

# Mitochondria-specific antioxidant supplementation does not influence endurance exercise training-induced adaptations in circulating angiogenic cells, skeletal muscle oxidative capacity or maximal oxygen uptake

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## Key points

- Reducing excessive oxidative stress, through chronic exercise or antioxidants, can decrease the negative effects induced by excessive amounts of oxidative stress. Transient increases in oxidative stress produced during acute exercise facilitate beneficial vascular training adaptations, but the effects of non-specific antioxidants on exercise training-induced vascular adaptations remain elusive.
- Circulating angiogenic cells (CACs) are an exercise-inducible subset of white blood cells that maintain vascular integrity.
- We investigated whether mitochondria-specific antioxidant (MitoQ) supplementation would affect the response to 3 weeks of endurance exercise training in CACs, muscle mitochondrial capacity and maximal oxygen uptake in young healthy men.
- We show that endurance exercise training increases multiple CAC types, an adaptation that is not altered by MitoQ supplementation. Additionally, MitoQ does not affect skeletal muscle or whole-body aerobic adaptations to exercise training.
- These results indicate that MitoQ supplementation neither enhances nor attenuates endurance training adaptations in young healthy men.

**Abstract** Antioxidants have been shown to improve endothelial function and cardiovascular outcomes. However, the effects of antioxidants on exercise training-induced vascular adaptations remain elusive. General acting antioxidants combined with exercise have not impacted circulating angiogenic cells (CACs). We investigated whether mitochondria-specific antioxidant (MitoQ) supplementation would affect the response to 3 weeks of endurance exercise training on CD3<sup>+</sup>, CD3<sup>+</sup>/CD31<sup>+</sup>, CD14<sup>+</sup>/CD31<sup>+</sup>, CD31<sup>+</sup>, CD34<sup>+</sup>/VEGFR2<sup>+</sup> and CD62E<sup>+</sup> peripheral blood mononuclear cells (PBMCs), muscle mitochondrial capacity, and maximal oxygen uptake ( $V_{O_2\max}$ ) in healthy men aged  $22.1 \pm 0.7$  years, with a body mass index of  $26.9 \pm 0.9$  kg m<sup>-2</sup>, and  $24.8 \pm 1.3\%$  body fat. Analysis of main effects revealed that training induced 33, 105 and 285% increases in CD14<sup>+</sup>/CD31<sup>+</sup>, CD62E<sup>+</sup> and CD34<sup>+</sup>/VEGFR2<sup>+</sup> CACs, respectively, and reduced CD3<sup>+</sup>/CD31<sup>-</sup> PBMCs by 14%. There was no effect of MitoQ on CAC levels. Also independent of MitoQ supplementation, exercise training significantly increased quadriceps muscle mitochondrial capacity by 24% and  $V_{O_2\max}$  by roughly 7%. In conclusion, endurance exercise training induced increases in multiple CAC types, and this adaptation is not modified by MitoQ supplementation. Furthermore, we demonstrate that a mitochondrial-targeted antioxidant does not influence skeletal muscle or whole-body aerobic adaptations to exercise training.

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**Abbreviations** BMI, body mass index; CAC, circulating angiogenic cell; CD, cluster differentiation; CD62E, E-selectin; FFM, fat-free mass; FITC, fluorescein isothiocyanate; MDA, malondialdehyde; MitoQ, mitochondria-targeted ubiquinone antioxidant; NIRS, near infrared spectroscopy; PBMC, peripheral blood mononuclear cell; PE, phycoerythrin; ROS, reactive oxygen species; VEGFR2, vascular endothelial growth factor receptor 2;  $V_{O_{2max}}$ , maximal oxygen consumption.

## Introduction

Reactive oxygen species (ROS) are important signalling molecules with roles in both health and disease. At physiological concentrations, ROS play a regulatory role in vascular homeostasis, beta oxidation, glucose uptake and myokine production (Quintero *et al.* 2006; Zhang & Gutterman, 2007; Silveira *et al.* 2008; Scheele *et al.* 2009; Finkel, 2012; Merry & McConell, 2012; Freed & Gutterman, 2013). Importantly, transient increases in ROS produced during exercise facilitate beneficial vascular and skeletal muscle adaptations to exercise training (Lauer *et al.* 2005; Ji *et al.* 2006; Ushio-Fukai, 2006; Powers *et al.* 2011; Nikolaidis *et al.* 2012). Minimizing excessive oxidative stress, via chronic exercise or exogenous antioxidants, can attenuate the deleterious effects induced by excessive amounts of ROS (Dudgeon *et al.* 1998; Kojda & Hambrecht, 2005; Jablonski *et al.* 2007; Zhang & Gutterman, 2007; Widlansky & Gutterman, 2011).

The effects of antioxidant supplementation on training-induced changes in vascular function are unclear (Polytarchou & Papadimitriou, 2004; Lauer *et al.* 2005; Polytarchou & Papadimitriou, 2005; Ristow *et al.* 2009; Wray *et al.* 2009; Theodorou *et al.* 2011), but literature suggests non-specific antioxidants do not alter training-induced changes in circulating angiogenic cells (CACs) (Balestrieri *et al.* 2008; Fiorito *et al.* 2008a,b). CACs are an exercise-inducible subset of peripheral blood mononuclear cells (PBMCs) with endothelial-specific antigens and angiogenic characteristics thought to facilitate endothelial repair by homing to sites of damage and integrating into the endothelial monolayer or releasing paracrine factors (Asahara *et al.* 1997; Kim *et al.* 2011; Lansford *et al.* 2016). Importantly, the maintenance of vascular integrity is thought to be contingent on the number and function of CACs (Koutroumpi *et al.* 2012). Exercise training enhances CACs and their regenerative capacity (Koutroumpi *et al.* 2012; Landers-Ramos *et al.* 2015), but the effect of mitochondria-specific antioxidant supplementation on training-induced changes in CACs is unknown.

The mitochondria-targeted antioxidant MitoQ is engineered to accumulate to the matrix surface of the inner mitochondrial membrane and exert beneficial effects through oxidation of ubiquinol to ubiquinone and subsequent re-reduction, thereby reducing electron backup and ROS formation (Smith & Murphy, 2010). Moreover, the ubiquinone moiety appears to have some

beneficial effects on the vascular endothelium. For example, evidence from animal and *in vitro* studies has demonstrated that MitoQ administration mitigates intracellular PBMC oxidative stress, prevents endothelial apoptosis and re-establishes endothelial function in disease states (Dhanasekaran *et al.* 2004; Graham *et al.* 2009; Marthandan *et al.* 2011; Gioscia-Ryan *et al.* 2014). However, the efficacy of MitoQ supplementation is uncertain (Doughan & Dikalov, 2007; Gane *et al.* 2010; Snow *et al.* 2010). The majority of studies indicate that non-specific exogenous antioxidant supplementation does not alter or can even blunt exercise training adaptations in muscle (Ristow *et al.* 2009; Wray *et al.* 2009; Nikolaidis *et al.* 2012). Interestingly, however, our group recently reported that a resveratrol/piperine supplement in combination with endurance training enhanced skeletal muscle oxidative capacity compared to training alone in the human forearm (Polley *et al.* 2016). Collectively, the mixed evidence on whether antioxidant supplements alter training adaptations indicates that further studies are warranted.

Therefore, the present study investigated the effect of endurance exercise training with and without MitoQ supplementation on CACs, skeletal muscle oxidative capacity and maximal oxygen uptake. Consistent with previously observed effects of non-specific antioxidant supplementation during aerobic training (Balestrieri *et al.* 2008; Fiorito *et al.* 2008a; Ristow *et al.* 2009; Wray *et al.* 2009; Theodorou *et al.* 2011) and MitoQ's putative mechanism, it was hypothesized that MitoQ would either attenuate or have no effect on training-induced adaptations in CACs, muscle oxidative capacity or maximal oxygen uptake.

## Methods

### Ethical approval

The University of Georgia Institutional Review Board approved all study procedures, which conformed to *Declaration of Helsinki* standards, and subjects provided written informed consent prior to participation.

### Screening

Twenty 18–40-year-old men free of cardiovascular or metabolic diseases, participating in less than 45 min of vigorous activity 3 days per week, or less than 60 min

of moderate activity 4 days per week for the previous 6 months were recruited. As previously described, vigorous activity was defined as activities requiring greater than or equal to six metabolic equivalents (e.g. running), and moderate activity was defined as activities requiring between three and six metabolic equivalents (e.g. brisk walking) (Pescatello *et al.* 2014). Subjects were excluded based on the following: any known food allergies, body mass index (BMI) < 20 or > 35 kg m<sup>-2</sup>, currently smoking or previous use within 2 years, currently taking more than three pharmacological agents including prescription, non-prescription, cardiovascular and/or metabolic drugs or oral antioxidants  $\geq 300$  mg day<sup>-1</sup> within 120 days of enrollment. A 7-day washout period was required for subjects currently taking oral antioxidants at doses between 25 and 300 mg day<sup>-1</sup> and any multivitamin or mineral supplements.

### Testing protocol

Baseline and post-training visits consisted of a blood draw and assessments of body composition, skeletal muscle mitochondrial capacity and maximal oxygen consumption ( $V_{O_{2max}}$ ). Subjects arrived in the laboratory between 06.00 and 09.00 h for their baseline visit in a fasted state (> 12 h) and refrained from exercise, alcohol consumption and caffeine ingestion for the preceding 24 h. Blood samples were obtained using standard venipuncture techniques. Dual-energy X-ray absorptiometry was used to assess body composition (iDXA, GE Healthcare, Pittsburg, PA, USA). Skeletal muscle mitochondrial oxidative capacity was measured as previously described (Ryan *et al.* 2012, 2014). Briefly, near infrared spectroscopy (NIRS) was used to measure metabolic recovery kinetics in the left vastus lateralis muscle following contractions induced by neuromuscular electrical stimulation. The recovery kinetics of muscle oxygen consumption were fitted to a mono-exponential curve where the rate constant (min<sup>-1</sup>) derived from the curve is directly related to the muscles' maximal oxidative capacity (Ryan *et al.* 2014). Whole-body  $V_{O_{2max}}$  was assessed via a progressive cycle ergometer protocol. To elicit  $V_{O_{2max}}$  within 6–12 min, the first stage commenced at a workload between 150 and 200 W and increased by 25 W every 2 min until subjects could no longer maintain a pedal cadence of 60 r.p.m. Expired gas (Parvo Medics TrueOne 2400, Parvo Medics, Salt Lake City, UT, USA) and heart rate (Polar, Polar Electro Inc., Lake Success, NY, USA) were collected throughout the graded exercise test.  $V_{O_2}$  was considered maximal if a plateau was achieved (< 250 ml min<sup>-1</sup> increase in  $V_{O_2}$  with increased work). If a plateau was not apparent, the following criteria verified maximal effort: respiratory exchange ratio > 1.15 and peak heart rate within 10 beats min<sup>-1</sup> of age-predicted maximum. In a randomized and double

blind fashion, subjects were assigned to consume a placebo or MitoQ pill every morning, with breakfast, throughout study enrollment. Further, subjects were instructed to maintain normal exercise routines and abstain from alcohol. Placebo pills contained rice flour (Arrowhead Mills, The Hain Celestial Group, Inc., Lake Success, NY, USA). MitoQ (Antipodean Pharmaceuticals Inc., San Francisco, CA, USA; obtained from Amazon.com, Inc., Seattle, WA, USA) capsules were prescribed in accordance with the manufacturer's recommended dose of 10 mg daily. Baseline measurements were repeated 24–48 h after the last training session. For post-testing measurements, subjects ingested their assigned pill with water before blood sampling, but otherwise remained in a fasted state and did not consume caffeine for the previous 24 h.

### Exercise training

Subjects completed three exercise sessions during the first week of training, with each session consisting of 45 min of stationary cycling at an intensity of 50–60%  $V_{O_{2max}}$ . During the second and third weeks, training load increased to 60 min sessions at 60–70%  $V_{O_{2max}}$  on 5 days per week. Research assistants supervised all exercise-training sessions and monitored intensity by heart rate and ratings of perceived exertion.

### Lipid peroxidation assay

EDTA-collected plasma samples were prepared by centrifugation and stored at  $-80^{\circ}\text{C}$  until examined in duplicate for concentrations of malondialdehyde (MDA) using the TBARS Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA), according to the manufacturer's instructions. Absorbance values read at 530 nm yielded a standard curve ( $R^2 = 0.9956$ ) to determine the amount of MDA in each sample.

### CAC preparation and data acquisition

PBMCs were isolated from 10 ml of EDTA-collected blood samples (Vacutainer K<sub>2</sub>EDTA, Becton Dickinson, Franklin Lakes, NJ, USA). After centrifugation on a density gradient (Ficoll, GE Healthcare, Pittsburg, PA, USA), cells were washed with PBS three times and characterized with the following fluorescein isothiocyanate (FITC) and phycoerythrin (PE) conjugated cluster differentiation (CD) antibody combinations: FITC-CD3/PE-CD31, FITC-CD14/PE-CD31, FITC-CD34/PE-VEGFR2 (vascular endothelial growth factor receptor 2), and PE-CD62E (E-selectin) (all BD Biosciences, Becton Dickinson, Franklin Lakes, NJ, USA). After fluorochrome staining, PBMCs were incubated in the dark and fixed. Fluorescence was acquired from 50 000 positive events via flow cytometry (CyAn ADP, Beckman Coulter,

**Table 1. Subject characteristics**

	Combined ( <i>n</i> = 20)		Placebo ( <i>n</i> = 10)		MitoQ ( <i>n</i> = 10)	
	Baseline	After training	Baseline	After training	Baseline	After training
Age (years)	22.1 ± 0.7	–	20.8 ± 0.7	–	23.4 ± 0.9	–
Height (m)	1.75 ± 0.01	–	1.74 ± 0.02	–	1.76 ± 0.02	–
Mass (kg)	81.9 ± 2.8	81.7 ± 2.7	82.3 ± 4.9	81.9 ± 4.8	81.6 ± 3.0	81.6 ± 3.0
BMI (kg m <sup>-2</sup> )	26.9 ± 0.9	26.8 ± 0.9	27.4 ± 1.7	27.3 ± 1.7	26.3 ± 0.8	26.3 ± 0.7
Body fat (%)	24.8 ± 1.3	23.9 ± 1.3*	24.8 ± 2.1	23.9 ± 2.0	24.1 ± 1.7	24.0 ± 1.7
Fat mass (kg)	20.1 ± 1.6	19.3 ± 1.5*	20.5 ± 2.7	19.5 ± 2.6	19.7 ± 1.7	19.0 ± 1.6
FFM (kg)	62.4 ± 1.7	62.8 ± 1.7	62.4 ± 2.5	62.8 ± 2.5	62.5 ± 2.4	62.8 ± 2.4
Lean mass (kg)	59.3 ± 1.6	59.6 ± 1.6	59.3 ± 2.4	59.7 ± 2.3	59.3 ± 2.3	59.6 ± 2.3
MDA (μM)	9.4 ± 0.6	8.4 ± 0.6	9.6 ± 0.6	9.1 ± 0.9	9.2 ± 1.1	7.7 ± 0.8

Values are mean ± SEM. BMI, body mass index; FFM, fat-free mass = mass – fat mass; lean mass = mass – fat mass – bone; MDA, malondialdehyde.

\*Statistically significant effect of training ( $P < 0.05$ ).

Hialeah, FL, USA). Data were gated from unstained and single-stained controls in respective populations and analysed in FlowJo v.10.0.08 (FlowJo, LLC, Ashland, OR, USA), as previously described (Shill *et al.* 2016).

### Statistics

Statistical analysis was performed in SPSS, Version 23.0 (IBM Corp., Armonk, NY, USA). Data were analysed using two-factor repeated measures ANOVA and Fisher's Least Significant Difference tests for simple effects. Data are presented as mean ± SEM. Statistical significance was accepted at  $P < 0.05$ . Approaching statistical significance was defined as  $P \leq 0.10$ .

### Results

Subject characteristics are presented in Table 1. MitoQ did not produce any adverse side effects among study participants, and no adverse events occurred during exercise training. Exercise training adherence was 99% (258/260 sessions completed during the study). Additionally, placebo/MitoQ pill compliance was 100%, as monitored through daily questionnaires. No interactions between training and MitoQ were observed ( $P > 0.05$ ); therefore, main effects of training are reported.

Training did not elicit significant changes in body mass, BMI, fat-free mass (FFM), lean mass or MDA concentrations (Table 1) ( $P > 0.05$ ). Body fat percentage and fat mass were reduced by 4% after training (Table 1) ( $P < 0.05$ ). Training induced 7% increases in absolute (Fig. 1A) and relative (Fig. 1B)  $V_{O_2\max}$  ( $P < 0.05$ ).  $V_{O_2\max}$  relative to FFM increased by 6% (Fig. 1C) ( $P < 0.05$ ). Additionally, training increased quadriceps muscle mitochondrial capacity by 24% (Fig. 1D) ( $P < 0.05$ ).

Training induced 105 and 285% increases in CD62E<sup>+</sup> (Fig. 2A) and CD34<sup>+</sup>/VEGR2<sup>+</sup> (Fig. 2B) PBMCs, respectively ( $P < 0.05$ ). A 33% increase in CD14<sup>+</sup>/CD31<sup>+</sup> cells approached statistical significance after training (Fig. 3A) ( $P = 0.056$ ). Training did not alter CD14<sup>+</sup>/CD31<sup>-</sup> or CD14<sup>-</sup>/CD31<sup>+</sup> PBMCs (Fig. 3B, C) ( $P > 0.05$ ). Exercise training reduced CD3<sup>+</sup>/CD31<sup>-</sup> PBMCs by 14% (Fig. 4A) ( $P < 0.05$ ). Training did not alter CD3<sup>+</sup>/CD31<sup>+</sup>, CD3<sup>-</sup>/CD31<sup>+</sup> or CD3<sup>+</sup> PBMCs (Fig. 4B–D) ( $P > 0.05$ ).

### Discussion

The present investigation demonstrates that supplementation of commercially available MitoQ at the manufacturer's recommended dose did not influence training-induced changes in CAC subpopulations or aerobic training adaptations in young healthy men. Additionally, our observations of training-induced increases in CD62E<sup>+</sup>, CD34<sup>+</sup>/VEGR2<sup>+</sup> and CD14<sup>+</sup>/CD31<sup>+</sup> cells, but a lack of change in CD3<sup>+</sup> or CD3<sup>+</sup>/CD31<sup>+</sup> cells, suggest that training-induced adaptations in CACs are heterogeneous among cell types. Finally, we demonstrate that MitoQ does not influence training-induced changes in skeletal muscle or whole-body aerobic capacity.

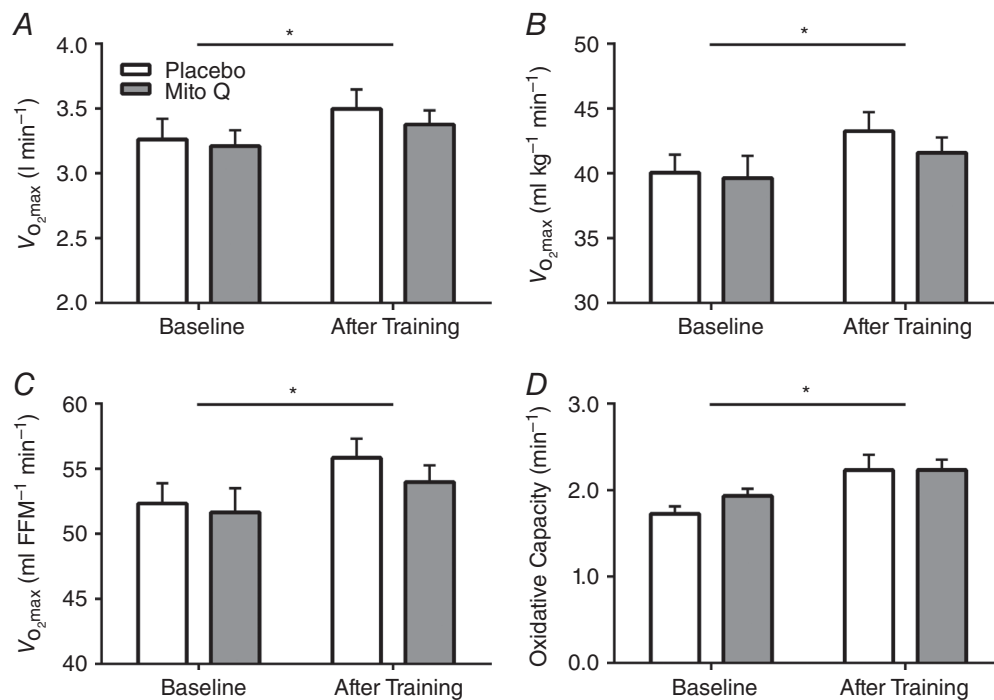
Vascular-derived mitochondrial ROS are cell-signalling molecules that facilitate CAC recruitment and function, contributing to the maintenance of vascular integrity (Schroder *et al.* 2009; Ushio-Fukai & Urao, 2009; Fleissner & Thum, 2011; Widlansky & Gutterman, 2011; Urao & Ushio-Fukai, 2013). While exercise-induced ROS have been shown to modulate CAC mobilization and angiogenesis, the present study demonstrates MitoQ does not influence the training-induced CAC enhancement (Polytarchou & Papadimitriou, 2004; Ushio-Fukai, 2006;

Urao *et al.* 2008; Fiorito *et al.* 2008*b*; Ushio-Fukai & Urao, 2009; Suvorava *et al.* 2010). Consistent with these results, previous studies have found CACs were not altered by antioxidant supplementation during exercise training (Balestrieri *et al.* 2008; Fiorito *et al.* 2008*a,b*). However, these studies used vitamins C and E, polyphenols and L-arginine, which do not specifically target mitochondrial ROS. Taken together, our current results and the available evidence support the notion that antioxidant supplements, mitochondrial-targeted or otherwise, do not alter training-induced effects on CACs.

Although mitochondria in contracting muscles have generally been considered the source of ROS during exercise (Kanter, 1994; Urso & Clarkson, 2003), accumulating evidence suggests mitochondrial bioenergetics are not responsible for producing excessive ROS, indicating an origin other than skeletal muscle mitochondria, such as NADPH- or xanthine oxidases (Herrero & Barja, 1997; Di Meo & Venditti, 2001; St-Pierre *et al.* 2002; Kozlov *et al.* 2005; Jackson *et al.* 2007; Powers & Jackson, 2008). Furthermore, CACs' predominant source of ROS is derived from sources other than mitochondria, for example NADPH-oxidases (Ushio-Fukai & Urao, 2009). Thus, non-mitochondrial ROS production during exercise could prevent MitoQ from being an effective modulator of exercise-induced

ROS, which may potentially explain our results. Moreover, although MitoQ did not influence training-induced changes in CACs, a main effect of training was noted in several CAC subpopulations. Our study is the first to report that short-term, aerobic exercise training affects multiple CACs including CD62E<sup>+</sup>, CD34<sup>+</sup>/VEGFR2<sup>+</sup> and CD14<sup>+</sup>/CD31<sup>+</sup> PBMCs, with a reduction in CD3<sup>+</sup>/CD31<sup>-</sup> PBMCs. As our study included only young healthy males, future studies are warranted to determine whether training enhances numbers of these cells in other populations (e.g. patients with chronic diseases) or alters their angiogenic function.

CD62E, an endothelial adhesion molecule, is critical to cell adhesion, angiogenesis and CAC recruitment to ischaemic tissue (Koch *et al.* 1995; Mazo *et al.* 1998; Oh *et al.* 2007). The present study documents, for the first time, an increase in CD62E<sup>+</sup> CACs after an endurance exercise training intervention. We recently reported that CD62E<sup>+</sup> PBMCs are an acute exercise-inducible cell type with angiogenic potential (Lansford *et al.* 2016). The cumulative effect of transient increases in CD62E<sup>+</sup> cells after each training session is a plausible explanation for our finding of enhanced basal CD62E<sup>+</sup> PBMCs after training. Although post-training blood samples were obtained 24–48 h after the final exercise session, it is nevertheless possible we captured a residual effect of



**Figure 1. Oxidative capacity before and after endurance training**

Absolute (A), relative (B), fat-free mass (C) and skeletal muscle oxidative capacity (D) before and after 3 weeks of endurance training in placebo (white) and MitoQ (grey) groups. FFM, fat-free mass (kg); skeletal muscle oxidative capacity data available on 19 subjects (Placebo = 10, MitoQ = 9). \*Statistically significant effect of training ( $P < 0.05$ ).

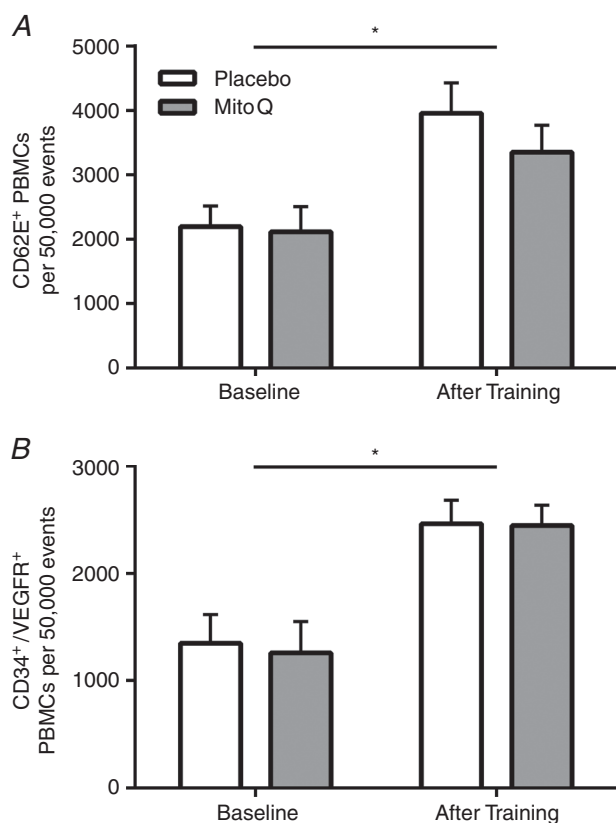
the final acute exercise bout rather than training effects *per se*. Future studies are necessary to determine the time course of acute exercise-induced increases in CD62E<sup>+</sup> PBMCs.

CD34<sup>+</sup> cell subpopulations, largely studied for their role in regenerative medicine, facilitate angiogenesis and rescue ischaemic tissue probably through paracrine signalling (Asahara *et al.* 1997, 1999; Weissman, 2000; Kim *et al.* 2011; Landers-Ramos *et al.* 2015). We demonstrate increased CD34<sup>+</sup>/VEGFR2<sup>+</sup> PBMCs after exercise training, consistent with previous investigations (Koutroumpi *et al.* 2012). Inherent antioxidative phenotypes in CD34<sup>+</sup> cells potentially explain why MitoQ did not impact changes in CD34<sup>+</sup>/VEGFR2<sup>+</sup> PBMCs with aerobic training (Dernbach *et al.* 2004).

Monocytes, identified by the CD14 surface antigen, migrate to sites of vascular injury and contribute to vascular homeostasis (Awad *et al.* 2006). Although we recently showed a decrease in CD14<sup>+</sup>/CD31<sup>+</sup> CACs after acute exercise (Lansford *et al.* 2016), the present study found an increase in CD14<sup>+</sup>/CD31<sup>+</sup> PBMCs after 3 weeks of aerobic training. Not all investigations have reported an

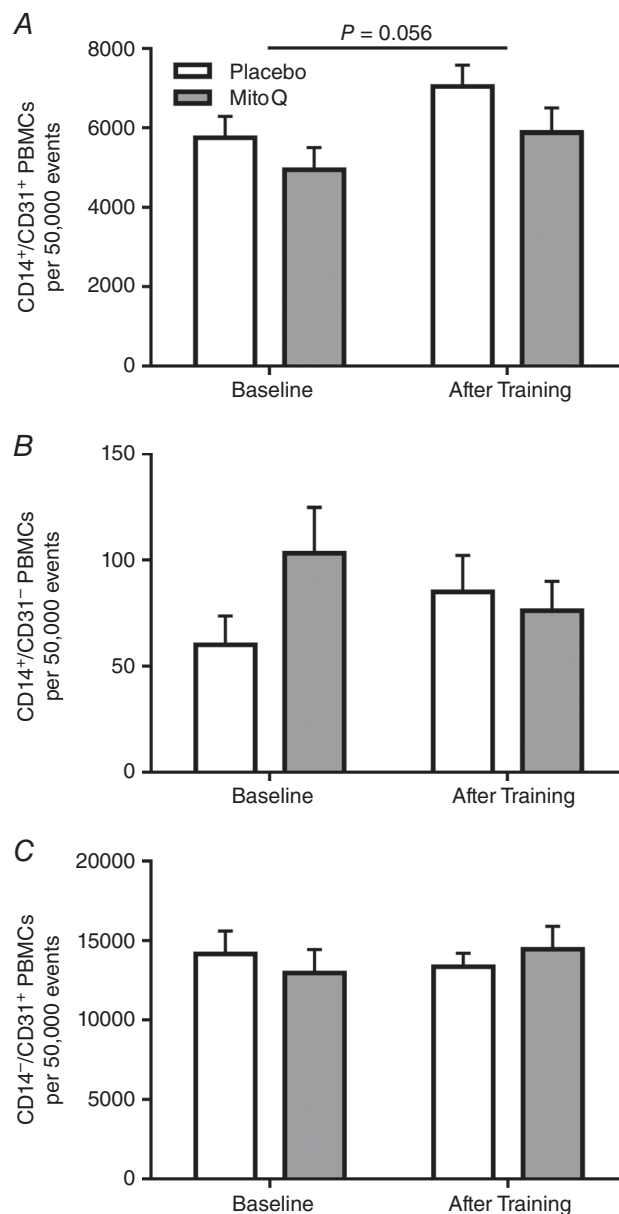
influence of exercise training on CD14<sup>+</sup> cells (Stewart *et al.* 2005; Czepluch *et al.* 2011). Enhanced CD14<sup>+</sup>/CD31<sup>+</sup> CACs after endurance exercise training may be indicative of an anti-inflammatory state and active endothelial repair (Harraz *et al.* 2001; Timmerman *et al.* 2008), but future studies are needed to address the influence of angiogenic monocytes on inflammation.

The influence of exercise training on CD3<sup>+</sup> cells appears to be mediated by training status, duration and intensity (Shore *et al.* 1999; Lancaster *et al.* 2004; Wang *et al.* 2011).



**Figure 2. CD62E<sup>+</sup> and CD34<sup>+</sup>/VEGFR2<sup>+</sup> CACs before and after endurance training**

CD62E<sup>+</sup> (A) and CD34<sup>+</sup>/VEGFR2<sup>+</sup> (B) PBMCs before and after 3 weeks of endurance training in placebo (white) and MitoQ (grey) groups. \*Statistically significant effect of training ( $P < 0.05$ ).



**Figure 3. CD14<sup>+</sup> PBMCs before and after endurance training**

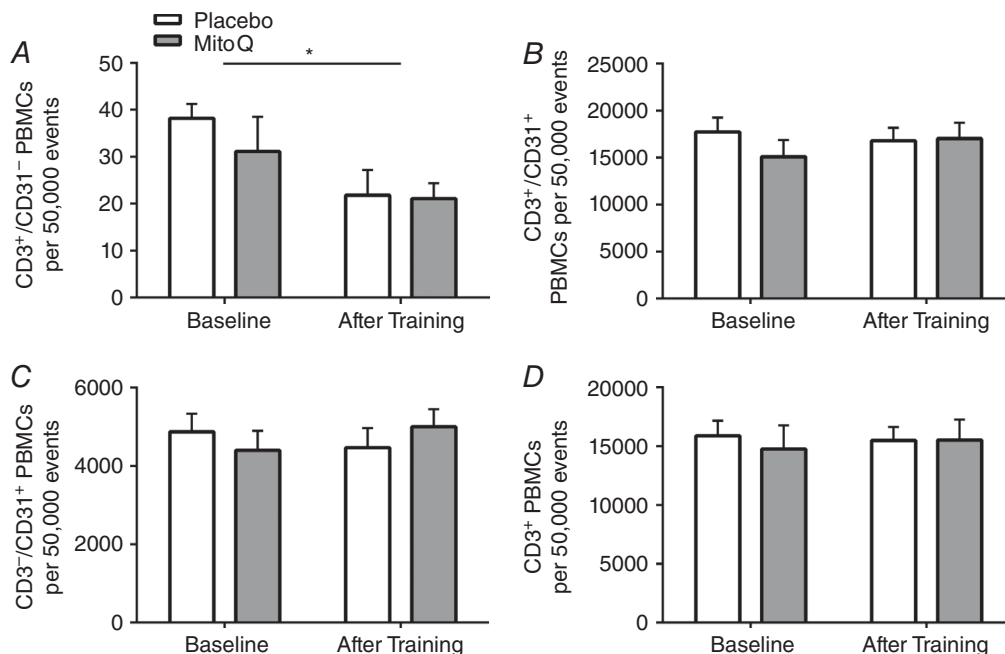
CD14<sup>+</sup>/CD31<sup>+</sup> (A), CD14<sup>+</sup>/CD31<sup>-</sup> (B) and CD14<sup>+</sup>/CD31<sup>+</sup> (C) PBMCs before and after 3 weeks of endurance training in placebo (white) and MitoQ (grey) groups. Brackets with P-values indicate data approaching statistical significance ( $P \leq 0.10$ ).

The present study observed no changes in CD3<sup>+</sup> cells, but a reduction in CD3<sup>+</sup>/CD31<sup>-</sup> PBMCs after exercise training. Although the precise mechanism underlying this effect of training remains unclear, it is possible that the training-induced decrease of CD3<sup>+</sup>/CD31<sup>-</sup> PBMCs was linked to a reduction in fat mass and associated systemic inflammation (Rajala & Scherer, 2003).

Our data indicate MitoQ supplementation neither enhances nor attenuates endurance training adaptations in skeletal muscle oxidative capacity and  $\dot{V}_{O_{2max}}$  in young healthy men. The aerobic stimulus produced a 24% increase in oxidative capacity, concurring with previous training studies in different muscles (Polley *et al.* 2016), and 6–7% increases in  $\dot{V}_{O_{2max}}$ . The effects of antioxidant supplementation on exercise-induced adaptations could be attributable to training intensity and duration, the dose of antioxidant, and the differential impact of exercise on systemic-tissue ROS production (Nikolaidis *et al.* 2012; Durand & Gutterman, 2014). The effect of antioxidant supplementation may also be influenced by the relationship between endogenous antioxidant scavenging capacity and the source of ROS responsible for exercise-induced training adaptations. Assuming MitoQ's aforementioned biochemical formula and molecular function were operative in the present study, the observed increases in  $\dot{V}_{O_{2max}}$  and mitochondrial capacity may have been driven by non-mitochondrial ROS production, supporting previous studies investigating the role of non-mitochondrial muscle ROS contributing to exercise

adaptations (Herrero & Barja, 1997; Di Meo & Venditti, 2001; St-Pierre *et al.* 2002; Kozlov *et al.* 2005; Jackson *et al.* 2007). It is also possible that the relatively lower dose of MitoQ administered in the present study, 10 mg day<sup>-1</sup> as recommended by the manufacturer *vs.* 20–80 mg day<sup>-1</sup> in previous clinical trials (Gane *et al.* 2010; Snow *et al.* 2010), was not sufficient to suppress the exercise-induced ROS in our study subjects. Importantly, neither exercise training nor MitoQ supplementation impacted plasma MDA concentrations, an indicator of oxidative stress, although we did not assay mitochondrial-derived ROS production in CACs or muscle biopsy samples. Future examinations incorporating these measures would be informative.

Our study has limitations that warrant mention. First, our study involved only young, healthy males. Accordingly, our findings on the influence of MitoQ on training-induced adaptations should not be extrapolated to other populations, e.g. women, older adults and/or patients with chronic diseases. Additionally, we did not measure levels of MitoQ in blood or muscle tissue. Such measurements would have provided insight into whether the 10 mg dose, as recommended by the manufacturer and therefore used in the present study, could induce biological effects. Future studies should address these limitations by examining other study populations and incorporating muscle biopsies and/or CAC oxidative stress measurements into their experimental approach.



**Figure 4. CD3<sup>+</sup> PBMCs before and after endurance training**

CD3<sup>+</sup>/CD31<sup>-</sup> (A), CD3<sup>+</sup>/CD31<sup>+</sup> (B), CD3<sup>-</sup>/CD31<sup>+</sup> (C) and CD3<sup>+</sup> (D) PBMCs before and after 3 weeks of endurance training in placebo (white) and MitoQ (grey) groups. \*Statistically significant effect of training ( $P < 0.05$ ).

In conclusion, the present study indicates that CAC subpopulations and aerobic training adaptations are not affected by supplementing commercially available MitoQ, at the manufacturer's recommended dose, during exercise training in young healthy men. We provide the first evidence of a heterogeneous CAC adaptation to endurance exercise training not limited to cells of progenitor origin. Specifically, we demonstrate an increase in CD62E<sup>+</sup>, CD34<sup>+</sup>/VEGFR2<sup>+</sup> and CD14<sup>+</sup>/CD31<sup>+</sup> CACs and a reduction in CD3<sup>+</sup>/CD31<sup>-</sup> PBMCs after training. Moreover, our study is the first to use NIRS to non-invasively detect a training-induced increase in the oxidative capacity of the human vastus lateralis. We extend previous findings of anti-oxidant effects on training adaptations by demonstrating that mitochondria-targeted antioxidant administration neither enhances nor attenuates short-term aerobic training adaptations in muscle oxidative capacity or whole-body  $\dot{V}_{O_{2max}}$ .

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## Additional information

### Competing interests

No conflicts of interest, financial or otherwise, are declared by the author(s).

### Author contributions

N.T.J. conception and design of research; D.D.S., W.M.S., T.B.W. and K.A.L. performed experiments; D.D.S. and W.M.S. analysed data; D.D.S., W.M.S., T.B.W., K.K.M. and N.T.J. interpreted results; D.D.S. prepared figures; D.D.S., W.M.S. and T.B.W. drafted manuscript; D.D.S., W.M.S., T.B.W., K.A.L., K.K.M. and N.T.J. edited and revised manuscript; D.D.S., W.M.S., T.B.W., K.A.L., K.K.M. and N.T.J. approved final version of manuscript.

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