



Effect of six weeks ubiquinol supplementation on mitochondrial respiratory function and exercise capacity in healthy males

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Abstract

Coenzyme Q10 (CoQ₁₀) is an integral component of the mitochondrial electron transfer system. Most studies have administered the oxidised form of CoQ₁₀ (ubiquinone) and observed no effects on mitochondrial respiratory function or endurance exercise performance. The reduced form of CoQ₁₀, ubiquinol (UQH₂), has greater bioavailability than ubiquinone, but the effects of UQH₂ supplementation on mitochondrial respiratory function and exercise capacity are unclear. Fifty-four healthy, recreationally active males were randomised to receive either 300 mg·day⁻¹ UQH₂ or placebo (PLA) for 6 weeks in a double-blind independent-group design. Before and after the supplementation period, skeletal muscle mitochondrial respiration variables and protein content of the mitochondrial leak proteins, adenine nucleotide translocase1+2 (ANT1+2) and uncoupling protein-3 (UCP-3), were assessed. In addition, participants completed a severe-intensity cycle test to exhaustion to assess time to the limit of tolerance (T_{Lim}) and oxygen uptake ($\dot{V}O_2$) kinetics. Compared to pre-supplementation and PLA, UQH₂ supplementation increased plasma [CoQ₁₀] ($P < 0.05$), and lowered inverse respiratory control ratio (Pre-PLA: 0.064 ± 0.034 vs. Post-PLA: 0.072 ± 0.026 ; Pre-UQH₂: 0.073 ± 0.039 vs. Post-UQH₂: 0.044 ± 0.019 ; $P < 0.05$), suggestive of improved oxidative phosphorylation coupling efficiency. There were no differences in ANT1+2 or UCP-3 protein content post-supplementation compared to pre-supplementation between groups ($P > 0.05$). End-exercise $\dot{V}O_2$, change in $\dot{V}O_2$ between 2 min and end-exercise, and T_{Lim} were not different between groups post-supplementation ($P > 0.05$). Six-weeks UQH₂ supplementation increased plasma [CoQ₁₀] and oxidative phosphorylation coupling efficiency, but did not alter mitochondrial leak proteins, T_{Lim} or $\dot{V}O_2$ kinetics during severe-intensity exercise in healthy, active males.

Keywords Mitochondrial respiratory efficiency · Exercise performance · Coenzyme Q10 · $\dot{V}O_2$ kinetics · Dietary supplement

Abbreviations

ADP	Adenosine diphosphate	CI–CIV	Mitochondrial protein complexes I–IV
ANT1+2	Adenine nucleotide translocase1+2	CI _L	Leak respiration through CI
BioPS	Biopsy preservation solution	CI _p	ADP-stimulated respiration through CI
		CI+II _p	ADP-stimulated respiration through CI+II

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	combined
CI+II _E	ETS capacity through CI+II
CII _E	ETS capacity through CII
CIV _E	ETS capacity through CIV
CIV _{res}	CIV reserve capacity
CoQ10	Coenzyme Q10
CS	Citrate synthase
E	Electron transfer system capacity
ETS	Mitochondrial electron transfer system
FCR	Respiratory flux control ratios
FOX-1	Ferrous oxidation in xylenol orange
GET	Gas exchange threshold
HDL	High-density lipoproteins
H ₂ O ₂	Hydrogen peroxide
inv-RCR	Inverse respiratory control ratio
L	Leak respiration
LCR	Leak control ratio
LDL	Low-density lipoprotein cholesterol
LOOH	Lipid hydroperoxides
MIR05	MIR05 respiration medium
OXPHOS	Oxidative phosphorylation
O ₂	Oxygen
P	ADP-stimulated respiration
PCR	Phosphorylation control ratio
PLA	Placebo
ROS	Reactive oxygen species
SCR	Substrate control ratio
T _{Lim}	Time to the limit of tolerance
UCP-3	Uncoupling protein-3
UQH ₂	Ubiquinol
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
$\dot{V}O_2 SC$	Oxygen uptake slow component

Introduction

Oxidative phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) in the mitochondrial electron transfer system (ETS) is the principal energy pathway used during endurance exercise (Bangsbo et al. 1990; Hargreaves and Spriet 2020). Coenzyme Q10 (CoQ₁₀) is a lipophilic molecule ubiquitously expressed in cell membranes, including the inner mitochondrial membrane, where it functions as an essential component of the mitochondrial ETS by undergoing continuous redox cycles (Rauchová 2021). The oxidised form of CoQ₁₀, ubiquinone, can be reduced to ubiquinol (UQH₂) by electrons transferred from mitochondrial complex I (via NADH), mitochondrial complex II (via FADH₂), glycerol 3-phosphate dehydrogenase (via glycolysis) and the electron-transferring flavoprotein (via fatty acid oxidation) (Gnaiger 2020). Subsequently, UQH₂

functions as a mobile electron carrier in the inner mitochondrial membrane, where it is oxidised by mitochondrial complex III, with the resultant ubiquinone able to recommence the aforementioned redox cycle (Rich and Maréchal 2010). Consequently, CoQ₁₀ availability is integral to preserve convergent electron transfer at the CoQ₁₀ junction (Q junction) and subsequent electron transfer along the remaining mitochondrial respiratory complexes. Indeed, impaired mitochondrial respiration, alongside compromised exercise capacity, is a characteristic feature of individuals with mitochondrial myopathies that lower mitochondrial CoQ₁₀ content. Importantly, these deficits can be abated with CoQ₁₀ supplementation (Artuch et al. 2006; Barca et al. 2016; Lalani et al. 2005).

Given that effective skeletal muscle mitochondrial respiration is an important determinant of endurance exercise performance in healthy adults (Granata et al. 2016 b; Jacobs et al. 2011), there has been great interest in dietary CoQ₁₀ supplements as potential ergogenic aids for endurance exercise (Fernandes et al. 2023; Sarmiento et al. 2016). Initial studies assessing the ergogenic potential of CoQ₁₀ supplementation on endurance exercise performance administered CoQ₁₀ as ubiquinone with most (Braun et al. 1991; Cooke et al. 2008; Laaksonen et al. 1995; Östman et al. 2012; Weston et al. 1997; Zhou et al. 2005), but not all (Gökbel et al. 2010; Ylikoski et al. 1997), reporting no ergogenic effects with ubiquinone supplementation. It is recognised that a limitation of CoQ₁₀ supplementation is the low bioavailability after oral ingestion (Bhagavan and Chopra 2006) with studies reporting no significant increase in muscle [CoQ₁₀] after ubiquinone supplementation (Cooke et al. 2008; Svensson et al. 1999). Importantly, however, the increase in plasma [CoQ10] after ubiquinone supplementation has been positively correlated with increased muscle [CoQ10], along with improved exercise performance (Cooke et al. 2008). Accordingly, supplementation with a more bioavailable form of CoQ10 may have greater potential to elicit ergogenic effects.

Initial studies administered CoQ10 as ubiquinone since UQH₂ is ready oxidised to ubiquinone. In 2007, a stable form of UQH₂ became commercially available (Hosoe et al. 2007). Compared to ubiquinone, UQH₂ has demonstrated greater bioavailability following oral ingestion with large ($g=1.04$; Langsjoen and Langsjoen 2014) to moderate ($g=0.6$; Zhang et al. 2018) effect sizes reported. As such, UQH₂ supplementation may have greater potential to increase muscle [CoQ10] and elicit exercise performance improvements compared to ubiquinone supplementation (Cooke et al. 2008). However, despite potential for greater bioavailability and ergogenic effects, few studies have assessed the effect of UQH₂ supplementation on exercise performance. Of the 3 studies conducted to date, 2 have

reported no improvement in endurance exercise performance after 200–300 mg/day UQH₂ supplementation for 4 weeks (Bloomer et al. 2012; Orlando et al. 2018). Conversely, 1 study reported a small increase (11.0%, $g=0.35$) in the cycling power output at a fixed blood lactate concentration (4 mmol/L) compared to placebo (8.5%) in elite cyclists following 6 weeks of supplementation with 300 mg/day of UQH₂ (Alf et al. 2013). Therefore, supplementation with at least 300 mg/day UQH₂ for a minimum of 6 weeks may be necessary to elicit an ergogenic effect during endurance exercise, but further research is required to verify this assertion.

Whilst it has been reported that 2 weeks supplementation with 200 mg/day UQH₂ could improve platelet respiration in recovery from COVID-19 (Sumbalová et al. 2022), in the only study to have assessed the effect of UQH₂ supplementation on mitochondrial respiration in human skeletal muscle, 6 weeks of supplementation with 200 mg/day UQH₂ did not improve leak respiration, maximal ADP-stimulated respiration or maximal ETS activity (Pham et al. 2020). However, UQH₂ supplementation did blunt the production of the reactive oxygen species (ROS), hydrogen peroxide (H₂O₂), during leak respiration (Pham et al. 2020). The ability of CoQ₁₀ to undergo redox cycles is not only important for electron transfer in the mitochondrial ETS, but also allows UQH₂ to donate electrons to and subsequently neutralise free radicals, which are highly reactive molecules that can damage cellular constituents, consequent to unpaired electrons in their outer orbital (Littarru and Tiano 2007). Although H₂O₂ is a non-radical ROS, it is synthesised in the mitochondria from the free radical ROS, superoxide ($\cdot\text{O}_2^-$), via dismutation of two $\cdot\text{O}_2^-$ molecules in a reaction catalysed by superoxide dismutase 2 (Powers and Jackson 2008). Mitochondrial ROS production results from premature electron-leaks in the ETS, which contributes towards the uncoupling of mitochondrial O₂ consumption at ETS complex IV from ATP synthesis at ETS complex V (Fang et al. 2020; Jastroch et al. 2010). Furthermore, ROS, or its derivatives such as the lipid peroxidation product, 4-hydroxy-2-nonenal (4-HNE), can activate mitochondrial leak proteins, adenine nucleotide translocase 1 + 2 (ANT1 + 2) and uncoupling protein-3 (UCP-3) (Echtay et al. 2003; Klingenberg and Winkler 1985). In addition, mitochondria-derived ROS could contribute to mitochondrial leak respiration by damaging mitochondrial membranes via lipid peroxidation leading to a dissipation of the proton motive force (Nicholls and Ferguson 2013). Whilst Pham et al. (2020) did not observe lower mitochondrial leak respiration after UQH₂ supplementation, despite lower H₂O₂ production, further research is required to assess whether supplementation with a higher dose of UQH₂ can attenuate mitochondrial leak respiration and modulate other respiratory states.

The purpose of the current study was to assess the effects of 6 weeks supplementation with 300 mg·day⁻¹ UQH₂ on exercise capacity, assessed via time to the limit of tolerance (T_{Lim}) during severe-intensity exercise, skeletal muscle mitochondrial respiration, and skeletal muscle protein content of ANT1+2 and UCP-3. It was hypothesised that UQH₂ supplementation would increase T_{Lim} during severe-intensity exercise and lower skeletal muscle mitochondrial leak respiration and ANT1+2 and UCP-3 protein content leading to improved mitochondrial respiratory coupling efficiency.

Methods

Participants

Fifty-four healthy, recreationally active males volunteered to participate in this study. Participants did not have any current or historical neuromuscular, haematological, or musculoskeletal issues. Participants were non-smokers and were not using any dietary supplements or medication prior to, or during the study. Approval of the experimental procedures were granted by the Loughborough University Ethics Approvals (Human Participants) Sub-Committee. Participants received information of the experimental procedures, and associated risks and benefits, prior to providing written informed consent. Participants were instructed to arrive for laboratory visits in a rested state, at least 3 h post-prandial, having avoided strenuous physical activity and alcohol ingestion for 24 h and caffeine ingestion for 12 h. In the 24 h before the first experimental trial (visit 3), participants recorded their diet and any habitual low-intensity physical activity to enable replication in the 24 h before the remaining experimental visits. Participants completed their laboratory visits at the same time of day (± 2 h) to minimise the influence of circadian variations on the measurements. Participants were instructed to maintain their normal diet and physical activity for the duration of the study. Females were excluded from participating in the current study based on the potential for biological- and hormone-mediated heterogeneity to influence the study outcome measures. Indeed, it has been recently reported that menstrual cycle phase can influence skeletal muscle mitochondrial leak respiration and H₂O₂ production (Nelson et al. 2025).

Experimental design

Over a 7–9 week period, participants attended the laboratory on 5 separate occasions (Fig. 1). During the first visit to the laboratory, participants completed a ramp incremental exercise test to determine peak oxygen uptake ($\dot{V}\text{O}_{2\text{peak}}$) and gas

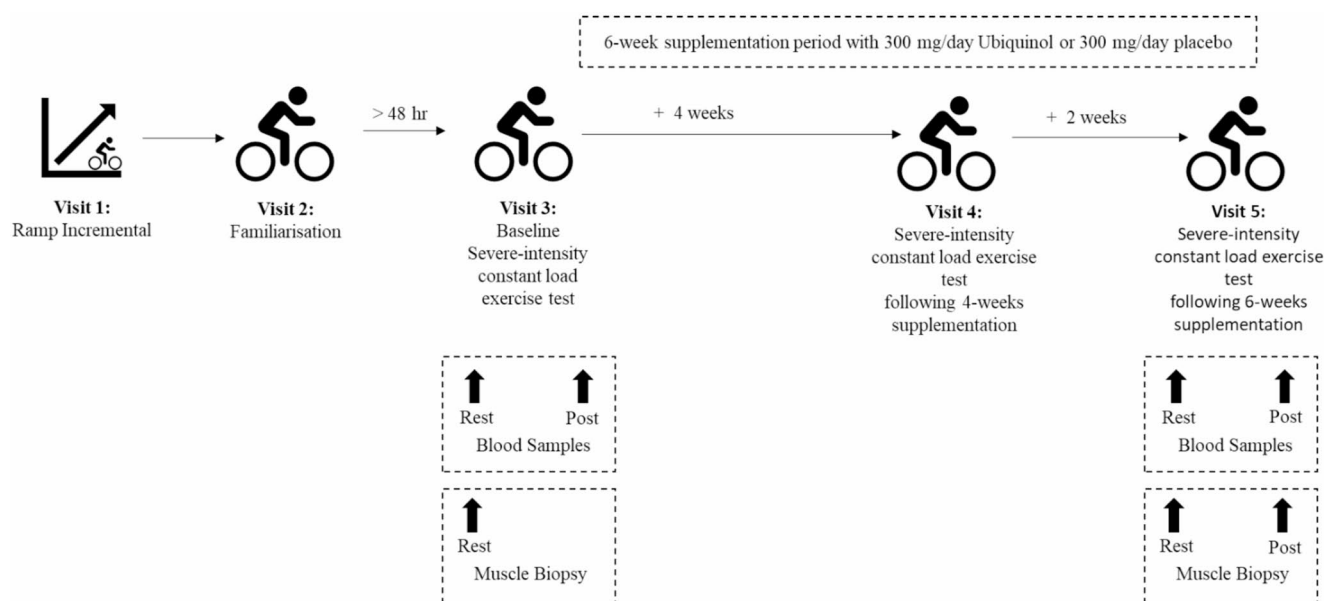


Fig. 1 Schematic diagram outlining the study design

exchange threshold (GET). On the second visit to the laboratory, participants were familiarised to the constant-load severe-intensity exhaustive exercise test. The third lab visit was the first experimental trial with baseline muscle biopsies obtained in participants who consented ($n=30$) prior to a baseline constant-load cycling exercise test to exhaustion ($n=54$). Subsequently, participants were stratified by $\dot{V}O_{2\text{peak}}$ and exercise performance, then randomly assigned to the ubiquinol (UQH₂; mean \pm SD: age, 24 \pm 4 year; height, 1.77 \pm 0.06 m; body mass, 76.7 \pm 10.4 kg; $\dot{V}O_{2\text{peak}}$, 44.2 \pm 4.8 mL \cdot kg⁻¹ \cdot min⁻¹) or placebo (PLA; mean \pm SD: age, 23 \pm 5 year; height, 1.78 \pm 0.07 m; body mass, 74.7 \pm 8.6 kg; $\dot{V}O_{2\text{peak}}$, 46.7 \pm 6.1 mL \cdot kg⁻¹ \cdot min⁻¹) supplementation groups, in a double-blind, independent groups experimental design (Fig. 2). Both participants and investigators were blinded to group assignment throughout the study, and placebo and ubiquinol capsules were identical in appearance, taste, odour and packaging. Allocation was concealed using opaque identical supplement containers, labelled A and B by an individual not involved in the study.

Participants were re-familiarised to the constant-load exercise tests after 4 weeks of a 6-week supplementation period (laboratory visit 4). A final constant-load exercise test was completed following a skeletal biopsy procedure (for the subsample who consented) after 6 weeks of supplementation, as described above for the third laboratory visit. All exercise tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode, Groningen, Netherlands). Preferred saddle and handlebar heights were recorded for each participant during visit 1 and replicated during subsequent visits.

Incremental test

To determine $\dot{V}O_{2\text{peak}}$ and GET, participants initially completed a ramp incremental exercise test. Following 5 min baseline cycling at 50 W using a self-selected pedal cadence (70–90 rpm), work-rate increased by 30 W \cdot min⁻¹ until the limit of tolerance (T_{Lim}). T_{Lim} was defined as the point of volitional exhaustion or when pedal cadence fell >10 rpm below the self-selected pedal cadence for >10 s. An online gas analyser (Vyntus CPX metabolic cart, Vyair Medical, Chicago, USA) was used to measure breath-by-breath gas exchange and ventilation data during the test. Throughout the test, participants wore a face mask and breathed through a low-resistance volume transducer assembly with gas concentration signals continuously sampled via a capillary line connected to the volume transducer assembly. The gas analyser was calibrated with gases of known concentration, and the turbine volume transducer was calibrated automatically and manually with a 3 L syringe. Breath-by-breath pulmonary gas exchange and ventilation data were collected continuously during the incremental test and averaged over consecutive 10 s periods, with $\dot{V}O_{2\text{peak}}$ determined as the highest 30 s rolling mean value attained during the test. The GET was determined from the following three criteria: (1) the first disproportionate increase in CO₂ production ($\dot{V}CO_2$) observed from individual plots of $\dot{V}CO_2$ vs. $\dot{V}O_2$; (2) an increase in the ventilatory equivalent for O₂ ($\dot{V}_E/\dot{V}O_2$) without an increase in the ventilatory equivalent for CO₂ ($\dot{V}_E/\dot{V}CO_2$); (3) an increase in end-tidal O₂ tension without a drop in end-tidal CO₂ tension. To account for the mean response time for $\dot{V}O_2$ during the ramp exercise protocol (i.e., the muscle-to-lung gas transit time) two-thirds of the

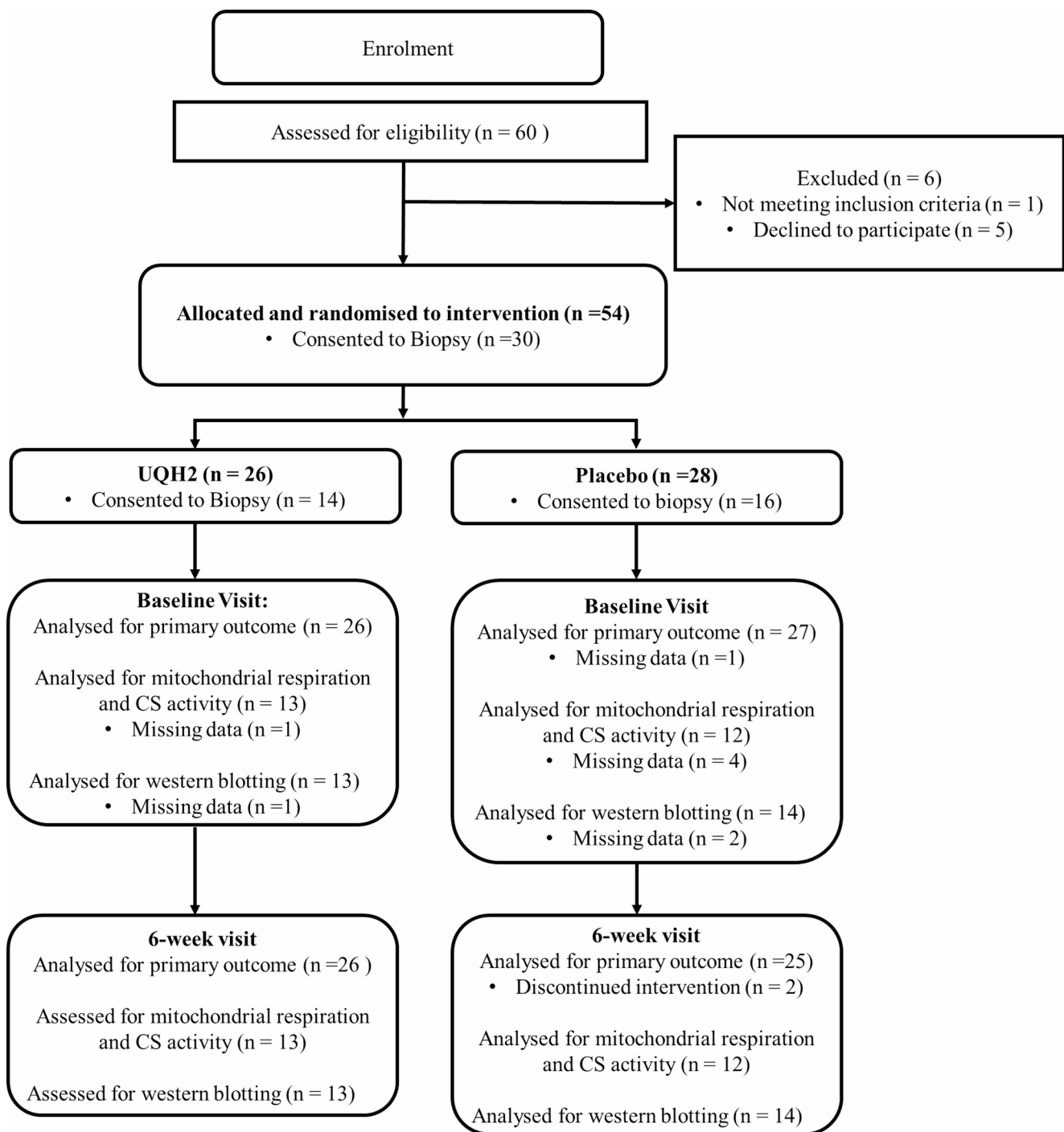


Fig. 2 Flow diagram of participant progress through a randomised controlled trial with two experimental conditions, including information on enrolment, allocation to study, follow-up, and data analysis. The

primary outcome was time to the limit of tolerance during a severe-intensity cycle test to exhaustion. Citrate synthase (CS)

ramp rate (20 W) was deducted from the work rates at GET and peak aerobic power (Whipp et al. 1981). Subsequently, the work rate at GET plus 70% of the difference in peak aerobic power and GET (Δ) (70% Δ) was calculated and administered in the subsequent severe-intensity step exercise tests.

Experimental trials

After arriving at the laboratory, participants rested in a supine position for 10 min and a venous blood sample was collected. A skeletal muscle biopsy was subsequently collected prior to completion of a constant-load step incremental

exercise test. For this test, participants completed 5 min of baseline cycling at 50 W, prior to an abrupt step increase in work rate to 70%Δ. Verbal encouragement was provided during the test as participants were instructed to maintain their preferred pedal cadence (70–90 rpm) until T_{Lim} , which was recorded using the same criteria outlined above for the ramp incremental test. T_{Lim} was recorded to the nearest second and was blinded to participants throughout the study. Upon completion, participants rested in a supine position for 10 min before a post-exercise venous blood sample was taken.

Ubiquinol supplementation

Upon random assignment to the UQH₂ or PLA group, participants consumed 300 mg·day⁻¹ of ubiquinol (Kaneka QH™, Kaneka corporation, Osaka, Japan) or placebo, respectively. This was spread evenly across the day in 100 mg doses consumed at breakfast, lunch, and dinner. Both the ubiquinol and placebo capsules contained edible oil, emulsifier, lecithin, modified food starch, carrageenan, caramel, and disodium phosphate encapsulated in a soft gel capsule. In addition to these ingredients, the ubiquinol and placebo capsules contained 100 mg ubiquinol or additional 100 mg edible oil, respectively. The purity of this ubiquinol supplement has been shown to be 96%, with ubiquinone and reduced coenzyme Q₁₀ constituting the main impurities (Hosoe et al. 2007). Supplementation adherence was monitored by intentionally undersupplying participants with the required capsules to complete the study. Prior to depletion of their supply, participants were asked if any additional supplements were required to complete the study, and if so, how many.

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Skeletal muscle analyses

Muscle sampling and fibre preparation

Muscle biopsies were obtained from the vastus lateralis. Briefly, local anaesthetic was administered (1% lidocaine hydrochloride), a small incision (~1–2 cm) was then made through the skin and fascia using a scalpel, before a biopsy needle with suction was used to obtain the sample. The sample was blotted on blotting paper to remove excess blood and initially inspected to remove connective tissue and fat. Subsequently the sample was split into two portions. The first portion was immersed in ice-cold biopsy preservation solution (BioPS) for subsequent high-resolution respirometry analysis. The second portion was immediately snap frozen in liquid nitrogen for later analysis of citrate synthase (CS) activity, as a valid marker of mitochondrial content (Larsen et al. 2012), and for the determination of protein content of adenine nucleotide translocase 1 & 2 (ANT1+2), mitochondrial uncoupling protein 3 (UCP3) and oxidative phosphorylation (OXPHOS) complexes.

Mitochondrial respiration protocol

Two 26-gauge needle tips were used to mechanically separate the muscle fibres under a light microscope, whilst immersed in BioPS. Upon separation, permeabilization of the plasma membrane was achieved by agitating the muscle fibre bundles for 30 min at 4 °C in BioPS containing 50 µg/ml saponin. The muscle fibre bundles were then washed in 2 mL of respiration medium (MIR05) containing, 0.5 mM EGTA, 3 mM MgCl₂, 60 mM potassium-lactobionate, 20 mM taurine, 10 mM KH₂PO₄, 20 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 110 mM sucrose, and 1 g/L bovine serum albumin essentially fatty acid-free (pH 7.1). Muscle fibre bundles were removed from MIR05 and placed on filter paper for 2 s, then moved to a dry piece of filter paper for a further 2 s, weighed, and placed in fresh MIR05 (2 mL). Approximately 1–3 mg wet weight of muscle was added to 2 mL of MIR05 at 37 °C in the chamber of a high-resolution respirometer (Oxygraph-2k, Oroboros, Innsbruck, Austria) and mitochondrial respiration was assessed in quadruplicate. A hyper-oxygenated environment (200–500 µM O₂) was maintained throughout the measurements with reoxygenation of the chamber achieved via direct injection of pure O₂ to mitigate against

oxygen diffusional limitation. The average coefficient of variation for measurement of CI+II_p respiration was 12.8%.

A substrate-uncoupler-inhibitor-titration protocol (SUIT 8) was used to determine leak respiration (I_L), ADP-stimulated respiration (I_P) and electron transfer system capacity (I_E) in mitochondrial protein complexes I – IV (CI – CIV). Steady states of O₂ flux were marked on DatLab 7.3 (Orbocor, Innsbruck, Austria) following titrations in the following sequence: Pyruvate (5 mM) and Malate (2 mM) were added, in the absence of adenylates, enabling determination of leak respiration through CI (CI_L). Subsequently, saturating concentrations of ADP (5 mM) were added to assess maximum ADP-stimulated respiration through CI (CI_P). Cytochrome *c* (10 μM) was then added to assess the integrity of the outer mitochondrial membrane, with samples deemed to be compromised if respiration increased $\geq 15\%$; where this was violated, samples were removed from the final analysis. Glutamate (10 mM) was added to ensure maximal CI_P respiration was achieved. Maximal ADP-stimulated respiration through CI+II combined (CI+II_P) was determined following the addition of succinate (10 mM). A series of stepwise titrations of carbonyl cyanide *m*-chlorophenyl hydrazone (0.5–1.5 μM) were added to uncouple respiration, enabling the assessment of ETS capacity through CI+II (CI+II_E). Rotenone (0.5 μM) was added to inhibit CI and obtain ETS capacity through CII (CII_E). Next, the addition of antimycin A (2.5 μM) inhibited complex III (CIII) allowing calculation of residual oxygen consumption, representing nonmitochondrial oxygen consumption, for the correction of mitochondrial respiratory parameters. Artificial electron donors (Ascorbate, 2 mM) and N, N,N',N'-tertamethyl-*p*-phenylenediamine (TMPD) (0.5 mM) were added to measure ETS through complex IV (CIV_E). Finally, all mitochondrial respiration was inhibited and for the calculation of autoxidation of the O₂k electrode facilitated following the addition of sodium azide (≥ 100 mM), allowing correction of CIV_E. Respirometry values were averaged across the four experimental runs and expressed relative to muscle fibre wet weight and termed mass-specific mitochondrial respiration. Mass-specific respiration was also expressed relative to CS activity for correction to mitochondrial content (Larsen et al. 2012) and termed mitochondrial-specific respiration. Respiratory flux control ratios (FCR) were calculated and described in Eqs. 1–5.

$$\text{LCR} = \frac{\text{CI}_L}{\text{CI} + \text{CII}_E} \quad (1)$$

$$\text{PCR} = \frac{\text{CI} + \text{CII}_P}{\text{CI} + \text{CII}_E} \quad (2)$$

$$1/\text{RCR} = \frac{\text{CI}_L}{\text{CI} + \text{CII}_P} \quad (3)$$

$$\text{SCR} = \frac{\text{CI}_P}{\text{CI} + \text{CII}_P} \quad (4)$$

$$\text{CIV}_{\text{res}} = \frac{\text{CI} + \text{CII}_P}{\text{CIV}_E} \quad (5)$$

Where LCR is the leak control ratio, PCR is the phosphorylation control ratio, inv-RCR is the inverse respiratory control ratio, SCR is the substrate control ratio and CIV_{res} is the CIV reserve ratio.

Preparation of whole-muscle lysates

Approximately 10–20 mg of snap frozen muscle tissue was added to ice cold lysis buffer (1:10 w/v) containing PBS, 0.2% Triton X-100 and protease and phosphatase inhibitor cocktail (Fisher Scientific, Loughborough, UK). Samples were then blitzed twice for 2 min at 20 Hz using a tissue lyser (Tissue Lyser II, Qiagen, UK), rotated for 60 min at 4 °C and centrifuged at 1200 *xg* for 10 min. The resulting supernatant was frozen at -80 °C. The Pierce 660 protein assay (Fisher Scientific, Loughborough, UK) was used to determine, in triplicate, the protein concentration of the thawed muscle homogenate. The muscle homogenate was used for CS activity analysis.

Citrate Synthase Activity

The following were added to a flat-bottomed 96-well plate to determine CS activity in triplicate: 40 μL of 3 mM acetyl CoA, 25 μL of 1 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) solution, 160 μL of 100 mM Tris buffer containing 1% Triton-X (pH 8.3) and 10 μL of a 1 mg·mL⁻¹ muscle homogenate. Immediately after the addition of 15 μL of 10 mM oxaloacetate to all wells, the plate was shaken for 30 s and read every 30 s for 6 min at 412 nm at 30 °C using a microplate reader (Varioskan Lux, ThermoFisher, Loughborough, UK). Absorbance values were corrected to blank wells and pathlength corrections applied. CS activity was determined over a 3 min period following exclusion of the initial min of the reaction and reported as mol·h⁻¹·kg protein⁻¹.

Immunoblot analysis

A portion of homogenate was then mixed with 4x Laemmli sample buffer (Bio-rad, Herts, UK), 0.1% β-mercaptoethanol (Sigma, Dorset, UK) and deionised water to the required concentration. Samples were then boiled for 5 min at 95 °C

for ANT1+2, and UCP3 but not Total OXPHOS. The appropriate sample load was determined prior to analysis for each antibody (Total OXPHOS, 20 µg; ANT1+2, 10 µg; UCP3, 10 µg) and loaded on to 4–20% (Total OXPHOS) or 4–15% (UCP3 & ANT1+2,) stain-free TGX polyacrylamide gels (Bio-rad, Herts, UK) and separated by gel electrophoresis at 150 V for ~1 h. Protein was transferred to PVDF membranes using a Trans-Blot turbo system (Bio-rad, Herts, UK). Membranes underwent 3 × 5 min washes in Tris-buffered saline with tween before being blocked in EveryBlot blocking buffer (Bio-rad, Herts, UK) for 5 min and then washed again as before. Membranes were incubated overnight at 4 °C with the primary antibodies: Total OXPHOS 1:2000 (AB110411, abcam, Cambridge, UK), ANT1-2 1:1000 (AB220408, abcam, Cambridge, UK) and UCP3 1:1000 (AB3046, abcam, Cambridge, UK). Primary antibodies were diluted in EveryBlot blocking buffer. Membranes were washed as before and incubated with anti-mouse (Total OXPHOS; 7076, Cell Signalling Technology, London UK) or anti-rabbit (ANT1+2 & UCP3; 7074, Cell Signalling Technology, London UK) horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Secondary antibodies were diluted 1:10000, in Everyblot blocking buffer. Membranes were washed as before and incubated with enhanced chemiluminescence substrate (ClarityMax, Bio-Rad, Herts, UK) for 5 min. Visualisation of the membranes was achieved using a ChemiDocTM imaging system (Bio-rad, Herts, UK). Image analysis software (Image Lab v. 4.0, Bio-Rad, Herts, UK) was used for densitometric analysis of protein bands with the target signal expressed relative to total lane protein on a stain free gel.

Venous blood analyses

Venous blood sampling and treatment

Venous blood was collected into lithium-heparin and serum separator vacutainers. Lithium-heparin vacutainers were immediately spun at 1620 g and 4 °C for 10 min. Serum separator vacutainers were left at room temperature for 30 min prior to centrifugation at 1620 g and 4 °C for 10 min to obtain serum.

Plasma coenzyme Q₁₀ content

Lithium-heparin plasma (100 µL) was added to deoxygenated 1-propanol (300 µL) and butylated hydroxytoluene (40 µL; 10 mg/mL in ethanol) and then vortexed for 10 s before being snap frozen in liquid nitrogen. Kaneka Techno Research Corporation (Osaka, Japan) analysed the

concentration of plasma UQ and UQH₂ using liquid chromatography (LC)-mass spectrometry (MS)/MS method as described previously (Suzuki et al. 2021). Kaneka Research Corporation were blinded to the experimental conditions and this analysis was undertaken once all other analysis for the study had been completed.

Serum lipid hydroperoxides

Serum lipid hydroperoxides (LOOH) were measured spectrophotometrically using a modification of the Wolff et al. (1994) method as described previously (Williamson et al. 2020). The ferrous oxidation in xylenol orange (FOX-1) reagent containing ammonium ferrous sulphate (250 µM), sorbitol (100 mM), xylenol orange (100 µM), and sulphuric acid (25 mM) was used. Briefly, 0–5.0 µmol·L⁻¹ of hydrogen peroxide was used to form a standard curve. Serum (90 µL) was added to FOX-1 reagent (900 µL). After a 30 min incubation in the dark, 200 µL of this solution was added to a 96 well plate, and immediately read using a microplate reader (Varioskan Lux, ThermoFisher, Loughborough, UK) at 560 nm and then every 2 min for 6 min to confirm the reaction rate had saturated.

Blood lipid profile

Serum triglycerides, high-density lipoproteins (HDL), total cholesterol were analysed spectrophotometrically using a semi-automatic analyser (Pentra C400, Horiba Medical, Northampton, UK). The Friedewald equation was used to estimate low-density lipoprotein cholesterol (LDL) (Friedewald et al. 1972).

Measurements

Breath-by-breath pulmonary gas exchange and ventilation were measured continuously using an online gas analyser (Vyntus CPX; Carefusion, California, USA) as described above for the ramp incremental test. Errant breaths falling more than 4 standard deviations outside the local mean were removed during initial examination of the data. The magnitude of the $\dot{V}O_2$ slow component ($\dot{V}O_2$ SC) was inferred from the difference in $\dot{V}O_2$ between 120 ± 10 s and T_{lim} (averaged over final 30 s).

Statistical analysis

Sample size was estimated with a priori power analysis using G*Power (G*Power 3.1.97; Kiel, Germany). Since the effects of UQH₂ supplementation on our primary outcome (T_{lim} during severe-intensity cycling exercise) had not been assessed previously, sample size to detect a supplement x

time interaction effect for two supplements (UQH₂ vs. PLA) and two time points (Pre & Post-supplementation) was estimated on the basis of a small effect size (0.20), a statistical power of 0.80, a significance level of 0.05. Inputting these values into G*Power revealed that a sample size of 52 was required. Statistical analysis was conducted using jamovi (version 2.3.28; Jamovi, Sydney, Australia). Data were tested for normality by visual inspection of Q-Q plots of residuals, residuals histogram and Shapiro-Wilk tests. Linear mixed models (2 [supplement: UQH₂ vs. PLA] x 2 [time: Pre & Post-supplementation]) were performed to determine differences in T_{Lim}, plasma CoQ₁₀ content variables, mitochondrial respiratory function variables, CS activity, LOOH and serum blood lipid variables. Effect sizes were calculated using partial eta squared (n_p^2) for linear mixed models outcomes and interpreted as small (0.01–0.05), moderate (0.06–0.13), and large (≥ 0.14). Where significant supplement \times time interaction effects were observed, Holm-Bonferroni corrected independent *t*-tests (between group comparisons) and paired *t*-tests (within group comparisons) were used for *post-hoc* analysis. Independent samples *t*-tests were used to determine differences between UQH₂ and PLA in fold change in protein content of ANT1+2, UCP3 and OXPHOS complexes. Effect sizes were calculated and reported for all *t*-tests using Hedge's *g* bias correction and interpreted as 'small' (0.20–0.49), 'moderate' (0.50–0.79), and large (≥ 0.80). All data are presented as mean \pm SD. Significance was accepted at $P \leq 0.05$.

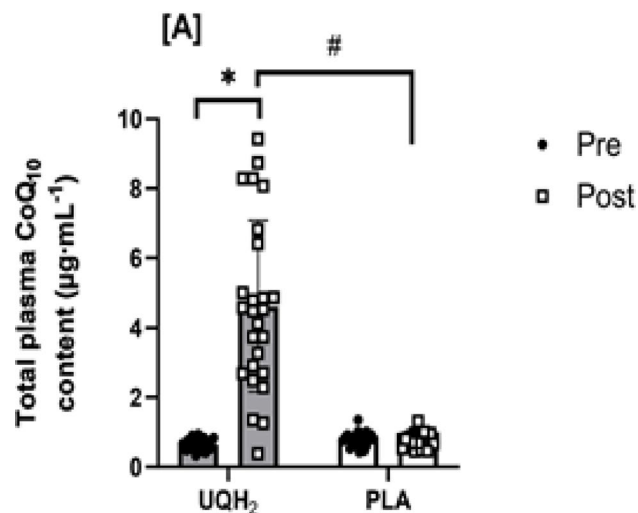


Fig. 3 Total Coenzyme Q₁₀ (CoQ₁₀) content in plasma pre and post supplementation with ubiquinol (UQH₂; $n=26$ at both timepoints) or placebo (PLA; $n=24$ at Pre and $n=22$ at Post). *indicates a statistically significant change ($P < 0.05$) pre to post within groups # indicates a statistically significant change ($P < 0.0001$) at Post between groups. Statistical significance was accepted as $P \leq 0.05$

Results

All participants requested additional capsules in line with the amount undersupplied, self-reported that they consumed all supplements as instructed, and returned empty capsule pots. No adverse side effects were reported in either the UQH₂ or PLA supplementation conditions.

Plasma CoQ₁₀ profile

There was a supplement \times time interaction effect ($P < 0.0001$; $n_p^2 \geq 0.17 \leq 0.58$) and main effects of supplement ($P \leq 0.002$; $n_p^2 \geq 0.17 \leq 0.52$) and time ($P < 0.0001$; $n_p^2 \geq 0.43 \leq 0.58$) for plasma total CoQ₁₀ (Fig. 3), UQH₂, UQ, total CoQ₁₀/Cholesterol and total CoQ₁₀/LDL. These measures were significantly higher Post compared to Pre following UQH₂ ($P < 0.0001$, $g > 2.0$) but did not change following PLA supplementation ($P > 0.05$). In addition, these measures were higher at Post in UQH₂ compared to PLA supplementation ($P < 0.0001$, $g > 2.0$). There was no supplement \times time interaction effect ($P = 0.37$; $n_p^2 = 0.02$) or no main effect of supplement ($P = 0.34$; $n_p^2 = 0.02$), but a main effect for time ($P = 0.04$; $n_p^2 = 0.08$), for UQH₂/Total CoQ₁₀ (Table 1).

Serum lipid profile

There was no supplement \times time interaction effect ($P \geq 0.51 \leq 0.79$; $n_p^2 < 0.01$), or no main effect of time ($P \geq 0.07 \leq 0.95$; $n_p^2 < 0.01$) or supplement ($P \geq 0.55 \leq 0.81$; $n_p^2 < 0.01$), for total cholesterol, triglycerides, LDL, and HDL concentration (Table 1).

Citrate synthase activity

There was no supplement \times time interaction effect ($P = 0.51$; $n_p^2 = 0.02$), or main effects of time ($P = 0.11$; $n_p^2 = 0.11$) or supplement ($P = 0.21$; $n_p^2 = 0.07$), for CS activity (Fig. 4).

Mitochondrial respiratory function

There was a supplement \times time interaction effect ($P = 0.004$; $n_p^2 = 0.32$), but no main effect of time ($P = 0.48$; $n_p^2 = 0.02$) or supplement ($P = 0.20$; $n_p^2 = 0.07$), for mass-specific CI_L respiration. Post-hoc tests revealed that CI_L respiration was lower in the UQH₂ group (4.3 ± 2.3 pmol O₂·s⁻¹·mg⁻¹) compared to the PLA group (8.0 ± 3.4 pmol O₂·s⁻¹·mg⁻¹) POST supplementation ($P = 0.024$, $g = 0.87$). CI_L was lower Post compared to Pre (7.1 ± 3.8 pmol O₂·s⁻¹·mg⁻¹) in the UQH₂ group ($P = 0.016$, $g = 0.85$) (Fig. 5A). There was a supplement \times time interaction effect ($P = 0.011$; $n_p^2 = 0.27$), but no main effects of time ($P = 0.364$; $n_p^2 = 0.04$) or supplement ($P = 0.247$; $n_p^2 = 0.06$), for mitochondrial-specific

Table 1 Plasma Coenzyme Q₁₀ and serum lipid profiles pre and post supplementation with ubiquinol or Placebo

	Ubiquinol			Placebo		
	Pre	Post	Mean diff	Pre	Post	Mean diff
<i>Coenzyme Q10 profile</i>						
Total CoQ ₁₀ (μg·mL ⁻¹)	0.65±0.16	4.62±2.46**	3.97±2.38	0.79±0.20	0.76±0.20	-0.01±0.17
Ubiquinol (μg·mL ⁻¹)	0.59±0.15	4.35±2.35**	3.76±2.29	0.69±0.18	0.68±0.21	0.01±0.17
Ubiquinone (μg·mL ⁻¹)	0.06±0.04	0.27±0.17**	0.21±0.14	0.10±0.08	0.08±0.06	-0.02±0.06
Ubiquinol/Total CoQ ₁₀ (%)	89.8±6.1	92.6±5.2	2.74±3.44	88.8±7.3	90.0±10.3	1.05±8.59
Total CoQ ₁₀ /Cholesterol	0.17±0.04	1.16±0.60**	0.99±0.58	0.20±0.04	0.20±0.06	0.01±0.05
Total CoQ ₁₀ /LDL	0.31±0.10	2.21±1.14**	1.89±1.11	0.37±0.12	0.39±0.23	0.02±0.22
<i>Serum lipid profile</i>						
Total Cholesterol (mmol·L ⁻¹)	3.92±0.87	3.96±0.67	0.04±0.45	4.05±0.83	4.02±0.75	-0.03±0.47
Triglycerides (mmol·L ⁻¹)	0.94±0.35	1.10±0.54	0.16±0.47	1.00±0.36	1.09±0.64	0.08±0.43
LDL (mmol·L ⁻¹)	2.21±0.68	2.14±0.55	-0.07±0.36	2.25±0.77	2.18±0.73	-0.07±0.39
HDL (mmol·L ⁻¹)	1.29±0.30	1.34±0.27	0.04±0.13	1.34±0.24	1.34±0.29	0.01±0.22

Values are Mean±SD. Total CoQ₁₀, total coenzyme Q₁₀

LDL low-density lipoprotein; HDL, high density lipoprotein

n = 26 in all measures in the ubiquinol group at both timepoints. In the placebo group *n* = 24 at Pre and *n* = 23 at Post for Total CoQ₁₀, Ubiquinol, Ubiquinone, and Ubiquinol/Total CoQ₁₀ measures and *n* = 24 at Pre and *n* = 22 at Post and *n* = 27 at Pre and *n* = 24 at Post for serum lipid profile measures

*Indicates statistically significant difference from PRE value,

indicates statistically significant difference between groups at POST time point. Statistical significance was accepted as *P* ≤ 0.05

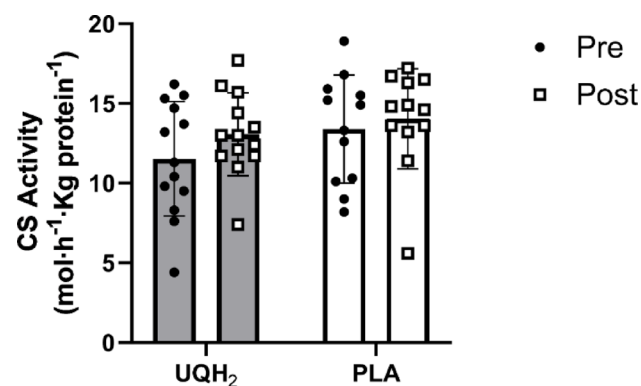


Fig. 4 Citrate synthase (CS) activity as a biomarker of mitochondrial content pre and post ubiquinol (UQH₂; *n* = 13 at both time points) or placebo (PLA; *n* = 12 at both time points) supplementation

CI_L respiration. Post-hoc tests revealed that mitochondrial-specific CI_L respiration was lower Post (0.34±0.19 pmol O₂·s⁻¹·mg⁻¹/CS activity) compared to Pre (0.57±0.33 pmol O₂·s⁻¹·mg⁻¹/CS activity) in the UQH₂ group (*P* = 0.026, *g* = 0.80) but was not different in the UQH₂ group compared to the PLA (0.62±0.34 pmol O₂·s⁻¹·mg⁻¹/CS activity) group POST supplementation (*P* = 0.08, *g* = 0.69) (Fig. 5B).

There was no supplement × time interaction effect (*P* ≥ 0.18 ≤ 0.99; *n_p*² ≤ 0.08), or no time (*P* ≥ 0.34 ≤ 0.94; *n_p*² ≤ 0.07) and supplement (*P* ≥ 0.59 ≤ 0.99; *n_p*² ≤ 0.01) main effects, for mass-specific or mitochondrial-specific CI_P, CI+II_P, CI+II_E or CIV_E respiration (Fig. 5). There was no supplement × time interaction effect (*P* = 0.21; *n_p*² = 0.07) or no main effect for time (*P* = 0.66; *n_p*² < 0.01), but a main effect for supplement (*P* = 0.03; *n_p*² = 0.20), for

mass-specific CII_E respiration (Fig. 4A). However, there was no supplement × time interaction effect (*P* = 0.25; *n_p*² < 0.06), or no main effect of time (*P* = 0.22; *n_p*² = 0.07) or supplement (*P* = 0.27; *n_p*² = 0.05), for mitochondrial specific CII_E respiration (Fig. 5B).

Flux control ratios

There was a supplement × time interaction effect (*P* = 0.02; *n_p*² = 0.22) for 1/R_{CR}, but no main effects of time (*P* = 0.15; *n_p*² = 0.09) or supplement (*P* = 0.348; *n_p*² = 0.04). Post-hoc tests revealed 1/R_{CR} was lower at Post (0.044±0.019) compared to Pre (0.073±0.039) supplementation in the UQH₂ group (*P* = 0.014, *g* = 0.88) (Fig. 5C), but was not significantly different Post supplementation in the UQH₂ group compared to the PLA group (0.072±0.026, *P* = 0.16, *g* = 1.20). There was a supplement × time interaction effect (*P* = 0.02; *n_p*² = 0.22), but no main effects of time (*P* = 0.18; *n_p*² = 0.08) or supplement (*P* = 0.27; *n_p*² = 0.06), for L_{CR}. Post-hoc tests revealed L_{CR} was lower POST (0.038±0.016) compared to PRE (0.063±0.034) in the UQH₂ group (*P* = 0.02, *g* = 0.88) (Fig. 5C).

There were no supplement × time interaction effects (*P* ≥ 0.49 ≤ 0.64; *n_p*² ≤ 0.02), or no main effects for time (*P* ≥ 0.29 ≤ 0.71; *n_p*² ≤ 0.05) or supplement (*P* ≥ 0.08 ≤ 0.32; *n_p*² ≤ 0.13), for PCR, SCR, and CIV_{Res} (Fig. 5C).

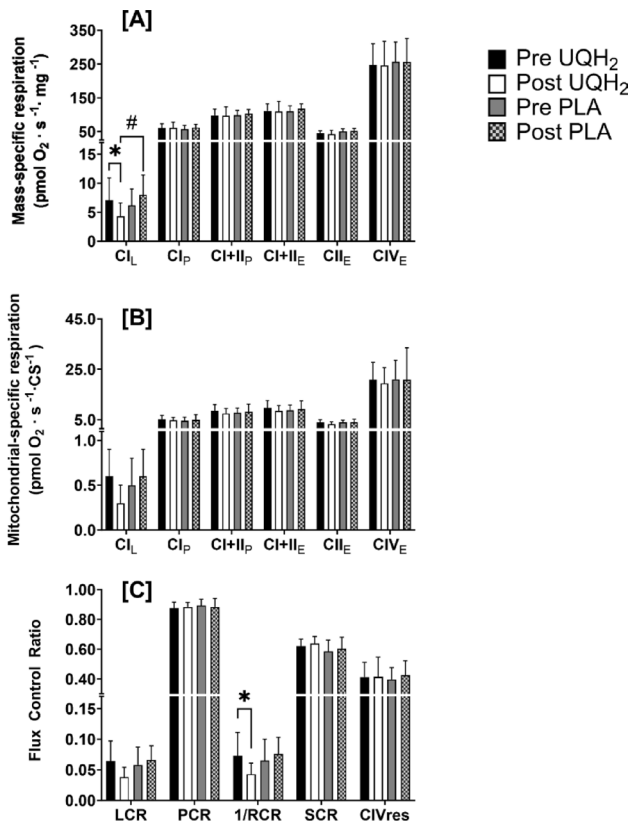


Fig. 5 Mitochondrial mass-specific respiration (A), mitochondrial-specific respiration (B) and flux control ratios (FCRs) (C) pre (Pre UQH₂) and post (Post UQH₂) ubiquinol supplementation and pre (Pre PLA) and post (Post PLA) placebo supplementation. UQH₂; *n* = 13 at both timepoints for panel A-C, PLA; *n* = 12 at both timepoints for panels A-C. *Indicates statistically significant change from Pre to Post supplementation within groups and # indicates statistically significant difference Post supplementation between groups. Statistical significance was accepted as *P* ≤ 0.05

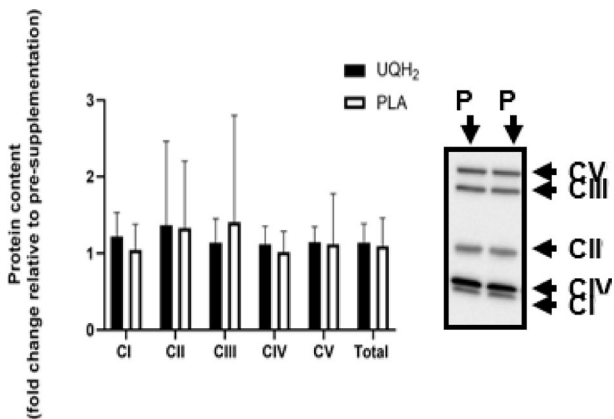


Fig. 6 Fold change compared to pre-supplementation of the protein content of mitochondrial complex I (CI), II (CII), III (CIII), IV (CIV), V (CV) and total mitochondrial OXPHOS complex content (Total) following ubiquinol (UQH₂; *n* = 13) supplementation and placebo (PLA; *n* = 14). A representative blot from one participant is shown on the right

Immunoblot analysis

There were no differences in fold change in protein content from PRE to POST supplementation between groups in any of the individual OXPHOS complexes (*P* ≥ 0.171, *g* = 0.05–0.53; Fig. 6), ANT1+2 (*P* = 0.247, *g* = 0.44; Fig. 7A) or UCP3 (*P* = 0.609, *g* = 0.19; Fig. 7B).

Performance measures

T_{Lim}

There was no supplement x time interaction effect (*P* = 0.79; *n_p*² < 0.01) or no main effect of effect supplement (*P* = 0.44; *n_p*² = 0.01), but there was a main effect of time (*P* = 0.03; *n_p*² = 0.08), for T_{Lim} (Fig. 8).

Pulmonary oxygen uptake

There was no supplement x time interaction effect (*P* = 0.25; *n_p*² = 0.03), or no main effects of time (*P* = 0.11; *n_p*² = 0.05) or supplement (*P* = 0.34; *n_p*² = 0.02), for end-exercise $\dot{V}O_2$ averaged over the final 30s of the constant-load exercise tests (Fig. 9).

There was no supplement x time interaction effect (*P* = 0.38; *n_p*² = 0.02) or main effect of supplement (*P* = 0.53; *n_p*² < 0.01), but there was a main effect of time (*P* = 0.01; *n_p*² = 0.12), for $\dot{V}O_2$ SC (Fig. 10).

Serum lipid hydroperoxides (LOOH)

There was no supplement x time interaction effect (*P* ≥ 0.37 ≤ 0.89; *n_p*² ≤ 0.04) or no main effect for supplement (*P* ≥ 0.17 ≤ 0.58; *n_p*² ≥ 0.01 ≤ 0.08), but there was a main effect for time (*P* < 0.004; *n_p*² = 0.32 ≤ 0.50), for LOOH both pre and post supplementation (Fig. 11).

Discussion

The principal original finding of the current study, consistent with our experimental hypothesis, was that 6 weeks UQH₂ lowered CIL respiration and 1/RCR suggesting improved mitochondrial oxidative phosphorylation coupling efficiency. However, contrary to our experimental hypothesis, this improved mitochondrial respiratory coupling efficiency was not mediated by lower skeletal muscle ANT1+2 and UCP-3 protein content and did not translate to improved T_{Lim} during severe-intensity exercise. These findings indicate that UQH₂ supplementation was effective at improving mitochondrial respiratory coupling efficiency but not enhancing severe-intensity exercise T_{Lim}.

Fig. 7 Fold change compared to pre-supplementation of the protein content of adenine nucleotide translocase 1+2 (ANT1+2; A) and mitochondrial uncoupling protein 3 (UCP3; B) following ubiquinol (UQH₂; *n* = 13) supplementation and placebo (PLA; *n* = 14). Representative blots from one participant are shown for each protein

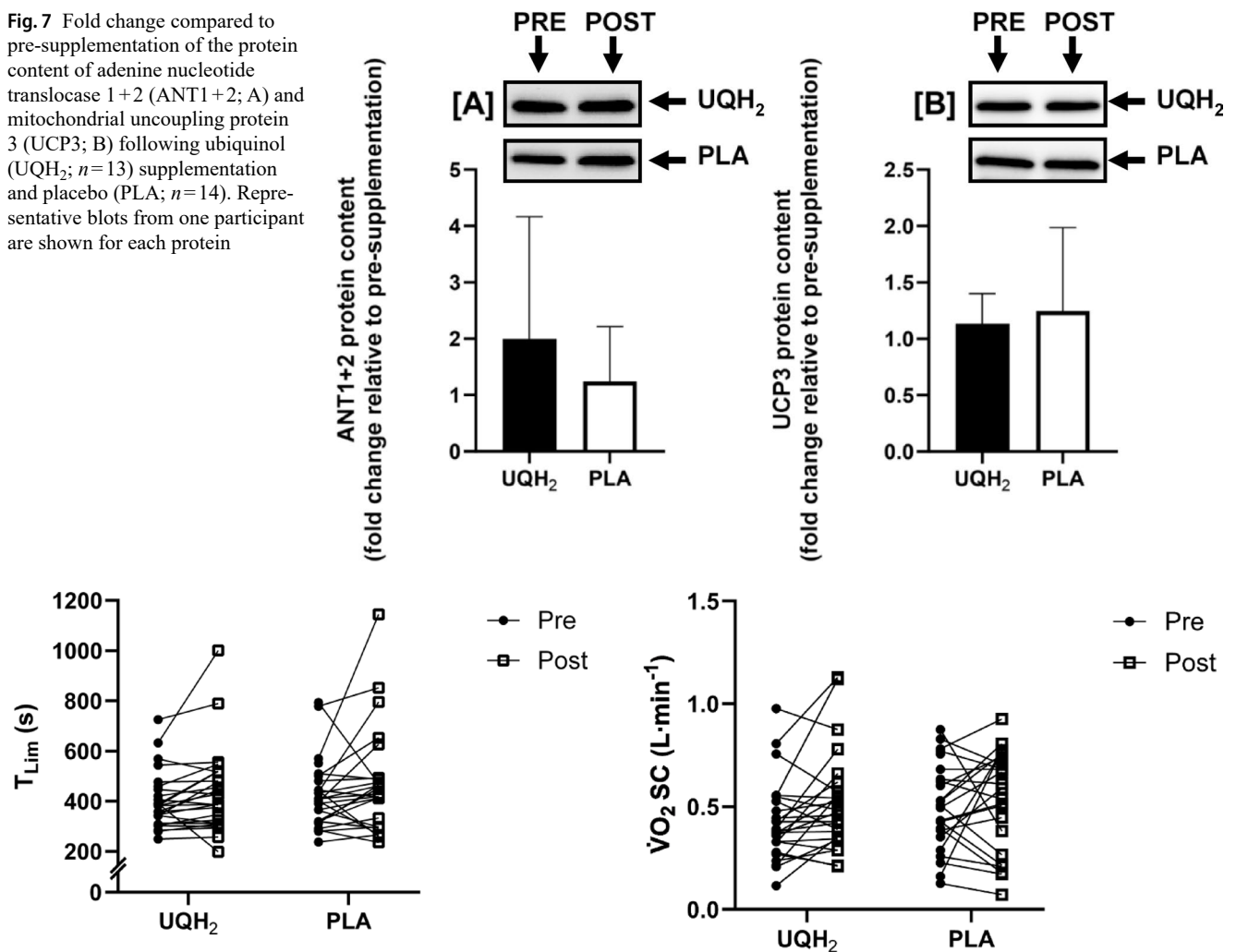


Fig. 8 Time to reach the limit of tolerance (T_{Lim}) during the severe intensity constant-load cycling test pre and post 6-weeks supplementation with ubiquinol (UQH₂; *n* = 26 at both timepoints) or placebo (PLA; Pre, *n* = 27, Post, *n* = 25)

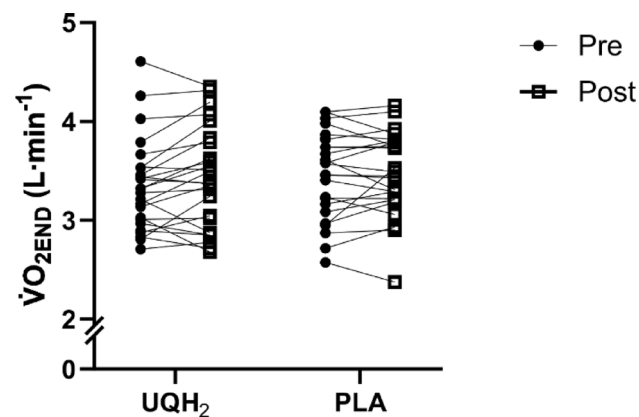


Fig. 9 Oxygen uptake averaged over the final 30 s of the constant load exercise test ($\dot{V}O_{2END}$) Pre and Post 6-weeks supplementation with ubiquinol (UQH₂; *n* = 25 at both timepoints) or placebo (PLA; Pre, *n* = 26, Post, *n* = 24)

Fig. 10 Oxygen uptake ($\dot{V}O_2$) slow component ($\dot{V}O_{2SC}$) assessed as the difference in $\dot{V}O_2$ between $\dot{V}O_2$ averaged over the final 30 s of the constant load test ($\dot{V}O_{2END}$) and at 120 ± 15 s Pre and Post 6-weeks supplementation with ubiquinol (UQH₂; *n* = 25 at both timepoints) or placebo (PLA; Pre, *n* = 26, Post, *n* = 24)

Consistent with previous studies, UQH₂ supplementation increased plasma [CoQ10] (Langsjoen and Langsjoen 2014; Zhang et al. 2018). The principal original findings from this study were that 6 weeks supplementation with 300 mg/day UQH₂ lowered 1/RCR and LCR. Lower 1/RCR and LCR indicate that leak respiration compromised a smaller fraction of maximal ADP-stimulated respiration and maximal ETS activity after UQH₂ supplementation, with lower 1/RCR suggesting that the efficiency of mitochondrial oxidative phosphorylation was improved. These findings were underpinned by lower CI_L respiration, concomitant with no change in CI_P , $CI+II_P$, $CI+II_E$ nor CII_E after UQH₂ supplementation. In the only other study to have assessed the effect of UQH₂ supplementation on mitochondrial respiratory variables in human skeletal muscle, Pham et al. (2020) reported that 6 weeks supplementation with 200 mg/day

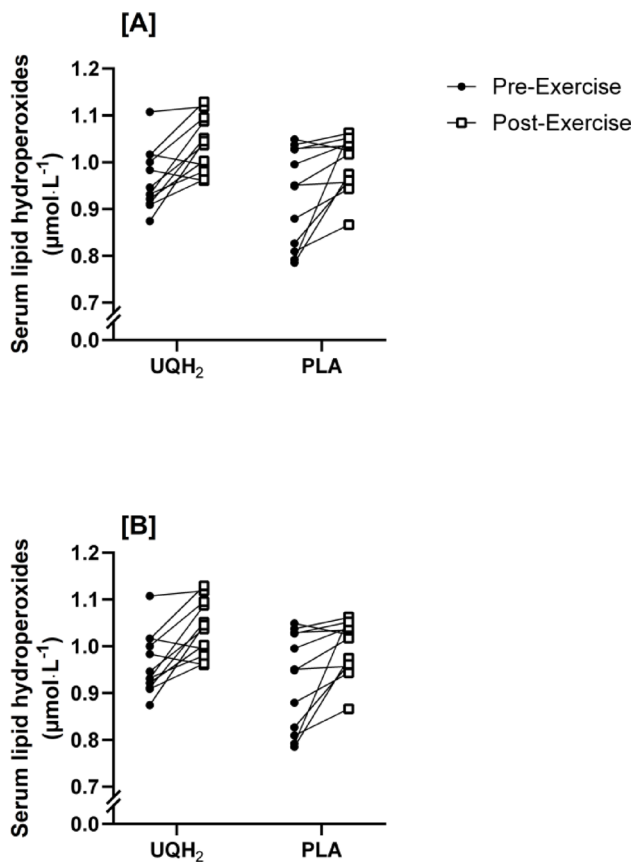


Fig. 11 Pre- and post-exercise serum lipid hydroperoxides both pre (A) and post supplementation (B), in the ubiquinol (UQH₂; $n=12$ at both timepoints) and placebo (PLA; $n=12$ at both timepoints) group

UQH₂ did not alter CI_L , $\text{CI}+\text{II}_L$, $\text{CI}+\text{II}_P$ nor $\text{CI}+\text{II}_E$. It is possible, therefore, that the higher daily dose of UQH₂ administered in the current study compared with Pham et al. (2020) accounted for the between-study difference in CI_L . However, both the current study and the study of Pham et al. (2020) did not observe improvements in $\text{CI}+\text{II}_P$ or $\text{CI}+\text{II}_E$ respiratory rates, or muscle CS activity, nor the protein content of mitochondrial respiratory complexes I-V as markers of mitochondrial content.

The lowering in CI_L respiration after UQH₂ supplementation occurred independent of altered skeletal muscle protein content of the mitochondrial leak proteins, ANT1+2 and UCP-3. It is possible that the lower CI_L respiration in the current study was linked to lower ETS electron leak as ROS, and therefore improved efficiency of mitochondrial electron handling, as previously reported after UQH₂ supplementation (Pham et al. 2020). Lower ROS formation after UQH₂ supplementation could also lower leak respiration by mitigating oxidative damage, via lipid peroxidation, to the mitochondrial membranes (Nicholls and Ferguson 2013) with ROS and lipid peroxidation derivatives also able to acutely increase ANT1+2 and UCP-3 activity (Echtay

et al. 2003; Klingenberg and Winkler 1985). Although circulating lipid hydroperoxides, as systemic markers of lipid peroxidation (Fogarty et al. 2011), were not altered, it cannot be excluded that UQH₂ supplementation attenuated lipid peroxidation in the muscle mitochondria, or attenuated muscle mitochondrial ROS production, in the current study. Indeed, the previous observation of lower mitochondrial H₂O₂ emission per unit respiration during leak respiration after UQH₂ supplementation occurred independent of changes in plasma TBARS and urinary isoprostanes as systemic markers of lipid peroxidation (Pham et al. 2020). Another candidate mechanism for the lower CI_L respiration after UQH₂ supplementation in the current study is lower electron slip, whereby electrons are transferred along the mitochondrial ETS in the absence of H⁺ translocation into the mitochondrial intermembrane space (Fang et al. 2020; Jastroch et al. 2010).

Despite enhanced mitochondrial oxidative phosphorylation efficiency, UQH₂ supplementation did not increase severe-intensity exercise T_{Lim} . The lack of an ergogenic effect of UQH₂ supplementation in the current study is consistent with some (Bloomer et al. 2012; Orlando et al. 2018), but not all (Alf et al. 2013; Moreno-Fernandez et al. 2023), previous studies. Moreover, and in keeping with the findings of the current study, some previous studies have also dissociated improved mitochondrial oxidative phosphorylation efficiency from exercise performance enhancements (Ghiarone et al. 2019; Jacobs et al. 2012; Peden et al. 2022). Whilst it is not possible to exclude that a longer duration of supplementation and/or a higher UQH₂ dose could have elicited an ergogenic effect, the UQH₂ supplementation regime in the current study has been previously reported to enhance endurance performance (Alf et al. 2013). In addition to a lack of effect on severe-intensity exercise T_{Lim} , UQH₂ supplementation did not alter the change in $\dot{V}\text{O}_2$ between 2 min and end-exercise, as a marker of exercise economy, or end-exercise $\dot{V}\text{O}_2$ which is equivalent to $\dot{V}\text{O}_{2\text{max}}$ during severe-intensity exercise (Burnley and Jones 2007). Previous studies have also dissociated changes in mitochondrial oxidative phosphorylation efficiency from exercise economy and $\dot{V}\text{O}_{2\text{max}}$ enhancements (Ghiarone et al. 2019; Jacobs et al. 2012). Given that 200 mg/day UQH₂ supplementation for 2 weeks improved resistance exercise performance (Moreno-Fernandez et al. 2023), whereas 300 mg/day UQH₂ supplementation for 6 weeks was required to improve endurance performance (Alf et al. 2013), it is possible that UQH₂ supplementation may hold greater ergogenic potential for resistance exercise performance and recovery, but this requires further research as the conclusions from these studies may have been based on a Type I statistical error.

Whilst the current study offers original insights into the effects of UQH₂ supplementation on mitochondrial respiration, and physiological and performance responses during severe-intensity exercise, there are some limitations. First, muscle and mitochondrial [CoQ₁₀] were not measured, meaning it is not possible to confirm whether the observed improvements in mitochondrial oxidative phosphorylation efficiency were due to increased mitochondrial [CoQ₁₀], or mediated by other mechanisms. In addition, mitochondrial ROS production, muscle lipid peroxidation markers and ANT1+2 and UCP-3 activation were not assessed and would have provided further insight into the mechanisms underpinning improved mitochondrial oxidative phosphorylation efficiency after UQH₂ supplementation in the present study. Further research is required to resolve the mechanisms for lower mitochondrial CI_L respiration and improved oxidative phosphorylation coupling efficiency after UQH₂ supplementation. Moreover, given that mitochondrial oxidative phosphorylation coupling efficiency is more likely to be compromised by prolonged endurance exercise and is accompanied by a loss in exercise economy in such settings (Fernström et al. 2007), further research is required to assess whether UQH₂ supplementation may confer greater ergogenic potential for longer duration lower intensity exercise. It is also recognised that not pre-registering the trial publicly, and the possibility for inflated risk of type I statistical errors given then number of measures undertaken, are limitations of our study. The lack of female participants is also an important limitation of the current study which impedes external validity of our findings. Including women would likely have introduced additional biological- and hormonal-heterogeneity related to menstrual-cycle stage and/or hormonal contraceptive use, and the study was not powered to examine sex-by-treatment interactions or phase-specific responses. In that context, restricting enrolment to men was a design choice intended to preserve statistical precision for the primary outcomes rather than an assertion that women cannot be studied in this setting. Therefore, the results of the current study cannot be generalised to females and further research is required to assess the potential ergogenic and metabolic benefits of UQH₂ supplementation in females.

In conclusion, short-term UQH₂ supplementation increased plasma [CoQ₁₀] and lowered CI_L respiration and 1/RCR suggesting improved mitochondrial oxidative phosphorylation coupling efficiency. These observations occurred independent of changes in skeletal muscle ANT1+2 and UCP-3 protein content as well as muscle CS activity and protein content of mitochondrial complexes I-V. However, UQH₂ supplementation did not affect $\dot{V}O_2$ or T_{Lim} during severe-intensity exercise. Therefore, while short-term UQH₂ supplementation improved skeletal muscle mitochondrial oxidative phosphorylation coupling

efficiency, it did not translate to improved exercise economy, $\dot{V}O_{2peak}$ or T_{Lim} during severe-intensity cycling exercise in healthy, recreationally active adults.

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Author contributions JA and SB conceived and designed the study. JA, SB and NA undertook data collection. JA, SB and MF analysed the data. JA wrote the manuscript. All authors read and approved the manuscript.

Data availability Data are available upon reasonable request.

Declarations

Conflict of interest This study formed part of JA's doctoral research which was match funded by Kaneka Pharma Europe and Loughborough University. Kaneka Pharma Europe had no role in the interpretation of findings or manuscript preparation. Kaneka Pharma Europe analysed plasma samples for CoQ₁₀ concentrations but were blinded throughout the process.

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