Mitochondria-targeted antioxidant supplementation improves 8 km time trial performance in middle-aged trained male cyclists

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Abstract

Background

Exercise increases skeletal muscle ROS production, which may contribute to the onset of muscular fatigue and impair athletic performance. Mitochondria-targeted antioxidants such as MitoQ are becoming popular amongst active individuals as they are designed to accumulate within mitochondria and may provide targeted protection against exercise-induced oxidative stress. However, the effect of MitoQ supplementation on cycling performance is currently unknown. Here we investigate whether MitoQ supplementation can improve cycling performance measured as time to complete an 8 km time trial.

Method

In a randomised, double-blind, placebo-controlled crossover study, 19 middle-aged (age: 44 ± 4 years) recreationally trained (VO_{2peak}: 58.5 ± 6.2 ml·kg·min^{-1}, distance cycled per week during six months prior to study enrollment: 158.3 ± 58.4 km) male cyclists completed 45 min cycling at 70% VO_{2peak} followed by an 8 km time trial after 28 days of supplementation with MitoQ (20 mg/day) and a placebo. Free F_2-isoprostanes were measured in plasma samples collected at rest, after 45 min cycling at 70% VO_{2peak} and after completion of the time trial. Respiratory gases and measures of rate of perceived exertion (RPE) were also collected.

Results

Mean completion time for the time trial was 1.3% faster with MitoQ (12.91 ± 0.94 min) compared to placebo (13.09 ± 0.95 min, P = 0.04 95% CI [0.05, 2.64], *d* = 0.2). There was no difference in RPE during the time trial between conditions (P = 0.82) despite average power output during the time trial being higher following MitoQ supplementation (280 ± 53 W) compared to placebo (270 ± 51 W, P = 0.04). Plasma F_2-isoprostanes were lower on completion of the time trial following MitoQ supplementation (35.89 ± 13.6 pg/ml) compared to placebo (44.7 ± 16.9 pg/ml P = 0.03).

Conclusion

These data suggest that MitoQ supplementation may be an effective nutritional strategy to attenuate exercise-induced increases in oxidative damage to lipids and improve cycling performance.

Trial registration

This study was registered with the Australia New Zealand Clinical Trial Registry (ACTRN12619000451101) on 19th March 2019.

Introduction

Oxygen-derived radical and non-radical species, collectively referred to as reactive oxygen species (ROS), are continuously produced by skeletal muscle (1). High levels of ROS production in cells may overwhelm the endogenous antioxidant defense network resulting in damage to cellular proteins, lipids and DNA and impaired cellular function. Chronic oxidative stress is deleterious to muscle function (2) and has been associated with the pathogenesis of several diseases, including cardiovascular disease (3) and type 2 diabetes (4). Interestingly, while regular exercise is known to promote a wide range of health benefits, it also results in an acute increase in ROS levels systemically and in skeletal muscle (1).

Despite the traditional view that ROS are primarily harmful molecules, more recent evidence has shown that transient and physiological exercise-induced increases in skeletal muscle ROS production are required for optimal contractile function (5, 6), and mediate several acute and chronic exercise adaptations through the activation of redox-sensitive signalling pathways (7). This likely explains why some studies have shown that supplementation with general antioxidants can attenuate training-induced improvements in performance (8–10). However, acute exposure to $O_2{\cdot}^-$ and H_2O_2 can impair sarcoplasmic reticulum calcium release and myofibrillar calcium sensitivity resulting in the development of muscular fatigue (11–14). Thus, increasing the capacity of skeletal muscle to neutralise ROS by using exogenous antioxidant supplements has received much attention as a potential strategy to delay the onset of muscular fatigue and improve athletic performance. Several general antioxidants such as N-acetylcysteine and vitamin C and E have been studied for their effectiveness as ergogenic aids. While the results have been mixed, if not mostly disappointing (15, 16), there is some evidence to support the use of antioxidant supplements to improve performance. Vitamin C and N-acetylcysteine have been shown to improve performance when they are used to reverse a particular antioxidant deficiency (17). Furthermore, N-acetylcysteine appears to inhibit muscular fatigue when infused intravenously during (13, 14) or immediately before exercise (18), and acute oral supplementation with N-acetylcysteine has been shown to improve performance during repetitive handgrip exercise (19) and repeated bouts of running (20).

Multiple enzymes and organelles within skeletal muscle generate ROS during exercise. NADPH oxidase enzymes are thought to be the primary source of exercise-induced $O_2{\cdot}^-/H_2O_2$ since the increase in ATP demand during exercise decreases $O_2{\cdot}^-$ production by mitochondria (16, 21). While total mitochondrial $O_2{\cdot}^-$ production decreases during exercise, mitochondria continue to produce $O_2{\cdot}^-$ at complex I under conditions mimicking aerobic exercise (21) and this may contribute to the development of fatigue. Coenzyme Q10 supplementation has gained considerable attention as a dietary strategy with the potential to improve athletic performance as coenzyme Q10 plays an essential role in mitochondrial bioenergetics and acts as an antioxidant within plasma membranes (22). However, accumulation of coenzyme Q10 within mitochondria is limited due to its high molecular weight and low aqueous solubility, which may explain why studies investigating the ergogenic effects of coenzyme Q10 supplementation have produced mixed results (16).

Mitochondria-targeted coenzyme Q10, known as Mitoquinone (MitoQ), accumulates in mitochondria owing to the addition of a lipophilic triphenylphosphonium cation (23, 24). Within mitochondria, MitoQ is reduced by complex II to ubiquinol, which prevents lipid peroxidation directly by acting as a chain-breaking antioxidant and indirectly by recycling the α-tocopheroxyl radical to its active form (25, 26). Williamson et al. (2020) recently showed that MitoQ protects against exercise-induced increases in mitochondrial DNA damage; however, the effects of MitoQ on athletic performance are unknown. The aim of this study was to investigate whether MitoQ supplementation could improve the time taken to complete an 8 km cycling time trial. It was hypothesised that MitoQ would improve cycling time trial performance compared to placebo.

Methods

Participants

Twenty-two healthy middle-aged (35–50 years) recreationally trained (defined as cycling > 100 km per week for the preceding six months) male cyclists were recruited via advertisements. Participants were non-smokers, free of injury and chronic illnesses including cardiovascular and metabolic disease, not taking medication that may influence cycling performance and had not taken antioxidant supplements within eight weeks of study enrollment. The study was limited to males in order to avoid any interference from fluctuations in hormone levels during the menstrual cycle (27). All participants provided written informed consent before the commencement of the study. The study was approved by the Northern Health and Disability Ethics Committee (New Zealand) (18/CEN/136) on 3rd September 2018, registered with the Australia New Zealand Clinical Trial Registry (ACTRN12619000451101) on 19th March 2019 and conducted in accordance with the ethical standards laid down in the Declaration of Helsinki.

Experimental design

The study was a double-blind, placebo-controlled crossover design (Fig. 1). Participants attended the laboratory at The University of Auckland, New Zealand on five occasions over 15 weeks between March and December 2019 to complete an incremental test to exhaustion (VO_{2peak}), two familiarisation rides and two performance trials on a stationary cycle ergometer (Velotron, RacerMate, Seattle, WA). The performance trial consisted of 45 min cycling at a fixed workload eliciting 70% VO_{2peak} followed by an 8 km time trial. All tests were performed under normal laboratory conditions (temperature: 22.18 ± 1.24 °C, relative humidity: 39.82 ± 6.18%) and were supervised by the same researcher who was blinded to the experimental condition and provided consistent encouragement across trials.

Incremental test to exhaustion (VO_{2peak})

On the first laboratory visit, participants completed an incremental ramped exercise test on a cycle ergometer to determine VO_{2peak}. The handlebars and seat were positioned to match that of the participant's own bike, and this setup was the same for all subsequent trials. The test protocol started at 125 W and increased at a rate of 25 W min^{-1} until voluntary exhaustion. Participants pedalled at a self-selected cadence between 70 and 90 rpm, and the test ended when cadence dropped below 70 rpm for more than 10 s. VO_{2peak} was determined as the highest 30-s average value attained using a metabolic system (Parvo Medics True One 2400, Sandy, UT) and workload corresponding to VO_{2peak} was recorded.

Supplementation

In a double blind, placebo-controlled design, participants were randomised by an independent researcher to two groups, which would determine the order in which they received MitoQ (mitoquinone mesylate; 20 mg/day) and an identical placebo (tapioca powder, precipitated silica and microcrystalline cellulose 101). Order and sequence effects were controlled for through counterbalancing. Participants were instructed to consume one tablet per day orally 30 min before breakfast for 28 days before completing the first performance trial. After a 6-week washout period, participants crossed over into the other treatment group before completing the second performance trial. Supplementation adherence was monitored by oversupplying the participants with tablets and counting the number of tablets returned at each performance trial.

Performance trial

Participants arrived at the laboratory at 8 am having abstained from alcohol consumption and exercise for the preceding 48 and 24 h, respectively. Participants rested in a supine position for 15 min before the collection of a resting blood sample. A standardised warm-up was completed, which involved cycling for 5 min at 100 W, followed by 1 min at 50, 60, 70, 80 and 90% peak power output (determined during the VO_{2peak} test), then 5 min at 100 W. Participants then cycled at a workload that elicited 70% VO_{2peak} for 45 min. Rate of perceived exertion (RPE) was measured using the Borg scale (6–20), and respiratory gases were collected at 15- and 30-min for 3 min. Participants then immediately completed an 8 km time trial, which they were instructed to finish in the shortest time possible. The time trial mode of the cycle ergometer allowed for the use of self-selected gearing. Visual feedback, including distance and time elapsed, speed, power and cadence was provided for the first 0.8 km of the time trial, after which the only feedback visible was the distance completed. During the time trial, RPE was measured at 2, 4 and 6 km. Blood samples were collected immediately after completion of the 45 min cycling at 70% VO_{2peak} and the time trial (Fig. 1b).

Blood sample collection and analysis

On arrival at the laboratory, a cannula was inserted into an antecubital forearm vein. At each blood sampling time point, 10 ml of blood was collected into a vacutainer tube containing EDTA, whilst 5 ml of blood was collected into a tube containing EDTA, butylated hydroxytoluene and reduced glutathione for later analysis of plasma F_2-isoprostanes. Blood samples were centrifuged at 2000 g for 10 min at 4 °C and plasma was recovered and stored at -80 °C for later analysis. Plasma lactate, glucose, non-esterified fatty acid (NEFA) and triglyceride concentration were measured using a Roche C311 autoanalyser (Roche, Mannheim, Germany) by enzymatic colorimetric assay. Free F_2-isoprostanes were measured in plasma using a commercial ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer’s instructions.

Dietary intake

Participants were instructed to consume their habitual diet and record their diet in a food diary for three days before the performance trials. The participant's food diary for the first performance trial was copied and returned to them so that they could replicate this diet for the second, crossover performance trial. Standard meals were provided for dinner (548 kcal, 75 g carbohydrate, 43 g protein, 9 g fat) the night before and breakfast (694 kcal, 90.4 g carbohydrate, 16.7 g protein, 23.3 g fat) the morning of both performance trials. Participants were instructed to consume the breakfast meal 90 min before the start of the performance trial.

Habitual training load

Participants were asked to maintain a consistent and habitual training load throughout the study and to prepare for each performance trial as if it was a competitive event. The duration and mean power output of all training sessions were recorded using the exercise tracking application Strava and the participant's own devices.

Statistical analyses

Statistical analyses were performed using Prism 8 (GraphPad Software, Version 8), with statistical significance determined as $P \leq 0.05$. Data are presented as means ± SD unless otherwise specified. Prior to analysis, data were assessed for normality using the D'Agostino & Pearson test, and all followed a Gaussian distribution. A paired-samples t-test was used to assess the difference in time taken to complete the time trial following MitoQ and placebo supplementation, which was the primary outcome measure in this study. Paired-samples t-tests were also used to analyse differences in power output during the time trial, heart rate during cycling at 70% VO_{2peak}, and average daily training duration, mean power output during each training session and adherence to the supplementation protocol during MitoQ and placebo supplementation phases. Two-way (time x treatment) repeated measures ANOVA with Holm-Sidak's post hoc analysis were used to analyse VO_2, RER, RPE, and carbohydrate and fat oxidation rates during cycling at 70% VO_{2peak}. Power output during each km of the time trial, RPE during the time trial and plasma glucose, NEFA, triglyceride, lactate and F_2-isoprostane concentration were also analysed using two-way (time x treatment) repeated measures ANOVA with Holm-Sidak's post hoc analysis. The relationship between resting F_2-isoprostanes and exercise-induced changes in F_2-isoprostanes and change in time to complete the time trial following MitoQ supplementation compared to placebo were analysed using linear regression. Linear regression was also used to analyse the association between power output during the time trial and plasma lactate concentration on completion of the time trial following MitoQ and placebo supplementation. Effect sizes were calculated using Cohen's d and interpreted using the following quantitative statements, > 0.2 small, > 0.5 medium and > 0.8 large. Uncertainty in the estimate of the effect on time trial performance is expressed as 95% confidence intervals (CI).

Results

Three participants withdrew from the study (Fig. 2) and results are presented for the 19 participants who completed all study visits. Participant characteristics are given in table 1, and training records indicated that participants cycled 158.3 ± 58.4 km per week on average during the six months prior to their enrollment in the study. Supplementation adherence was > 90% and there was no difference in supplementation adherence during MitoQ and placebo supplementation periods (placebo; 94 ± 10%, MitoQ; 95 ± 6% P = 0.80). No side effects of supplementation were reported. A similar average daily training duration (placebo; 41.56 ± 20.12 min, MitoQ; 46.33 ± 21.15 min, P = 0.17) and mean power output during each training session (placebo; 169 ± 56 W, MitoQ; 172 ± 43 W, P = 0.75) were recorded during the placebo and MitoQ supplementation phases.

Fixed-load cycling

During cycling at 70% VO_{2peak}, a similar VO_2, RER, carbohydrate and fat oxidation and RPE were observed following MitoQ and placebo supplementation (Table 2). Heart rate was not different between supplementation conditions (placebo; 151 ± 16 bpm, MitoQ; 153 ± 13 bpm, P = 0.34).

8 km cycling time trial performance

Time to complete the 8 km time trial was 1.3% faster following MitoQ supplementation (12.91 ± 0.94 min) compared to placebo (13.09 ± 0.95 min, P = 0.04, 95% CI [0.05, 2.64], *d* = 0.2, Fig. 3a-b), indicating improved performance following MitoQ supplementation. Consistent with this, 12 out the 19 participants completed the time trial faster following MitoQ supplementation compared to placebo (Fig. 3c). Average power output during the time trial was higher following MitoQ supplementation (280 ± 53 W) compared to placebo (270 ± 51 W, P = 0.04, Fig. 4a). Moreover, a significant main effect of treatment was observed when comparing average power output during each km of the time trial (P < 0.001, Fig. 4b). Despite the difference in power output, there was no difference in RPE between the supplementation conditions during the time trial (Fig. 4c).

Blood Measures

A complete set of blood samples were collected from 16 participants due to technical issues with cannulas for three participants (Fig. 2). Plasma glucose, NEFA and triglyceride concentration increased during exercise (main effect for time P ≤ 0.01), and this was not affected MitoQ supplementation (P > 0.05 for treatment and interaction effect; Fig. 5a-c). Plasma lactate concentration increased (P < 0.001) during exercise, and post-hoc analysis indicated that the main effect for treatment (P = 0.01) was a result of increased plasma lactate concentration post-exercise following MitoQ supplementation (12.09 ± 4.13 mmol/L) compared to placebo (10.27 ± 4.45 mmol/L, P = 0.04, Fig. 5d). Further analysis revealed that there was no significant difference in the slope of the linear regression lines for the association between power output during the time trial and plasma lactate concentration on completion of time trial following MitoQ vs placebo supplementation, indicating that the increased plasma lactate concentration during the MitoQ trial was the result of the increased power output.

Lipid peroxidation

A main effect of time and a time x treatment interaction were observed for plasma F_2-isoprostanes ($P < 0.05$, Fig. 6a), with post hoc analysis revealing that plasma F_2-isoprostanes were lower on completion of the time trial following MitoQ (35.89 ± 14.02 pg/ml) compared to placebo supplementation (44.68 ± 17.50 pg/ml), $P = 0.03$, Fig. 6a). There was no association between resting plasma F_2-isoprostanes or exercise-induced changes in plasma F_2-isoprostanes during the performance trial and change in time to complete the time trial following MitoQ supplementation compared to placebo ($P > 0.05$, Fig. 6b and c).

Discussion

The production of ROS by skeletal muscle during exercise is associated with the onset of muscular fatigue and a reduction in athletic performance (12). Our study provides evidence to suggest that oral supplementation of the mitochondria-targeted antioxidant MitoQ can improve cycling time trial performance in middle-aged, recreationally trained male cyclists. The improvement in time trial performance observed in this study occurred along with an attenuation of exercise-induced increases in plasma F_2-isoprostanes by MitoQ.

This is the first study to investigate the effect of MitoQ supplementation on cycling performance. Several studies have described improvements in cycling performance following supplementation with non-mitochondria-targeted coenzyme Q10 (28–31), which may improve performance by acting as an antioxidant within skeletal muscle mitochondria. However, accumulation of orally ingested coenzyme Q10 within mitochondria is limited by its poor bioavailability, which may explain why several others have failed to show improved performance following coenzyme Q10 supplementation (8, 32–35). Indeed, supplementation with coenzyme Q10 is often ineffective at increasing its concentration within skeletal muscle (36–38), and several studies have shown that coenzyme Q10 supplementation does not affect exercise-induced increases in systemic markers of oxidative stress (28, 39, 40). MitoQ has been shown accumulate in skeletal muscle following oral administration in mice (41). However, studies investigating the accumulation of MitoQ within skeletal muscle following oral supplementation in humans are needed.

The smallest worthwhile enhancement for cyclists competing in road time-trials has been reported to be 0.6% (42). Therefore, the 1.3% improvement in time trial performance observed following MitoQ supplementation in this study indicates a meaningful performance improvement. However, there was significant interindividual variability in the response to MitoQ, meaning we saw a small effect of MitoQ on time trial performance ($d = 0.2$). Individual redox status seems to be an important determinant of the efficacy of antioxidant supplementation to improve performance (43). We observed large interindividual variability in resting F_2-isoprostanes and the redox response to exercise. However, there was no correlation between resting F_2-isoprostanes or exercise-induced changes in F_2-isoprostanes and change in time to complete the time trial following MitoQ supplementation compared to placebo. It should be noted that this study was not statistically powered for this outcome measure and it is important to investigate this further given that the ergogenic effects of vitamin C and N-acetylcysteine may be limited to individuals in which a specific deficiency is reversed (17). Whether the ergogenic effects of MitoQ are limited to individuals with low levels of endogenous ubiquinone poses an interesting avenue for further investigation.

In contrast to our finding that MitoQ supplementation improves cycling performance, the mitochondria-targeted antioxidant SS-31 did not affect force production during fatiguing stimulation in isolated mouse skeletal muscle (44) or the rate of contractile force decline in intact single muscle fibres (45). Direct comparisons between SS-31 and MitoQ are difficult given that SS-31 binds to the mitochondrial lipid cardiolipin and protects against oxidative damage whereas MitoQ protects cell membranes by acting as a chain breaking antioxidant and recycling α-tocopherol (24, 46). However, the ergogenic effect of MitoQ may be related to the impact of MitoQ on peripheral tissues, which would not be observed in an *in vitro* model of muscle contraction. MitoQ has been shown to improve endothelial function in older individuals with endothelial dysfunction (47). Whether these effects translate to healthy, physically trained individuals during exercise has not been investigated. Alternatively, the effects of mitochondria-targeted antioxidant supplementation on performance may be dependent on the mode and intensity of muscular contraction. Exercise-induced ROS production by skeletal muscle is thought to increase in an intensity-dependent manner (48), which may explain why we saw an improvement in performance during high intensity exercise following MitoQ supplementation while the physiological response to cycling at 70% VO_{2peak} was unchanged. On the other hand, the higher plasma lactate concentration coupled with improved time trial performance with MitoQ suggests that MitoQ may improve performance by allowing for increased use of anaerobic metabolism during high intensity exercise, or by improving tolerance to lower pH in skeletal muscle. Future studies should aim to clarify whether the positive effects of MitoQ on performance and markers of exercise-induced oxidative stress are limited to exercise at a high intensity.

A major finding of the current study is that MitoQ attenuated the increase in plasma F_2-isoprostanes during the time trial, which may indicate a protective effect of MitoQ against exercise-induced lipid peroxidation within mitochondria. Mitochondrial lipids are essential for maintaining the integrity of mitochondrial membranes and proper function of mitochondria; however, they are prone to lipid peroxidation by free radicals (49). Therefore, by attenuating exercise-induced lipid peroxidation in mitochondria, MitoQ may preserve the structure and dynamics of mitochondrial membranes and the efficiency through which mitochondria can supply ATP for muscular contraction. However, plasma F_2-isoprostanes are a systemic marker of lipid peroxidation, meaning the post-exercise increase in plasma F_2-isoprostane levels may also be derived from non-mitochondrial sources. Similar to previous studies (50, 51), we saw no effect of MitoQ on pre-exercise markers of oxidative stress. However, given that the participants were healthy and exercise-trained, it seems likely that any changes in resting F_2-isoprostanes would have been difficult to detect (52). It appears that MitoQ may be most effective in lowering oxidative stress in situations where mitochondria are under stress (53).

Our study has limitations that should be acknowledged. Participants were all recreationally trained men and caution should be taken when extrapolating these results to elite athletes and females. Furthermore, the inclusion of a performance test at the start of each supplementation phase would have enabled us to ensure that any lasting effects of MitoQ supplementation were not carried over. A 6-week washout period has been shown to reverse the effects of MitoQ supplementation on mitochondrial H_2O_2 levels (50). However, we cannot be sure that MitoQ supplementation did not result in adaptations that may have lasted beyond the washout period. Participants also supplemented for 28 days while maintaining their habitual training meaning we cannot determine whether the improved cycling performance reflects an ergogenic effect of MitoQ on exercise performance alone or an interaction between MitoQ and training.

Conclusions

Taken together, our work provides evidence that MitoQ can attenuate exercise-induced increases in lipid peroxidation and improve cycling time trial performance in middle-aged, recreationally trained men. It appears that MitoQ may improve performance above the aerobic threshold by allowing for increased use of anaerobic metabolism, or by improving tolerance to lower pH in muscle. Further research is required to determine the mechanisms responsible for the ergogenic effect of MitoQ, understand the important factors that determine an individual’s response to MitoQ supplementation and identify optimal dosing strategies.

List Of Abbrieviations

H_2O_2

Hydrogen peroxide

NEFA

Non-esterified fatty acids

$O_2{\cdot}^-$

Superoxide

RER

Respiratory exchange ratio

ROS

Reactive oxygen species

RPE

Rate of perceived exertion

DECLARATIONS

Declarations

Ethics approval and consent to participate

All experiments were previously approved by the Northern Health and Disability Ethics Committee (New Zealand) (Reference number: 18/CEN/136) and conducted in accordance with the ethical standards laid down in the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

Data and publication materials are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests to disclose.

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Authors’ contributions

All authors conceived and designed the study, SCB performed the experiments and analysed the data, SCB and TLM interpreted the results of the experiments, SCB drafted the manuscript, and all authors edited and revised the manuscript and approved the final manuscript version.

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References

1. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev. 2008;88(4):1243–76.
2. Powers SK, Hudson MB, Nelson WB, Talbert EE, Min K, Szeto HH, et al. Mitochondria-targeted antioxidants protect against mechanical ventilation-induced diaphragm weakness. Critical care medicine. 2011;39(7):1749–59.
3. Incalza MA, D'Oria R, Natalicchio A, Perrini S, Laviola L, Giorgino F. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. Vascul Pharmacol. 2018;100:1–19.
4. Meo SD, Iossa S, Venditti P. Skeletal muscle insulin resistance: role of mitochondria and other. ROS sources. 2017;233(1):R15.
5. Mollica JP, Dutka TL, Merry TL, Lamboley CR, McConell GK, McKenna MJ, et al. S-Glutathionylation of troponin I (fast) increases contractile apparatus Ca2 + sensitivity in fast-twitch muscle fibres of rats and humans. The Journal of Physiology. 2012;590(6):1443–63.
6. Andrade FH, Reid MB, Westerblad H. Contractile response of skeletal muscle to low peroxide concentrations: myofibrillar calcium sensitivity as a likely target for redox-modulation. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2001;15(2):309–11.
7. Margaritelis NV, Paschalis V, Theodorou AA, Kyparos A, Nikolaidis MG. Redox basis of exercise physiology. Redox biology. 2020:101499.
8. Laaksonen R, Fogelholm M, Himberg JJ, Laakso J, Salorinne Y. Ubiquinone supplementation and exercise capacity in trained young and older men. Eur J Appl Physiol Occup Physiol. 1995;72(1–2):95–100.
9. Braakhuis AJ, Hopkins WG, Lowe TE. Effects of dietary antioxidants on training and performance in female runners. European journal of sport science. 2014;14(2):160–8.
10. Malm C, Svensson M, Ekblom B, Sjodin B. Effects of ubiquinone-10 supplementation and high intensity training on physical performance in humans. Acta Physiol Scand. 1997;161(3):379–84.
11. Allen DG, Lamb GD, Westerblad H. Skeletal Muscle Fatigue: Cellular Mechanisms. Physiol Rev. 2008;88(1):287–332.
12. Reid MB. Reactive Oxygen Species as Agents of Fatigue. Med Sci Sports Exerc. 2016;48(11):2239–46.
13. Medved I, Brown MJ, Bjorksten AR, Murphy KT, Petersen AC, Sostaric S, et al. N-acetylcysteine enhances muscle cysteine and glutathione availability and attenuates fatigue during prolonged exercise in endurance-trained individuals. J Appl Physiol. 2004;97(4):1477–85.
14. McKenna MJ, Medved I, Goodman CA, Brown MJ, Bjorksten AR, Murphy KT, et al. N-acetylcysteine attenuates the decline in muscle Na+,K+-pump activity and delays fatigue during prolonged exercise in humans. J Physiol. 2006;576(Pt 1):279–88.

15. Braakhuis AJ, Hopkins WG. Impact of Dietary Antioxidants on Sport Performance: A Review. Sports medicine (Auckland, NZ). 2015;45(7):939–55.
16. Mason SA, Trewin AJ, Parker L, Wadley GD. Antioxidant supplements and endurance exercise: Current evidence and mechanistic insights. Redox biology. 2020:101471.
17. Margaritelis NV, Paschalis V, Theodorou AA, Kyparos A, Nikolaidis MG. Antioxidant supplementation, redox deficiencies and exercise performance: A falsification design. Free Radic Biol Med. 2020.
18. Reid MB, Stokic DS, Koch SM, Khawli FA, Leis AA. N-acetylcysteine inhibits muscle fatigue in humans. J Clin Investig. 1994;94(6):2468–74.
19. Matuszczak Y, Farid M, Jones J, Lansdowne S, Smith MA, Taylor AA, et al. Effects of N-acetylcysteine on glutathione oxidation and fatigue during handgrip exercise. Muscle Nerve. 2005;32(5):633–8.
20. Cobley JN, McGlory C, Morton JP, Close GL. N-Acetylcysteine's attenuation of fatigue after repeated bouts of intermittent exercise: practical implications for tournament situations. Int J Sport Nutr Exerc Metab. 2011;21(6):451–61.
21. Goncalves RL, Quinlan CL, Perevoshchikova IV, Hey-Mogensen M, Brand MD. Sites of superoxide and hydrogen peroxide production by muscle mitochondria assessed ex vivo under conditions mimicking rest and exercise. J Biol Chem. 2015;290(1):209–27.
22. Frei B, Kim MC, Ames BN. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. Proceedings of the National Academy of Sciences. 1990;87(12):4879-83.
23. KELSO GF, PORTEOUS CM, HUGHES G, LEDGERWOOD EC, GANE AM, SMITH RAJ, et al. Prevention of Mitochondrial Oxidative Damage Using Targeted Antioxidants. Annals of the New York Academy of Sciences. 2002;959(1):263 – 74.
24. Murphy MP, Smith RA. Targeting antioxidants to mitochondria by conjugation to lipophilic cations. Annu Rev Pharmacol Toxicol. 2007;47:629–56.
25. James AM, Smith RAJ, Murphy MP. Antioxidant and prooxidant properties of mitochondrial Coenzyme Q. Arch Biochem Biophys. 2004;423(1):47–56.
26. Ingold KU, Bowry VW, Stocker R, Walling C. Autoxidation of lipids and antioxidation by alpha-tocopherol and ubiquinol in homogeneous solution and in aqueous dispersions of lipids: unrecognized consequences of lipid particle size as exemplified by oxidation of human low density lipoprotein. Proceedings of the National Academy of Sciences. 1993;90(1):45.
27. Joo MH, Maehata E, Adachi T, Ishida A, Murai F, Mesaki N. The relationship between exercise-induced oxidative stress and the menstrual cycle. European journal of applied physiology. 2004;93(1):82–6.
28. Bonetti A, Solito F, Carmosino G, Bargossi AM, Fiorella PL. Effect of ubidecarenone oral treatment on aerobic power in middle-aged trained subjects. J Sports Med Phys Fit. 2000;40(1):51–7.
29. Mizuno K, Tanaka M, Nozaki S, Mizuma H, Ataka S, Tahara T, et al. Antifatigue effects of coenzyme Q10 during physical fatigue. Nutrition. 2008;24(4):293–9.
30. Gökbel H, Gül I, Belviranl M, Okudan N. The Effects Of Coenzyme Q10 Supplementation on Performance During Repeated Bouts of Supramaximal Exercise in Sedentary Men. The Journal of

Strength Conditioning Research. 2010;24(1):97–102.

31. Alf D, Schmidt ME, Siebrecht SC. Ubiquinol supplementation enhances peak power production in trained athletes: a double-blind, placebo controlled study. J Int Soc Sports Nutr. 2013;10(1):24.
32. Bloomer RJ, Canale RE, McCarthy CG, Farney TM. Impact of Oral Ubiquinol on Blood Oxidative Stress and Exercise Performance. Oxidative Med Cell Longev. 2012;2012:465020.
33. Weston SB, Zhou S, Weatherby RP, Robson SJ. Does exogenous coenzyme Q10 affect aerobic capacity in endurance athletes? Int J Sport Nutr. 1997;7(3):197–206.
34. Porter DA, Costill DL, Zachwieja JJ, Krzeminski K, Fink WJ, Wagner E, et al. The effect of oral coenzyme Q10 on the exercise tolerance of middle-aged, untrained men. Int J Sports Med. 1995;16(7):421–7.
35. Snider IP, Bazzarre TL, Murdoch SD, Goldfarb A. Effects of coenzyme athletic performance system as an ergogenic aid on endurance performance to exhaustion. Int J Sport Nutr Exerc Metab. 1992;2(3):272–86.
36. Matthew Cooke MI, Buford T, Shelmadine B, Hudson G, Kerksick C, Rasmussen C. Mike Greenwood, Brian Leutholtz, Darryn Willoughby and Richard Kreider. Effects of acute and 14-day coenzyme Q10 supplementation on exercise performance. 2008.
37. Zhou S, Zhang Y, Davie A, Marshall-Gradisnik S, Hu H, Wang J, et al. Muscle and plasma coenzyme Q10 concentration, aerobic power and exercise economy of healthy men in response to four weeks of supplementation. J Sports Med Phys Fit. 2005;45(3):337–46.
38. Svensson M, Malm C, Tonkonogi M, Ekblom B, Sjodin B, Sahlin K. Effect of Q10 supplementation on tissue Q10 levels and adenine nucleotide catabolism during high-intensity exercise. Int J Sport Nutr. 1999;9(2):166–80.
39. Okudan N, Belviranli M, Torlak S. Coenzyme Q10 does not prevent exercise-induced muscle damage and oxidative stress in sedentary men. J Sports Med Phys Fit. 2018;58(6):889–94.
40. Östman B, Sjödin A, Michaëlsson K, Byberg L. Coenzyme Q10 supplementation and exercise-induced oxidative stress in humans. Nutrition. 2012;28(4):403–17.
41. Smith RA, Porteous CM, Gane AM, Murphy MP. Delivery of bioactive molecules to mitochondria in vivo. Proc Natl Acad Sci U S A. 2003;100(9):5407–12.
42. Paton CD, Hopkins WG. Ergometer Error and Biological Variation in Power Output in a Performance Test with Three Cycle Ergometers. Int J Sports Med. 2006;27(06):444–7.
43. Paschalis V, Theodorou AA, Margaritelis NV, Kyparos A, Nikolaidis MG. N-acetylcysteine supplementation increases exercise performance and reduces oxidative stress only in individuals with low levels of glutathione. Free Radic Biol Med. 2018;115:288–97.
44. Katz A, Hernandez A, Caballero DM, Briceno JF, Amezquita LV, Kosterina N, et al. Effects of N-acetylcysteine on isolated mouse skeletal muscle: contractile properties, temperature dependence, and metabolism. Pflug Arch: Eur J Physiol. 2014;466(3):577–85.

45. Cheng AJ, Bruton JD, Lanner JT, Westerblad H. Antioxidant treatments do not improve force recovery after fatiguing stimulation of mouse skeletal muscle fibres. J Physiol. 2015;593(2):457–72.
46. Zhao K, Zhao GM, Wu D, Soong Y, Birk AV, Schiller PW, et al. Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. J Biol Chem. 2004;279(33):34682–90.
47. Graham D, Huynh NN, Hamilton CA, Beattie E, Smith RAJ, Cochemé HM, et al. Mitochondria-Targeted Antioxidant MitoQ < sub > 10</sub > Improves Endothelial Function and Attenuates Cardiac Hypertrophy. Hypertension. 2009;54(2):322–8.
48. Parker L, McGuckin TA, Leicht AS. Influence of exercise intensity on systemic oxidative stress and antioxidant capacity. Clin Physiol Funct Imaging. 2014;34(5):377–83.
49. Xiao M, Zhong H, Xia L, Tao Y, Yin H. Pathophysiology of mitochondrial lipid oxidation: Role of 4-hydroxynonenal (4-HNE) and other bioactive lipids in mitochondria. Free Radic Biol Med. 2017;111:316–27.
50. Pham T, MacRae CL, Broome SC, D'souza RF, Narang R, Wang HW, et al. MitoQ and CoQ10 supplementation mildly suppresses skeletal muscle mitochondrial hydrogen peroxide levels without impacting mitochondrial function in middle-aged men. 2020.
51. Williamson J, Hughes CM, Cobley JN, Davison GW. The Mitochondria-Targeted Antioxidant MitoQ, Attenuates Exercise-Induced Mitochondrial DNA Damage. Redox biology. 2020:101673.
52. Gomez-Cabrera MC, Domenech E, Vina J. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic Biol Med. 2008;44(2):126–31.
53. Rossman MJ, Santos-Parker JR, Steward CAC, Bispham NZ, Cuevas LM, Rosenberg HL, et al. Chronic Supplementation With a Mitochondrial Antioxidant (MitoQ) Improves Vascular Function in Healthy Older Adults. Hypertension. 2018.

Tables

Table 1. Participant characteristics	
Characteristics	**Mean (n = 19)**
Age (years)	44 ± 4
Height (cm)	178.2 ± 4.8
Weight (kg)	78.5 ± 10.2
BMI (kg/m^2)	24.7 ± 2.4
VO_{2peak} ($ml \cdot kg \cdot min^{-1}$)	58.5 ± 6.2
Power output at VO_{2peak} (W)	398 ± 40
Power output eliciting 70% VO_{2peak} (W)	224 ± 28
Values are mean ± SD	

Table 2. Physiological response to cycling at a fixed load. VO_2, RER, carbohydrate and fat oxidation rates and RPE at 15 and 30 min during 45 min cycling at 70% VO_{2peak}.

	Placebo		**MitoQ**	
	15 min	**30 min**	**15 min**	**30 min**
VO_2 ($ml \cdot kg \cdot min^{-1}$)	40.6 ± 4.1	41.8 ± 4.4	40.5 ± 5.2	41.4 ± 5.2
RER	0.89 ± 0.04	0.90 ± 0.04	0.89 ± 0.03	0.89 ± 0.03
Carbohydrate oxidation ($g \cdot min^{-1}$)	2.84 ± 0.84	2.98 ± 0.82	2.74 ± 0.59	2.87 ± 0.60
Fat oxidation ($g \cdot min^{-1}$)	0.55 ± 0.23	0.54 ± 0.23	0.58 ± 0.17	0.56 ± 0.14
RPE	13.1 ± 1.3	13.8 ± 1.7	12.8 ± 1.6	13.7 ± 1.8
Values are mean ± SD; RER, respiratory exchange ratio; RPE, rate perceived exertion				

Figures

Figure 1

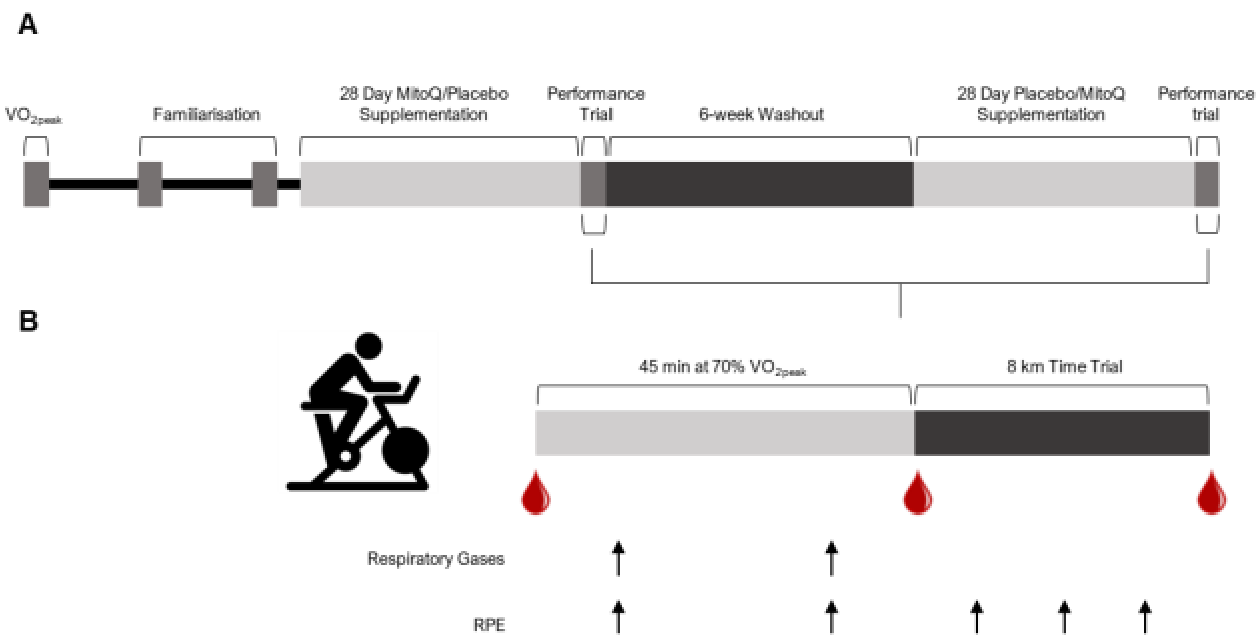

Figure 1

Study timeline (A) and performance trial procedures (B)

Figure 2

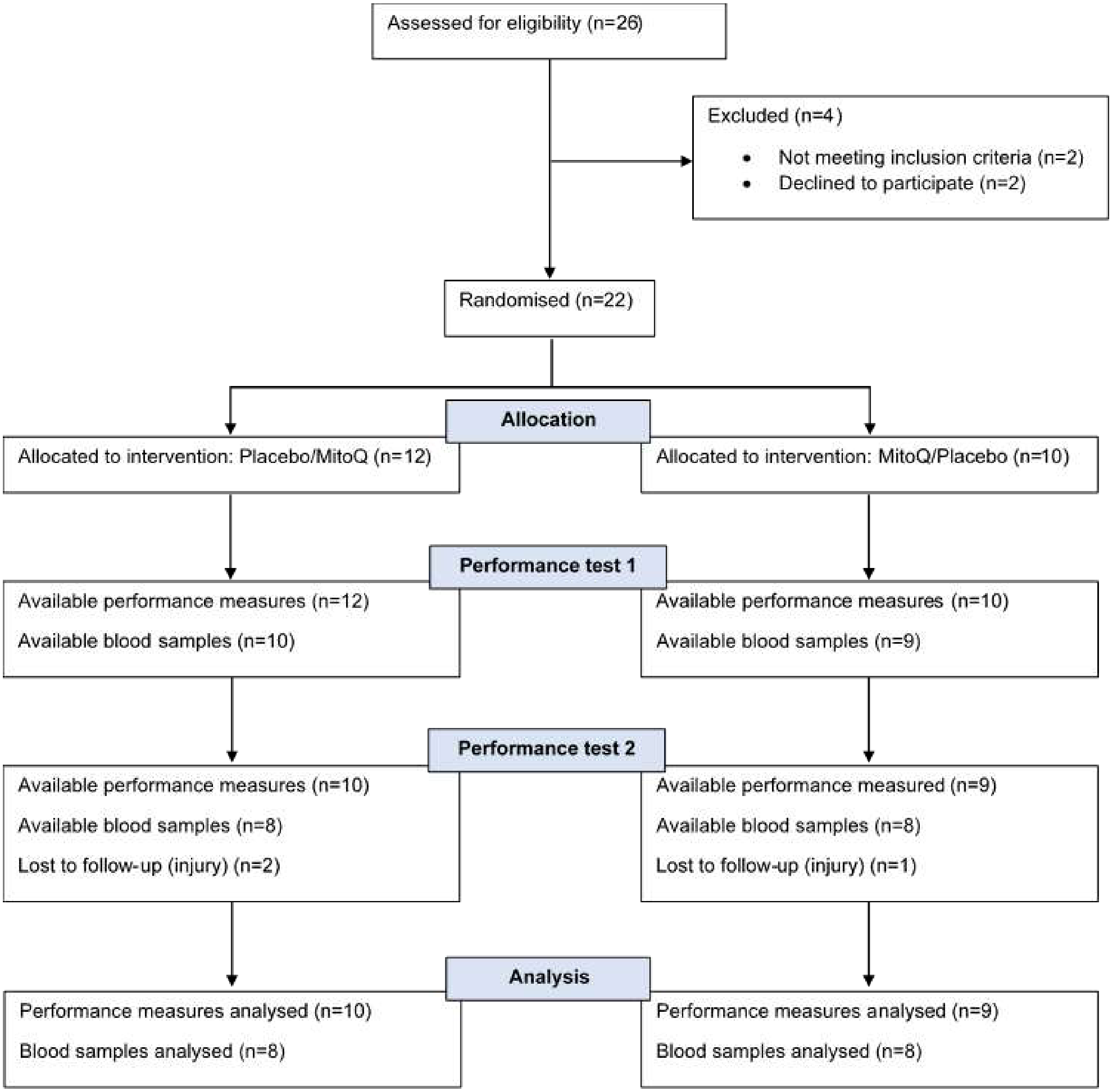

Figure 2

CONSORT flow chart. This figure shows the flow of patients through the trial according to the criteria recommended in the CONSORT Guidelines.

Figure 3

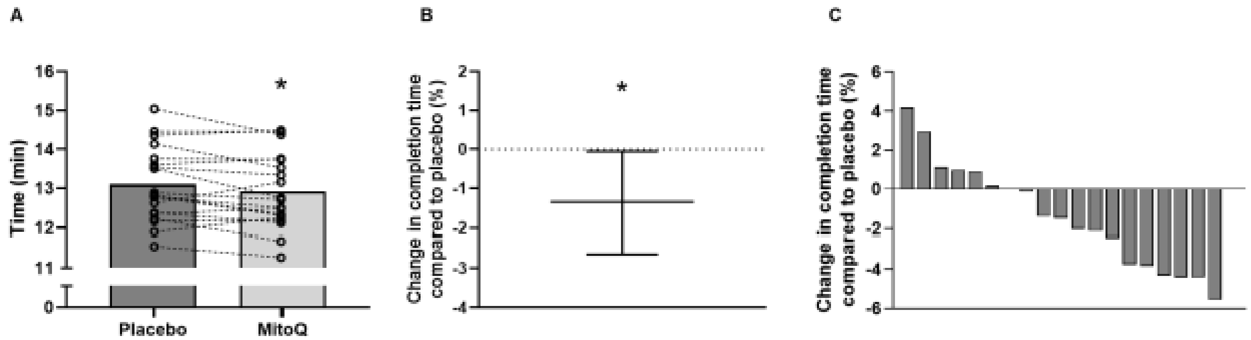

Figure 3

Mean time to complete the time trial following six weeks of placebo and MitoQ supplementation (A), percent change in time to complete time trial with MitoQ compared to placebo, data are presented at mean ± 95% CI (B), and individual percent change in time to complete the time trial with MitoQ compared to placebo (each grey bar represents one individual) (C). * P<0.05 vs placebo for paired-samples t-test, n=19.

Figure 4

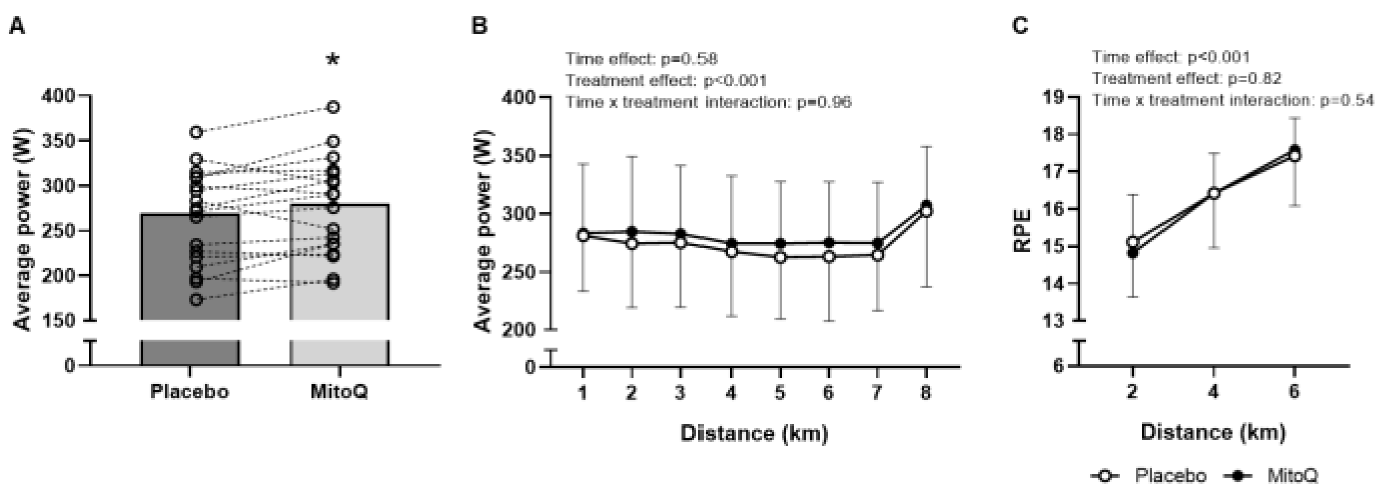

Figure 4

Mean power output during the time trial following six weeks of placebo and MitoQ supplementation (A), mean power output during each km of the time trial (B), mean RPE at 2, 4 and 6 km during the time trial (C). Data are presented as mean ± SD. Two-way repeated-measures ANOVA effects are given in figures and * P<0.05 vs placebo for paired-samples t-test, n=19.

Figure 5

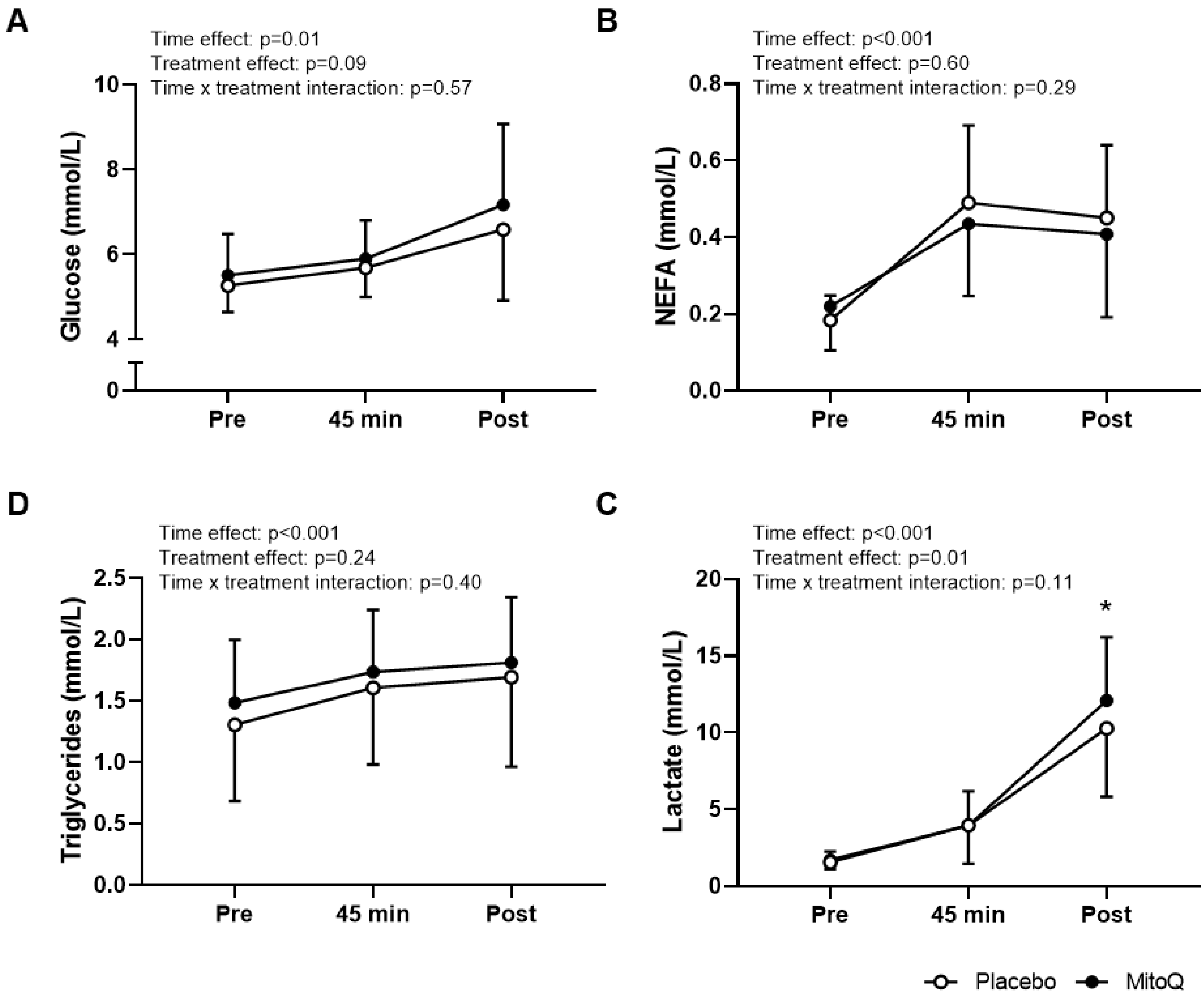

Figure 5

Mean plasma glucose (A), NEFA (B), triglyceride (C) and lactate (D) concentration at rest (pre), after 45 min cycling at 70% VO2peak (45 min) and after the time trial (post). Data are presented as mean ± SD. Two-way repeated-measures ANOVA effects are given in figures and * P<0.05 for post hoc vs placebo at the same time point, n=16.

Figure 6

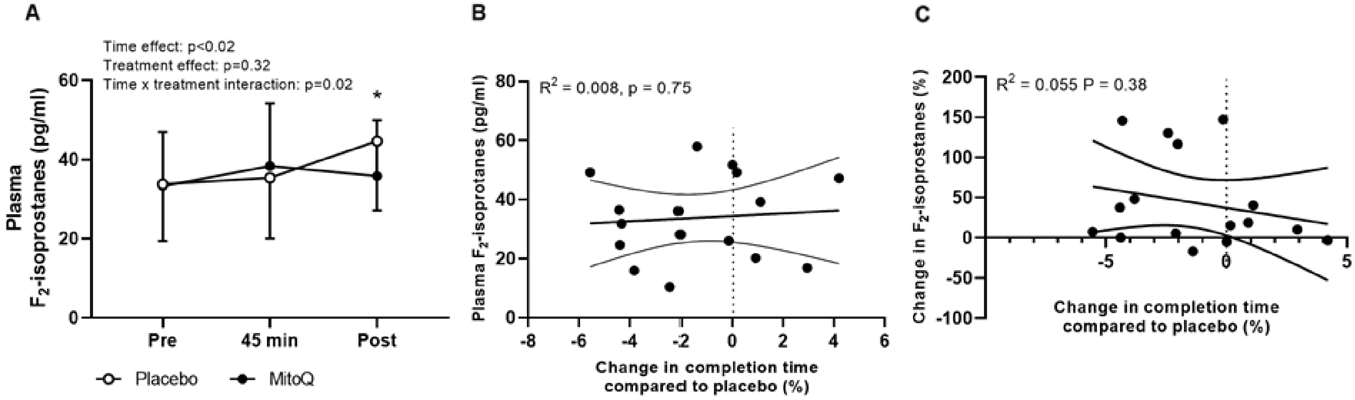

Figure 6

Mean plasma F2-isoprostane concentration at rest (pre), after 45 min cycling at 70% VO2peak (45 min) and after the time trial (post), data are presented as mean ± SD (A), correlation between change in time to complete the time trial following MitoQ supplementation and plasma F2-isoprostanes at rest following placebo supplementation (B), and correlation between change in time to complete the time trial following MitoQ supplementation and exercise-induced changes in plasma F2-isoprostanes following placebo supplementation (C). Two-way repeated-measures ANOVA effects are given in figures and * P<0.05 for post hoc vs placebo at the same time point, n=16.