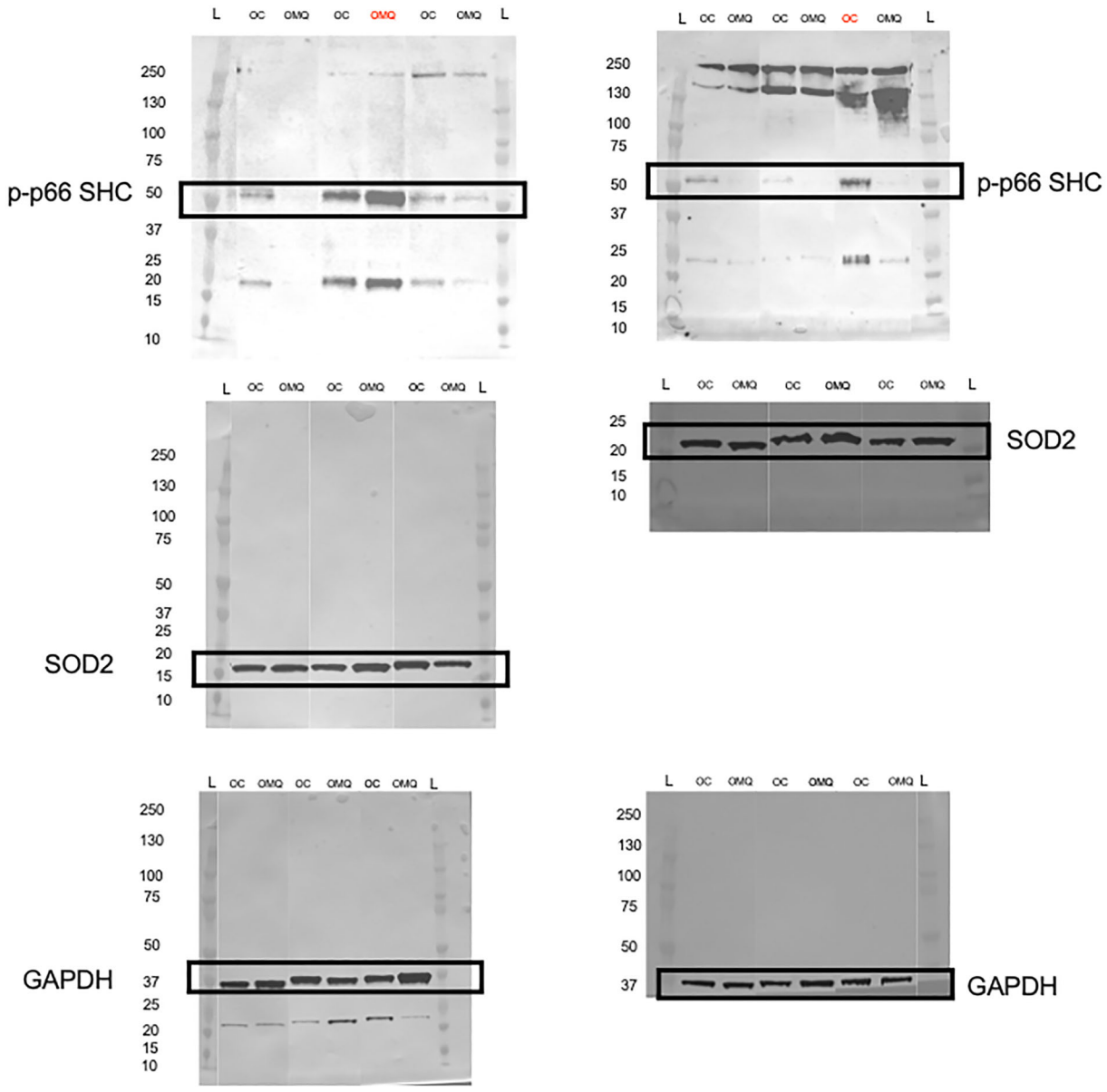

Figure A1.
Western blot images for targets p-p66SHC (phosphorylated SHC-transforming protein 1, p66Shc isoform) and SOD2 (superoxide dismutase 2, mitochondrial) and control GAPDH (glyceraldehyde 3-phosphate dehydrogenase) (Fig. 4*B* and *C*). Those lanes with a red header were identified as outliers within their respective groups: one each for the old control and old MitoQ groups in the p-p66 SHC measure and none reported in SOD2 (also reported in 'Methods'). Blots were cropped to put visually experimental groups one after another alternating.

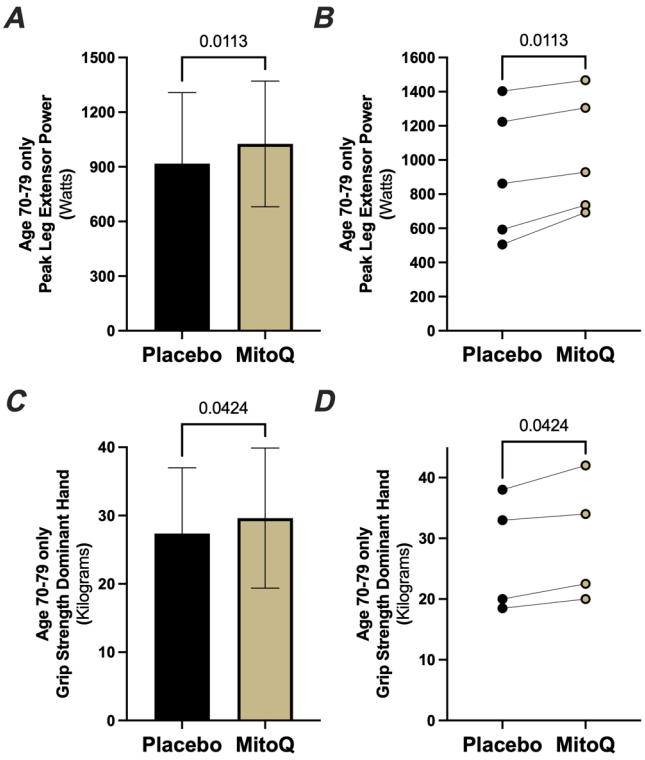

Figure A2.
Bar graph and individual data points for peak leg extensor power (*A* and *B*) and dominant hand grip strength (*C* and *D*) assessed after 6 weeks of placebo and after 6 weeks of chronic oral MitoQ supplementation in participants aged 70–79 years of age. $^*P < 0.05$. $N = 4$–5. Paired *t* tests. Data presented as the mean ± SD.

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Additional information

Data availability statement

Data are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Author contributions

M.J.R., J.R.S.P., R.A.G.R., M.C. and D.R.S. designed the initial studies. K.O.M., R.A.G.R., M.J.R., N.Z.B., S.D., L.M.C., J.R.S.P., D.A.H., J.N.J., Z.S.C. and J.M.D. acquired and analysed data. K.O.M., R.A.G.R., D.R.S. and M.J.R. interpreted data. K.O.M. created tables and figures. K.O.M. and M.J.R. wrote the initial draft. K.O.M., R.A.G.R, J.N.J, J.R.S.P, N.Z.B, S.D., L.M.C., D.A.H., J.M.D., Z.S.C., M.C., D.R.S. and M.J.R. edited and approved the final version of the manuscript submitted for publication.

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Keywords

inflammation, MitoQ, motor function, older adults, post-menopausal women, reactive oxygen species, skeletal muscle, superoxide

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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