Mitochondria-Targeted Antioxidant MitoQ\textsubscript{10} Improves Endothelial Function and Attenuates Cardiac Hypertrophy

Delyth Graham, Ngan N. Huynh, Carlene A. Hamilton, Elisabeth Beattie, Robin A.J. Smith, Helena M. Cocheme, Michael P. Murphy, Anna F. Dominiczak

Abstract—Mitochondria are a major site of reactive oxygen species production, which may contribute to the development of cardiovascular disease. Protecting mitochondria from oxidative damage should be an effective therapeutic strategy; however, conventional antioxidants are ineffective, because they cannot penetrate the mitochondria. This study investigated the role of mitochondrial oxidative stress during development of hypertension in the stroke-prone spontaneously hypertensive rat, using the mitochondria-targeted antioxidant, MitoQ\textsubscript{10}. Eight-week–old male stroke-prone spontaneously hypertensive rats were treated with MitoQ\textsubscript{10} (500 µmol/L; n = 16), control compound decyltriphenylenyphosphonium (decylTPP; 500 µmol/L; n = 8), or vehicle (n = 9) in drinking water for 8 weeks. Systolic blood pressure was significantly reduced by \textasciitilde25 mm Hg over the 8-week MitoQ\textsubscript{10} treatment period compared with decylTPP (F = 5.94; P = 0.029) or untreated controls (F = 65.6; P = 0.0001). MitoQ\textsubscript{10} treatment significantly improved thoracic aorta NO bioavailability (1.16±0.03 g/g; P = 0.002, area under the curve) compared with both untreated controls (0.68±0.02 g/g) and decylTPP-treated rats (0.60±0.06 g/g). Cardiac hypertrophy was significantly reduced by MitoQ\textsubscript{10} treatment compared with untreated control and decylTPP treatment (MitoQ\textsubscript{10}: 4.01±0.05 mg/g; control: 4.42±0.11 mg/g; and decylTPP: 4.40±0.09 mg/g; ANOVA P = 0.002). Total MitoQ\textsubscript{10} content was measured in liver, heart, carotid artery, and kidney harvested from MitoQ\textsubscript{10}-treated rats by liquid chromatography-tandem mass spectrometry. All of the organs analyzed demonstrated detectable levels of MitoQ\textsubscript{10}, with comparable accumulation in vascular and cardiac tissues. Administration of the mitochondria-targeted antioxidant MitoQ\textsubscript{10} protects against the development of hypertension, improves endothelial function, and reduces cardiac hypertrophy in young stroke-prone spontaneously hypertensive rats. MitoQ\textsubscript{10} provides a novel approach to attenuate mitochondrial-specific oxidative damage with the potential to become a new therapeutic intervention in human cardiovascular disease. (Hypertension. 2009;54:322-328.)

Key Words: hypertension • hyper trophy • mitochondria • antioxidant • endothelial function

Mitochondria are a major site of reactive oxygen species (ROS) generation within cells, and there is increasing evidence that mitochondrial oxidative stress contributes to a wide range of pathologies, including cardiovascular disease, neurodegeneration, and aging.\textsuperscript{1–5} Production of ROS by the mitochondrial respiratory chain (complexes I through III) occurs under normal physiological conditions\textsuperscript{2,3} and is caused by the 1-electron reduction of O\textsubscript{2} to superoxide (O\textsubscript{2}−) by the respiratory chain.\textsuperscript{6,7} This O\textsubscript{2}− goes on to produce a range of damaging ROS that lead to nonspecific modification of mitochondrial proteins, lipids, and nucleic acids, thereby altering mitochondrial function.\textsuperscript{3–5} Mitochondrial DNA is particularly susceptible to modification by ROS, and this damage can rapidly lead to functional changes in the cell, because it encodes 13 essential polypeptide components of the mitochondrial respiratory chain.\textsuperscript{4} Extensive evidence suggests that mitochondrial DNA damage occurs in cardiovascular disease in humans, animal models, and cellular models.\textsuperscript{3,7–9}

Mitochondria are normally protected from oxidative damage by a multilayer network of mitochondrial antioxidant systems.\textsuperscript{10,11} These include the mitochondrial matrix enzyme manganese superoxide dismutase, which converts the O\textsubscript{2}− anion to hydrogen peroxide, glutathione peroxidase, and peroxiredoxins 3 and 5, which readily convert hydrogen peroxide to water\textsuperscript{7,10} and ultimately prevent forms of mitochondrial oxidative damage, eg, lipid peroxidation. Modification of these antioxidant enzymes resulting from the knockout of manganese superoxide dismutase or glutathione peroxidase genes can significantly affect mitochondrial activity and ROS production and has been linked to hypertension and salt sensitivity in mice.\textsuperscript{12–15}

The precise contribution of mitochondria to the total ROS production in the vessel wall or other cardiovascular tissues...
remains unclear. Part of the problem relates to the limited efficacy of conventional antioxidants because of the difficulty of delivering them to mitochondria in situ. Furthermore, although specific antagonists of the respiratory chain, e.g., the complex I inhibitor rotenone or the complex III inhibitor antimycin, can modify mitochondria-derived ROS production, they have confounding effects on mitochondrial ATP production and membrane potential that make it difficult to interpret their effects on cellular ROS levels.16 Because mitochondria are a major source of intracellular ROS and are particularly vulnerable to oxidative damage, mitochondria-targeted antioxidants should be an effective therapeutic strategy to prevent or reduce the progression of cardiovascular and neurodegenerative disorders.1,17–19

A recently developed mitochondria-targeted ubiquinone, MitoQ10, overcomes the problem of direct delivery to the mitochondria. MitoQ10 is composed of a lipophilic triphenylphosphonium cation covalently attached to an ubiquinol antioxidant.17,19 Lipophilic cations can easily move through phospholipid bilayers without requiring a specific uptake mechanism; therefore, the triphenylphosphonium cation concentrates MitoQ10 several hundred-fold within the mitochondria, driven by the large mitochondrial membrane potential (Figure 1).1,17,19,20 Within mitochondria, MitoQ10 is reduced by the respiratory chain to its active ubiquinol form, which is a particularly effective antioxidant that prevents lipid peroxidation and mitochondrial damage.1,17,20–26

MitoQ10 is also orally bioavailable and has been shown to accumulate extensively in rat tissues after administration in the drinking water and to protect against tissue damage.17,27 Moreover, MitoQ10 has been shown to be effective against mitochondrial oxidative damage in vivo and in rodent models of sepsis and reperfusion injury.18,28

The stroke-prone spontaneously hypertensive rat (SHRSP) is a well-characterized experimental model for human essential hypertension that develops a number of cardiovascular complications, including cardiac hypertrophy, stroke, and endothelial dysfunction. We have demonstrated previously that endothelial dysfunction in the SHRSP is attributed to a marked but reversible imbalance between NO and O2 at the level of the vascular endothelium.29–35 Furthermore, similarly to humans, we have shown that oxidative stress and the resulting endothelial dysfunction in the SHRSP are accelerated by aging.36 The aim of this study was to investigate the contribution of mitochondria-specific oxidative stress to the development of cardiovascular disease in the SHRSP using the novel mitochondria-targeted antioxidant MitoQ10. The effect of oral administration of MitoQ10 was assessed in vivo and compared with the control compound decyltriphenylphosphonium (decylTPP), which is composed of the lipophilic triphenylphosphonium cation and aliphatic 10-carbon chain but lacks the ubiquinone moiety20 and thereby enables us to control for any nonspecific effects attributed to the accumulation of lipophilic cations within mitochondria that are not attributed to the prevention of oxidative damage. It is anticipated that these studies will inform new therapeutic interventions in human essential hypertension.

Methods

In Vivo Experimental Procedures

An inbred colony of SHRSPs has been maintained at the University of Glasgow since 1991, as described previously.37 From weaning, all of the rats were maintained on normal rat chow (rat and mouse No. 1 maintenance diet, Special Diet Services). All of the studies were conducted in accordance with United Kingdom Home Office regulations.

Eight-week-old male SHRSPs were allowed free access to tap water or tap water containing either MitoQ10 (500 μmol/L) or decylTPP (500 μmol/L) for 8 weeks. Drug solutions were prepared fresh every 3 days, protected from light, and stored at 4°C. In the comparison of MitoQ10 with untreated controls, systolic blood pressure was measured by tail-cuff plethysmography in conscious restrained rats (n=21), as described previously.38 An additional group of rats (n=16) was implanted with radio telemetry probes.
Ex Vivo Analysis
At sacrifice, rats were anesthetized with isoflurane, and blood samples were collected by cardiac puncture. Body weight, heart weight, and left ventricle plus septum weight were measured for the calculation of cardiac and left ventricular mass index. Aorta and mesenteric resistance arteries were taken for endothelial function tests assessed by organ bath pharmacology and wire myography (please see http://hyper.ahajournals.org). Oxidative stress status was assessed by analyzing O$_2^-$ generation in aortic rings using lucigenin chemiluminescence and in whole blood by electron paramagnetic resonance spectroscopy (please see http://hyper.ahajournals.org).

MitoQ10 Biodistribution
A range of tissues (heart, carotid arteries, kidney, and liver) were harvested and snap frozen in liquid nitrogen at sacrifice. The MitoQ10 content of the rat tissues was quantified by liquid chromatography-tandem mass spectrometry (please see http://hyper.ahajournals.org).

Statistical Analysis
All of the results are expressed as mean±SEM. Comparisons between groups were performed using ANOVA and Tukey’s simultaneous test, where appropriate. Comparisons of radiotelemetry data between MitoQ10 and decylTPP-treated groups were carried out by repeated-measures ANOVA, as described previously. F-statistics and P values corresponding with the main effects for strain are reported.

Results
Systolic blood pressure measured by tail-cuff plethysmography was significantly lower in MitoQ10-treated rats compared with vehicle-treated (tap water) controls (P=0.0001; F=65.6; repeated-measures ANOVA). An ∼25 mm Hg reduction in blood pressure was achieved after one week of MitoQ10 treatment, which was maintained throughout the remaining study period (Figure 2A). A similar level of blood pressure reduction (∼23 mm Hg) was observed between MitoQ10 and the control compound decylTPP when measured by radiotelemetry (P=0.029; F=5.94; Figure 2B). MitoQ10 treatment had no significant effect on body weight (measured at sacrifice) compared with untreated controls (Figure 3A). However, after 12 weeks of treatment, decylTPP rats had significantly lower body weight at sacrifice (246.4±5.7 g) than MitoQ10-treated rats (274.2±4.3 g; 95% CI: 1.79 to 53.79; Figure 3A). Cardiac mass index (Figure 3B) was significantly reduced by MitoQ10 treatment (4.01±0.06 mg/g) compared with decylTPP (4.36±0.09 mg/g; 95% CI: −0.652 to −0.056) and untreated controls (4.42±0.12 mg/g; 95% CI: −0.693 to −0.121), and a similar trend was observed for left ventricular mass index (3.05±0.07 mg/g; 3.29±0.09 mg/g, 95% CI: −0.53 to 0.06; and 3.28±0.12 mg/g, 95% CI: −0.56 to 0.07, respectively; Figure 3C). NO bioavailability in aorta from MitoQ10-treated rats (area under the curve [AUC]: 1.16±0.36 g/g) was significantly improved compared with decylTPP-treated (AUC: 0.60±0.29 g/g; 95% CI: 0.153 to 0.968) and control rats (AUC: 0.68±0.15 g/g; 95% CI: 0.121 to 0.849; Figure 4A). No significant difference in relaxation to carbachol was observed in aorta from MitoQ10 (AUC: 118.4±10.5 g/g), decylTPP (AUC: 128.4±11.9 g/g; 95% CI: −55.4 to 35.4), or control rats (AUC: 96.6±17.4 g/g; 95% CI: −23.5 to 67.2; Figure 4B). There was also no significant difference observed in NO bioavailability in mesenteric resistance arteries between control and MitoQ10-treated rats (pD2 [−logEC50]: MitoQ10, 5.49±0.12; control, 5.32±0.06; P=0.843). Figure 5A illustrates O$_2^-$ levels in aorta measured in the presence of 1 μmol/L of rotenone by lucigenin chemiluminescence in control and MitoQ10-treated rats. There was a trend toward reduced O$_2^-$ levels as a result of MitoQ10 treatment, but this did not reach statistical significance (control: 3.31±0.50 counts per minute (cpm)/mg wet weight, MitoQ10: 2.09±0.29 cpm/mg wet weight).
weight, ANOVA, \( P=0.06 \). The presence of rotenone did not significantly affect \( \text{O}_2^- \) levels (control + rotenone: 2.84±0.40 cpm/mg of wet weight; MitoQ10 + rotenone: 2.3±0.28 cpm/mg of wet weight). Oxidative stress status in whole blood measured by electron paramagnetic resonance spectrometry tended to be lower in MitoQ10-treated rats (104.8±11.4×10^5 cpm) compared with decyITPP-treated (116.9±13.6×10^5 cpm; 95% CI: −40.0 to 64.3) and untreated controls (115.0±16.7×10^5 cpm; 95% CI: −36.4 to 56.8), but this failed to reach significance (Figure 5B).

Total MitoQ10 content was measured in tissues/organs harvested from MitoQ10-treated rats by mass spectrometry (liquid chromatography-tandem mass spectrometry system; Figure 6). All of the organs analyzed demonstrated detectable levels of MitoQ10, with comparable levels of accumulation in vascular and cardiac tissues.

**Discussion**

In the present study we have demonstrated that oral administration of the mitochondria-targeted antioxidant MitoQ10 protects against the development of hypertension, improves endothelial NO bioavailability, and reduces cardiac hypertrophy in young SHRSPs. This protective mechanism appears to be attributed to the accumulation of ubiquinol within the mitochondria, because the control substance, decyITPP (the lipophilic cation used to target MitoQ10 to the mitochondria), had no beneficial action. The attenuation of blood pressure rise in young SHRSPs suggests that prevention of mitochondrial oxidative damage at an early age can provide significant hemodynamic beneficial effect. However, the finding that treatment did not completely prevent the development of hypertension indicates that mitochondrial oxidative damage is just one of a number of factors contributing to the underlying pathology.

DecyITPP was used as a control compound because it is known to be taken up in a very similar way to MitoQ10 in both cells in vitro and into organs in vivo after IV injection (C. M. Porteous et al, personal communication, 2009). Therefore, our finding that decyITPP had no beneficial action is a strong indication that the effects of MitoQ10 are attributable to the antioxidant action and not secondary to the accumulation of lipophilic cations within mitochondria.

The beneficial action of MitoQ10 on aortic NO bioavailability suggests that vascular endothelium is an important target organ for mitochondria-specific antioxidation. Endothelial dysfunction is tightly linked to the overproduction of ROS, development of oxidant stress, and inflammatory response in the endothelium, and increasing evidence suggests that mitochondria of the vascular endothelium play an impor-
tant role in these processes.\textsuperscript{47} Results from the present study provide further evidence to support a role for mitochondria-derived ROS in the control of endothelial function.

ROS also play a key role in the pathophysiology of cardiac hypertrophic remodeling and dysfunction through activation of integral signaling molecules.\textsuperscript{48} Although all of the current heart and left ventricular mass data have been adjusted for body weight, similar relationships have also been observed when adjusted for tibial length in the SHRSP. From the present data it is not possible to determine whether the significant reduction in cardiac hypertrophy in MitoQ-treated rats is a direct effect of MitoQ\textsubscript{10} or a secondary response to the reduction in blood pressure. Additional studies will be required to determine the exact mechanisms underlying the improvements in hemodynamic function and cardiac mass.

Despite improvements in systolic blood pressure and endothelial function, the current study was unable to demonstrate significant reductions in O$_2^\text{-}$ levels in the thoracic aorta from MitoQ-treated rats. However, the mitochondrial respiratory chain is just one of a variety of cellular enzyme systems that are potential sources of ROS. In vascular endothelial cells, 4 systems predominate in ROS generation, which include NAD(P)H oxidase, xanthine oxidase, uncoupled endothelial NO synthase, and mitochondrial electron leakage.\textsuperscript{49} Moreover, although the primary ROS produced by the mitochondria is O$_2^\text{-}$, as a charged species it is not readily diffusible across the mitochondrial membranes and is largely dismutated to H$_2$O$_2$ by manganese superoxide dismutase.\textsuperscript{7} Therefore, it is not entirely unexpected that mitochondria-targeted antioxidant treatment in the present study was unable to significantly reduce total O$_2^\text{-}$ levels in aortic rings and whole blood.

From studies with [\textsuperscript{3}H]MitoQ\textsubscript{10}, it is known that feeding 500 \textmu mol/L of MitoQ\textsubscript{10} in the drinking water of rodents leads rapidly over a few days to a steady-state distribution of the compound in organs, eg, the heart, liver, kidneys, and skeletal muscle.\textsuperscript{27} Liquid chromatography-tandem mass spectrometry results from the present study indicate that the steady-state levels in the heart are of the order of 30 to 40 pmol of MitoQ\textsubscript{10} per gram of wet weight, with similar uptake in the carotid artery to that of the heart. Therefore, in the present study, the range of tissue concentrations of MitoQ\textsubscript{10} is comparable to concentrations that have been demonstrated to protect cells in culture from oxidative damage.\textsuperscript{50}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{A, Superoxide production in aorta from control and MitoQ\textsubscript{10}-treated rats measured in the presence and absence of 1 \textmu mol/L of rotenone. There was a trend toward a reduction in O$_2^\text{-}$ levels in MitoQ\textsubscript{10}-treated rats (ANOVA, \textit{P}=0.06). The presence of rotenone did not significantly affect O$_2^\text{-}$ levels. B, O$_2^\text{-}$ production in whole blood in untreated control, decylTPP-treated, and MitoQ\textsubscript{10}-treated SHRSPs. O$_2^\text{-}$ production showed a trend toward reduced levels as a result of MitoQ\textsubscript{10} treatment but did not reach statistical significance.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Liquid chromatography-tandem mass spectrometry quantification of MitoQ\textsubscript{10} from tissues of rats fed \pm MitoQ\textsubscript{10}. Data are expressed in picomoles per gram of tissue (wet weight) and are mean \pm SEM of 3 rats for the heart samples and 4 rats for the carotid artery, liver, and kidney samples.}
\end{figure}
No significant adverse effects of MitoQ<sub>10</sub> administration were observed in this study. This is in line with previously published data where long-term oral administration in rodents showed no significant effects on a range of markers of mitochondrial function, whole-body metabolism, or behavior. In addition, there are also several studies showing no adverse effects of MitoQ administration to rodents by IP or IV delivery at doses that are effective at preventing pathology. It is possible that administering a mitochondria-targeted antioxidant, eg, MitoQ<sub>10</sub>, might lead to the downregulation of endogenous mitochondrial antioxidant defenses, eg, manganese superoxide dismutase, with the net effect that the mitochondria are no better protected in the presence of MitoQ than they would be in its absence. However, evidence from in vivo studies strongly suggests that MitoQ<sub>10</sub> is protective against liver damage in a small study of patients with chronic hepatitis C, and has been shown to be well tolerated with no serious adverse effects when taken for up to a year by patients with Parkinson’s disease.

**Perspectives**

MitoQ<sub>10</sub> provides a novel approach to attenuate mitochondrial-specific oxidative damage and has the potential to become a new therapeutic intervention in human cardiovascular disease.

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**Disclosures**

M.P.M. and R.A.J.S. hold intellectual property in the area of mitochondria-targeted antioxidants, and this is being commercialized by Antipodean Pharmaceuticals Inc, in which both have an interest.

**References**


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THE MITOCHONDRIA TARGETED ANTIOXIDANT MITOQ<sub>10</sub> IMPROVES ENDOTHELIAL FUNCTION AND ATTENUATES CARDIAC HYPERTROPHY.

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Short title: Mitochondria-targeted antioxidant in SHRSP
Expanded methods

Organ bath pharmacology
Thoracic aortae were cleaned of connective tissue and suspended in organ baths, as previously described for measurement of NO bioavailability [1]. Krebs’ buffer in which aortae were maintained, contained indomethacin (0.02 mmol/l) to inhibit any prostanoid-mediated effects. Isometric tension studies were performed using a force transducer and recorded using a MacLab dedicated computer. Contractile responses to 10 mmol/l KCl were examined, the baths washed out and tissues allowed to relax. Cumulative concentration-response curves to phenylephrine (PE) (10 mmol/l to 100 µmol/l) were constructed, first in the absence and again after washout, in the presence of 100 µmol/l N^G^-nitro-L-arginine methyl ester (L-NAME) to inhibit NO synthase. The increase in tension in the presence of L-NAME provides a measure of the effect of NO on basal tone, and was calculated for each ring over the full dose-response curve and expressed as area under the curve (AUC). In addition, the rings were pre-constricted to the EC₅₀ of PE and a concentration response curve for carbachol (10 nmol/l to 100 µmol/l) was obtained which provides a measure of stimulated NO release. Responses to phenylephrine were standardised against the initial contractile response to KCl.

Wire myograph methods
Resistance arteries were dissected from connective tissue and segments (approximately 2 mm in length) were mounted as ring preparations on two stainless steel wires on a four-channel small vessel myograph (Danish MyoTechnology, Aarhus, Denmark). One wire was attached to a force transducer and the other to a micrometer. Following a 30 min rest period vessels were set to a normalised internal diameter (L₁) to achieve optimal contraction. Internal diameter was calculated using the following equation L₁=0.0*L₁₀₀, (where L₁₀₀ was determined using the Laplace equation, P=T/r (P is effective pressure, T is wall tension and r is the internal radius). After further 60 min, contractile responses to 10 µmol/l KCl were examined, followed by washout. A cumulative concentration response curve to PE, 10nmol/l to100 mmol/l was performed first in the absence and again after washout, in the presence of L-NAME (100 µmol/l). The increase in tension in the presence of L-NAME provided a measure of the effect of NO on basal tone.

Vascular superoxide generation
Vascular superoxide generation was measured in aortic rings (3 mm) using lucigenin-enhanced chemiluminescence in a liquid scintillation counter (Tricarb 2100TR; Hewlett Packard, Palo Alto, California, USA) [2,3]. The lucigenin concentration was 5 mmol/l. A xanthine/xanthine oxidase calibration curve was used to quantify superoxide generation which was normalised to wet weight. Measurement of vascular superoxide was made in the presence and absence of the complex 1 inhibitor, rotenone after a 30 min preincubation (1 µmol/l).

EPR spectrometry
Oxidative stress status was assessed by analysing superoxide release from heparinised whole blood taken by cardiac puncture at sacrifice. Blood was kept on ice and processed within 30 min. Superoxide levels were detected by electron paramagnetic resonance (e-scan R; Bruker BioSpin GmbH, Rheinstetten Germany) with the spin probe 1-Hydroxy-3-carboxy- 2,2,5,5-tetramethylpyrrolidine (CPH; Noxygen, Elzach,
Germany) to a final concentration of 500 µM [4]. Immediately after addition of CPH, a 500 µl aliquot of blood is snap frozen in liquid nitrogen. A second 500 µl aliquot is snap frozen after incubation at 37°C for 60 minutes. Superoxide levels were measured in the snap frozen samples at 0 and 60 minutes and the rate of superoxide anion production was calculated as counts per minute. Instrument settings were: centre field of 3392 G, modulation amplitude of 5.08 G, sweep time of 10.49 s, sweep width of 120 G and 10 scans.

Analysis of MitoQ\textsubscript{10} biodistribution by LC/MS/MS

Heart, liver, carotid artery and kidney samples were weighed and snap-frozen upon removal, and stored at –80°C. Frozen tissue (100 mg wet weight, except for carotid arteries where samples were ~25 mg) was homogenised in 0.5 mL 50 mM Tris (pH 7), then 5 pmol of deuterated MitoQ\textsubscript{10} (\textit{d}_3-MitoQ\textsubscript{10}) was added as an internal standard. Standards of the appropriate rat tissue spiked with 0-100 pmol MitoQ\textsubscript{10} and 5 pmol \textit{d}_3-MitoQ\textsubscript{10}, were prepared and processed in parallel. MitoQ\textsubscript{10} was extracted from samples and standards into 2 x 1.5 mL 95% acetonitrile, 0.1% formic acid, and the pooled organic phases were dried under vacuum and resuspended in 200 µL of 60% acetonitrile, 0.1% formic acid. Portions (~150 µL) were transferred to glass autosampler vials (silanised; Chromacol Ltd, UK) for LC/MS/MS analysis.

The LC/MS/MS system consisted of a Waters Quattro Ultima triple quadrupole mass spectrometer (Waters) attached to a binary pump (model 1585; Jasco) and an HTC-PAL autosampler (CTC-Analytics). Liquid chromatography was performed at 30°C using a Luna 5µ Phenyl-Hexyl column (1 x 50 mm, 5 µm) with a Phenyl-Hexyl guard column (2 x 4 mm) (both from Phenomenex). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1 % formic acid in 95% acetonitrile (B) delivered as a linear gradient as follows: 0-2 min, 5% B; 2-3 min, 5-50% B; 3-5 min, 50-100% B; 5-10 min, 100% B; 10-12 min, 100-5% B; 12-20 min, 5% B. The flow rate was 50 µL/min and a 30 µL volume was injected into a 20 µL sample loop. An in-line divert valve was used to divert the eluent away from the mass spectrometer from 0-3 min and 15-20 min of the acquisition time. For mass spectrometry, electrospray ionisation in positive ion mode was employed. Multiple reaction monitoring (MRM) was used to detect the transitions of MitoQ\textsubscript{10} at \textit{m/z} 583.4 -> 441.3 and of \textit{d}_3-MitoQ\textsubscript{10} at \textit{m/z} 586.4 -> 444.3. The instrument parameters were: source spray voltage, 3 kV; ion source temperature, 100°C; collision energy, 50 V. Nitrogen was used as the curtain gas and argon as the collision gas. Data were analysed with MassLynx software.

References