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ORIGINAL PAPER

Coenzyme Q10 supplementation acts as antioxidant on dystrophic muscle cells

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Abstract

Increased oxidative stress is a frequent feature in Duchenne muscular dystrophy (DMD). High reactive oxygen species (ROS) levels, associated with altered enzyme antioxidant activity, have been reported in dystrophic patients and *mdx* mice, an experimental model of DMD. In this study, we investigated the effects of coenzyme Q10 (CoQ10) on oxidative stress marker levels and calcium concentration in primary cultures of dystrophic muscle cells from *mdx* mice. Primary cultures of skeletal muscle cells from C57BL/10 and *mdx* mice were treated with coenzyme Q10 (5 μM) for 24 h. The untreated *mdx* and C57BL/10 muscle cells were used as controls. The MTT and live/dead cell assays showed that CoQ10 presented no cytotoxic effect on normal and dystrophic muscle cells. Intracellular calcium concentration, H_2O_2 production, 4-HNE, and SOD-2 levels were higher in *mdx* muscle cells. No significant difference in the catalase, GPx, and Gr levels was found between experimental groups. This study demonstrated that CoQ10 treatment was able to reduce levels of oxidative stress markers, such as H_2O_2, acting as an antioxidant, as well as decreasing abnormal intracellular calcium influx in dystrophic muscles cells. This study demonstrated that CoQ10 treatment was able to reduce levels of oxidative stress markers, such as H_2O_2, acting as an antioxidant, as well as decreasing abnormal intracellular calcium influx in dystrophic muscles cells. Our findings also suggest that the decrease of oxidative stress reduces the need for upregulation of antioxidant pathways, such as SOD and GSH.

Keywords CoQ10 · Oxidative stress · *mdx* muscle cells · Intracellular calcium · Enzymatic antioxidant system

Introduction

Duchenne muscular dystrophy (DMD) is a lethal muscular disease, which affects about 1 in 3500–5000 male births (Kamdar and Garry 2016; Robinson-Hamm and Gersbach 2016) and is caused by an X-linked genetic mutation or deletion of the dystrophin scaffolding protein (Guiraud and Davies 2017). This protein has an important role in maintaining membrane muscle fiber integrity during contractions (Ervasti and Sonnemann 2008). Therefore, dystrophin absence may lead to membrane damage with consequent changes in intracellular calcium, leading to muscle fiber necrosis (Guiraud and Davies 2017).

In addition to high intracellular calcium concentration in DMD, oxidative stress driven by reactive oxygen species (ROS) produced in myofibers or released by inflammatory infiltrates is recognized as a key pathogenic event in DMD (Disatnik et al. 1998; Whitehead et al. 2006; Shkryl et al. 2009). Oxidative stress results in the imbalance between reactive oxygen/nitrogen species (ROS/RNS) and antioxidants in favor of the reactive species, promoting a disruption of redox signaling and molecular damage such as lipid peroxidation (Liguori et al. 2018). One such reactive aldehyde species, generated by lipid peroxidation, is 4-hydroxy-trans-2-nonenal (HNE), which increases in the skeletal muscle fibers in *mdx* mice, the experimental model of DMD (Macedo et al. 2015). At the same time, the concentration of hydrogen peroxide (H_2O_2), a strong oxidant, can also be higher in dystrophic muscle cells (Macedo et al. 2015).

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Further to oxidative stress being recognized as a key pathogenic event in DMD, there is a strong cross-talk between ROS and exacerbated intracellular calcium concentration in dystrophic muscles. Besides the lipid peroxidation contributing with the increase of intracellular calcium concentration in *mdx* and DMD muscles, it was also reported that ROS is part of the pathway that triggers increased stretch-activated channels (SACNSC) activity, which leads to a rise in ($[Ca^{2+}]i$) (Allen et al. 2010).

The high efficiency of CoQ10 as a lipid soluble antioxidant is established because of its localization, effective reactivation, and relatively high concentration (Bentinger et al. 2010). CoQ10 is a central component in the mitochondrial electron transport chain (ETC) located in the inner mitochondrial membrane where it transports electrons from complexes I and II to complex III to provide energy for proton translocation to the intermembrane space. It is a component of the mitochondrial respiratory chain complexes (Hernandez-Camacho et al. 2018), and is present in vegetables, fruit, and several other dietary sources (Littarru and Tiano 2007). CoQ10 supplementation has been reported as providing beneficial effects on cardiovascular, cancer, and neurodegenerative diseases (Flint Beal 2002; Lekli et al. 2008; Bahar et al. 2010). CoQ10 potential benefits are associated to its antioxidant and free radical scavenging properties (Bentinger et al. 2010).

Despite exhaustive clinical management and corticosteroid treatment, there is currently no effective treatment for DMD. Given the role that oxidative stress plays in DMD, its prevention can be a promising therapeutic strategy for muscular dystrophies.

In this study, we have examined the possible role of CoQ10 on oxidative stress markers and on the antioxidant defense system in primary skeletal muscle cell cultures derived from *mdx* mice. In addition, due to the deregulation of intracellular calcium content being an important factor involved in DMD pathogenesis, the calcium concentration was also verified.

Materials and methods

Animals and cell cultures

The research protocol was approved (#3587-1) by the Committee on the Ethics of Animal Experiments (CEUA) of UNICAMP, Sao Paulo, Brazil and the study was conducted in accordance with the guidelines of the Brazilian College for Animal Experimentation (COBEA). Both *mdx* (C57BL/10-Dmdmdx/PasUnib) and their normal counterparts of the same genetic background C57BL/10 (C57BL/10ScCr/PasUnib) mice were obtained from a breeding colony maintained by our institutional animal care facility and were used in all the experiments.

Primary skeletal muscle cell culture was prepared in parallel with male and female *mdx* and C57BL/10 mice (28 days old), using 4–6 mice per isolation in three independent cultures. Cultures were established in triplicate and experiments were carried out at least three times. To perform the primary culture of muscle cells, the hind limb muscles of mice were dissected and rapidly the muscles were isolated in DPBS containing 1% glucose (v/v) and 1% penicillin (v/v) to prepare primary skeletal muscle cell culture (Rando and Blau 1994). For 10 min the muscle tissue was crushed using scissors and the suspension was enzymatically digested with collagenase and trypsin solutions at 37 °C. The satellite cells (5×10^4 cells/cm^2) were plated in 1% Matrigel-coated dishes. The myoblasts were cultured in a proliferation and growth medium containing DMEM with glucose (5.5 mM), L-glutamine (2 mM), fetal bovine serum (10% v/v), horse serum (10% v/v), and penicillin/streptomycin (1% v/v) for 2 days. Myotube differentiation was induced by the addition of a fusion medium (FM) composed by DMEM with glucose (5.5 mM), L-glutamine (2 mM), and horse serum (10% v/v). The culture was maintained in a 5% CO_2 incubator at 37 °C. The differentiated muscle cells with contractile properties were observed at 7–8 days of culture in the FM. Skeletal muscle cell cultures at 7–8 days were used in all experiments and were divided in four experimental groups: Ctrl untreated (skeletal muscle cells from C57BL/10 mice that did not receive any treatment), CtrlCo (skeletal muscle cells from C57BL/10 mice treated with 5 μM of coenzyme Q10 for 24 h), mdx untreated (skeletal muscle cells from mdx mice that did not receive any treatment), and mdxCo (skeletal muscle cells from mdx mice treated with 5 μM of coenzyme Q10 for 24 h).

Cell viability

MTT assay

For quantification of mitochondrial metabolism and activity of the respiratory chain of cells, tetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT; Sigma) assay was used. In this experiment, the primary muscle cells were washed in PBS once and MTT solution was added (5 mg/ml, tetrazolium salt) and incubated for 4 h at 37 °C. After the incubation period, whole medium were discarded and MTT crystals were dissolved with acid isopropanol and the absorbance measured in spectrophotometer (Synergy H1, Hybrid Reader, Biotek Instruments, Winooski, VT, USA) at 570 nm with a 655-nm reference wave length to quantify the amount of formazan product, which reflects the number of cells in culture. Wells that did not contain cells were used as a zero point of absorbance.

Live/dead cell assay

The polyanionic dyecalcein-green is retained within live cells, producing an intense uniform green fluorescence. Ethidium homodimer-1 (EthD-1) enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells. All media were removed from primary skeletal muscle culture cells and replaced with a solution containing 5 μl of AM calcein (4 mM) and 20 μl of EthD-1 (2.5 mM) (Biotium, California, USA) diluted in 10 ml DMEM. Cells were overlaid with 100 μl of staining solution and left to incubate for 45 min at room temperature. Following this time, cells were photographed using a fluorescent inverted microscope (Nikon, Eclipse TS100/TS100F) connected to a Nikon camcorder at 20× magnification.

Fluo-4 assay for intracellular calcium content analyses

This assay used calcium-sensitive dye Fluo-4 (Molecular Probes, Oregon, USA) to measure intracellular calcium concentrations $[Ca^{2+}]i$. Cells were incubated with the dye Fluo-4 AM for 60 min at room temperature at a final concentration of 1 mmol/l (plus 0.005% Pluronic F-127 (Invitrogen, Oregon, USA) (Guatimosim et al. 2011). The intensities of fluo-4 fluorescence was monitored on a fluorescent inverted microscope (Nikon, Eclipse TS100/TS100F) for qualitative analyses. Quantitative measurements were performed using a spectrophotometer (Synergy H1, Hybrid Reader, Biotek Instruments, Winooski, VT, USA) at excitation and emission wavelengths of 494 and 516 nm, respectively.

Determination of H_2O_2 production

For determination of H_2O_2 levels, Amplex Red assay kit was used (Molecular Probes, Life Technologies, California, EUA) according to manufacturer's instructions. The Amplex UltraRed reagent (50 μM) and HRP (0.1 U/ml) were added for 60 min. The absorbance was determined at 530-(excitation) and 590-nm wavelength (emission). Measurements of ROS were previously calibrated using exogenous 10 μM H_2O_2 (positive control). All measurements were performed in phenol red-free culture medium (1 ml), pH 7.4, at 37 °C.

Western blotting

Cell cultures were washed thrice with PBS, before addition of lysis buffer (Tris–HCl, 100 mM, pH 7.5; EDTA, 10 mM, pH 8.0; sodium pyrophosphate, 10 mM; sodium fluoride, 0.1 mM; sodium orthovanadate, 10 mM; PMSF, 2 mM and aprotinin, 10 μg/ml). The cell extracts were sonicated for 30 s at 4 °C. Cell debris was removed by centrifugation at 11,000 rpm for 20 min at 4 °C and the cleared lysate was subjected to SDS-Page gel electrophoresis. An aliquot from the supernatant was used to determine the total protein content by the Bradford method. Thirty micrograms of total protein homogenate was loaded on 6–15% SDS-polyacrylamide gels. Following electrophoresis, the proteins were transferred onto nitrocellulose membranes using an iBlot Gel Transfer Device (Bio-Rad Laboratories, Hercules, California). All membranes were blocked with blocked for 2 h at room temperature with 5% skim milk/Tris-HCl buffer saline-Tween buffer (TBST; 10 mM Tris-HCl, pH 8, 150 mM NaCl, and 0.05% Tween 20). Primary antibodies (4-HNE, goat polyclonal, Santa Cruz Biotechnology, Santa Cruz, California; monoclonal anti-catalase antibody, mouse, Sigma-Aldrich, St. Louis, MO, USA; anti-SOD-2, rabbit, Sigma-Aldrich, St. Louis, MO, USA; anti-GSR (rabbit, Sigma-Aldrich, St. Louis, MO); anti-GPx1 (rabbit, Sigma-Aldrich, St. Louis, MO); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; rabbit polyclonal; Santa Cruz Biotechnology, Santa Cruz, California) were incubated overnight at 4 °C with gentle shaking. Then, membranes were incubated for 2 h at room temperature with the peroxidase-conjugated secondary antibodies purified goat, mouse, or rabbit IgG antibody (KPL), respectively. Membranes were washed 3 × 10 min with TBST after both incubations. All membranes were revealed using the SuperSignal West Pico Chemiluminescent Substrate kit (Pierce Biotechnology, Rockford, Illinois). To control protein loading, Western blot transfer, and nonspecific changes in protein levels, the blots were stripped and reprobed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Band intensities were quantified using ImageJ 1.38X (National Institutes of Health, Bethesda, Maryland) software.

Glutathione (GSH) content

Total GSH content was determined by Ellman's reaction using 5′5′-dithio-bis-2-nitrobenzoic acid (DTNB) as described by Anderson (1985). The intensity of the yellow color was read at 412 nm. The results were expressed as nmol per mg of protein.

Statistical analysis

Initially, we have used Kolmogorov and Smirnov test to evaluate the data normality, and then Anova one-way test followed by Tukey analysis to compare the effects of treatment. Data are expressed as median, minimum, first interquartile, third interquartile, and maximum values. The significance level of 5% ($p < 0.05$) was used. We have used the GrapPad Prims5 software package (GraphPad Software, CA, USA).

Results

C57BL/10 (normal) and *mdx* (dystrophic) muscles cells showed a similar time-course development, characterized by typical progression of proliferation to differentiation and fusion into thick, branching myotubes (Fig. 1). Coenzyme Q10 did not alter the morphology of skeletal muscle cells (Fig. 1).

Cell viability evaluated by MTT and live dead cell assays showed that coenzyme Q10 has no cytotoxic effect on C57BL/10 and *mdx* muscle cells (Fig. 2A, B). No significant difference in cell viability was found between the experimental groups (Fig. 2B).

No significant difference in intracellular calcium was found between Ctrl and CtrlCo groups (Fig. 3A, B). The *mdx* muscle cells showed a significant difference of 13.1% more fluorescence intensity of intracellular calcium when compared to the Ctrl group (Fig. 3A, B). In addition, the *mdx* muscle cells treated with coenzyme Q10 showed lesser fluorescence intensity of intracellular calcium compared to Ctrl, CtrlCo, and *mdx* groups (by 31.4%, 36.3%, and 44.4%, respectively), reaching levels significantly lower than ctrl (Fig. 3B).

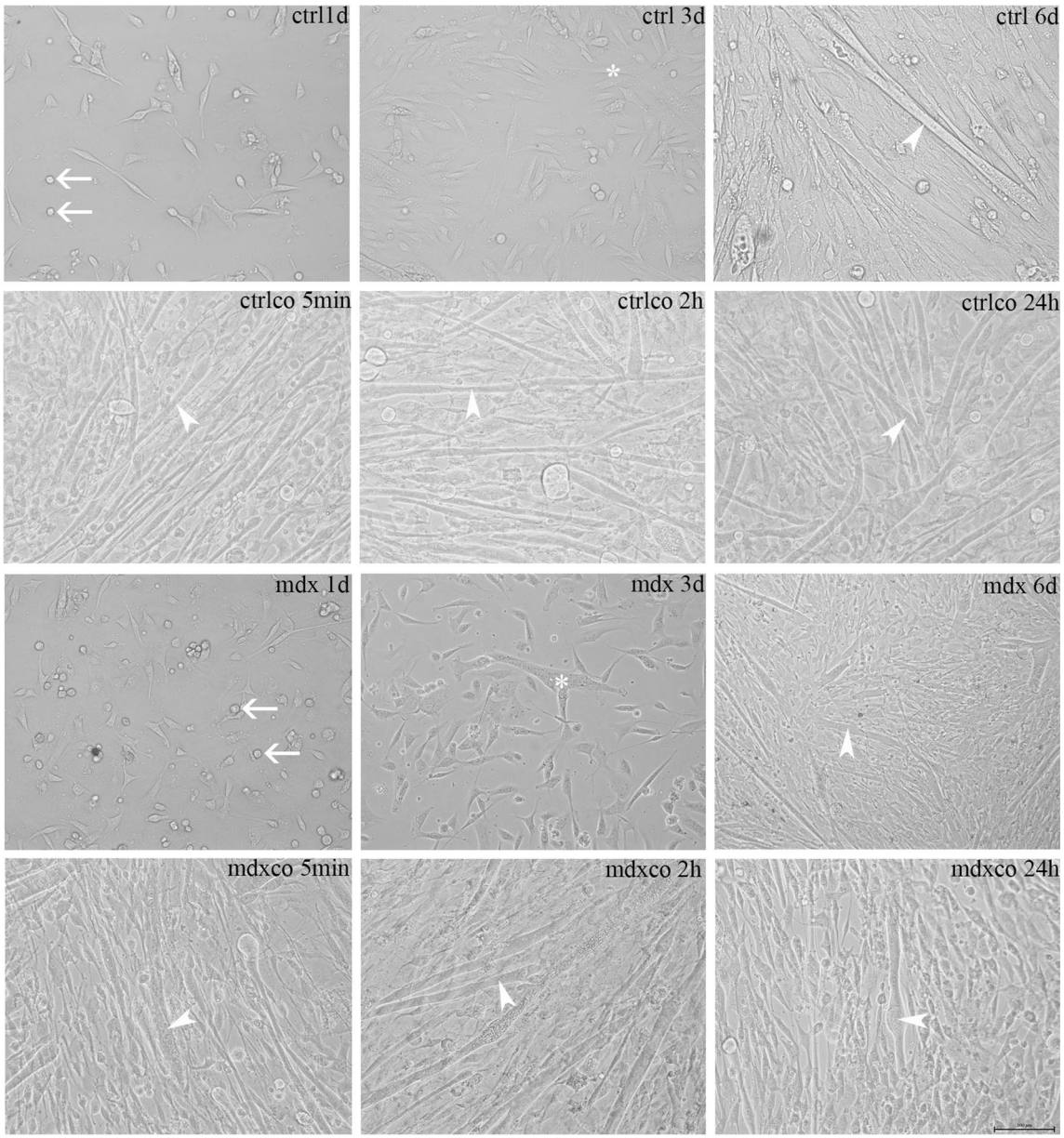

Fig. 1 Morphology of C57BL/10 muscle cells (ctrl), C57BL/10 muscle cells treated with coenzyme Q10 (ctrlco), untreated *mdx* muscle cells (*mdx*) and *mdx* muscle cells treated with coenzyme Q10 (mdxco). (1d) shows undifferentiated muscle cells (arrows), (3d) shows maturation process (asterisk), and (6d) shows complete morphological maturation of muscle cell (arrowhead). (5min) shows muscle cells (arrowhead) 5 min after coenzyme Q10 treatment, (2h) shows skeletal muscle cells (arrowhead) 2 h after coenzyme Q10 treatment, and (24h) shows muscle cells (arrowhead) 24 h after coenzyme Q10 treatment. Scale bar 100 μm

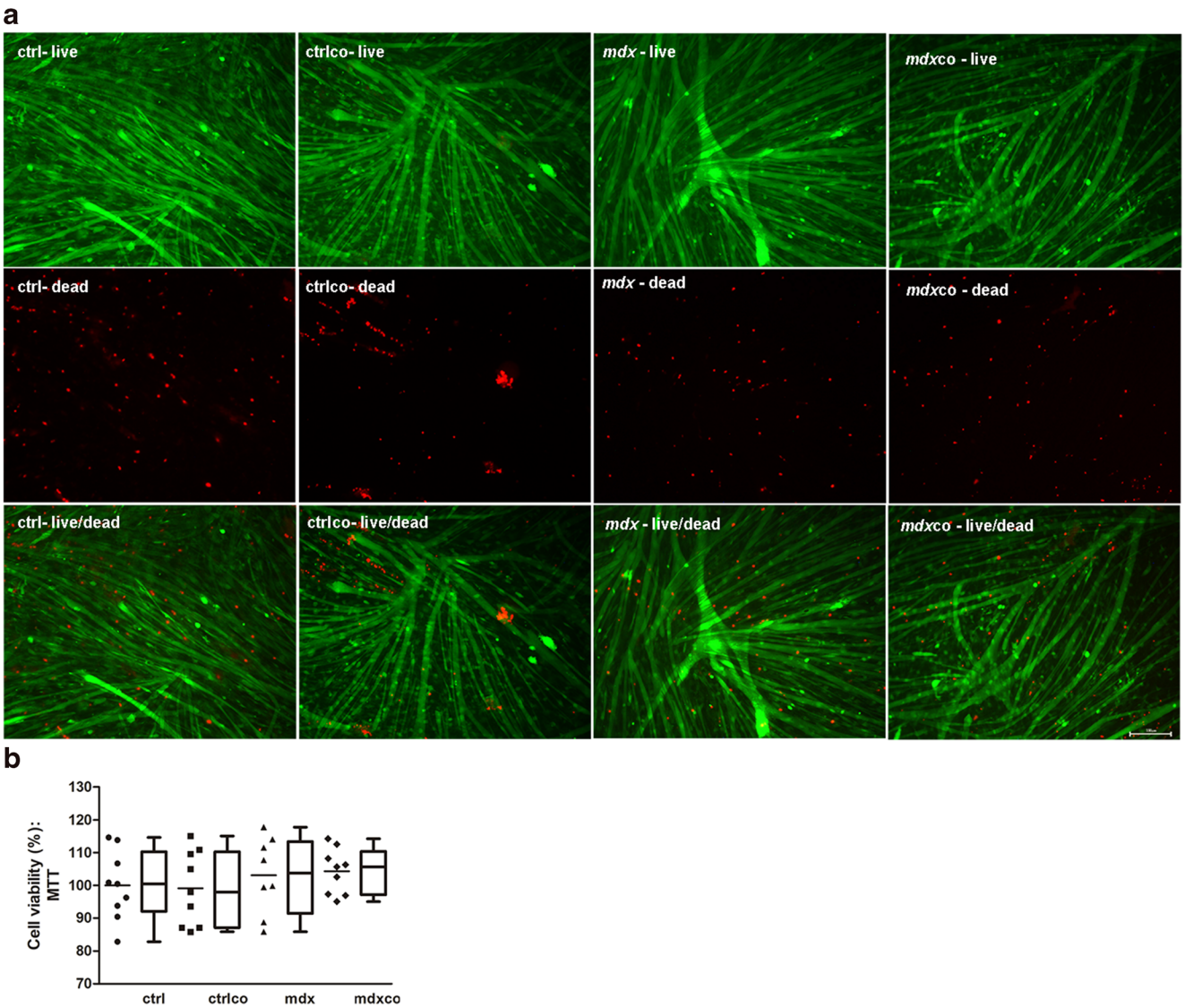

Fig. 2 In (**A**), fluorescence images of live/dead staining of C57BL/10 muscle cells (ctrl), C57BL/10 muscle cells treated with coenzyme Q10 (ctrlco), untreated *mdx* muscle cells (*mdx*) and *mdx* muscle cells treated with coenzyme Q10 (mdxco). Live cells in green. Dead cells in red. Scale bar 100 μm. In (**B**), cell viability was assessed by measurement of MTT assay in the muscle cells from Ctrl, Ctrlco, *mdx*, and *mdx*co ($n = 5$). Box-and-whisker plot (median, first, and third percentiles, range) displayed with the scatter plot of raw data

To test whether the coenzyme Q10 treatment affected oxidative stress in dystrophic muscle cells, we analyzed 4-hydroxynonenal (4-HNE)-protein adduct levels; H_2O_2 production; and catalase, GPx, GR, SOD2, and GSH content.

Representative immunoblots and quantification of 4-HNE-protein adducts are shown in Fig. 4A. Bands of 4-HNE-protein adducts from 17 to 170 kDa were detected in control and in dystrophic muscle cells. The 4-HNE-protein adducts were significantly different, presenting higher values in the CtrlCo, mdx, and mdxCo groups versus Ctrl group. Production of H_2O_2 was higher in *mdx* muscle cells than in control muscle cells (by 50%, Fig. 4B). Coenzyme Q10 treated groups demonstrated significantly lower H_2O_2 production. The CtrlCo group had 40.3% lower H_2O_2 production than Ctrl, as well as mdxCo muscle cells presented 42.1% lesser versus mdx untreated group. No statistically significant difference was found between the ctrl and mdxco groups, indicating that the treatment promoted the return of the H_2O_2 levels to the normal parameters (Fig 4B).

No significant difference in the catalase, GPx, and Gr levels was found between experimental groups (Fig. 5A, B, C). The SOD-2 levels were significantly different in the *mdx* muscle cells exhibiting values 30.9% higher compared to CtrlCo group. The mdxCo group showed lower levels of SOD-2, reaching levels closer to Ctrl and CtrlCo groups (by 19.5% lesser versus ctrl; Fig. 5D). Regarding GSH levels, a significant difference of 27.8% more was observed in CtrlCo muscle cells compared to the Ctrl group (Fig. 5E). In dystrophic muscle cells, coenzyme Q10 treatment promoted a significant difference in GSH levels, exhibiting lower values compared to

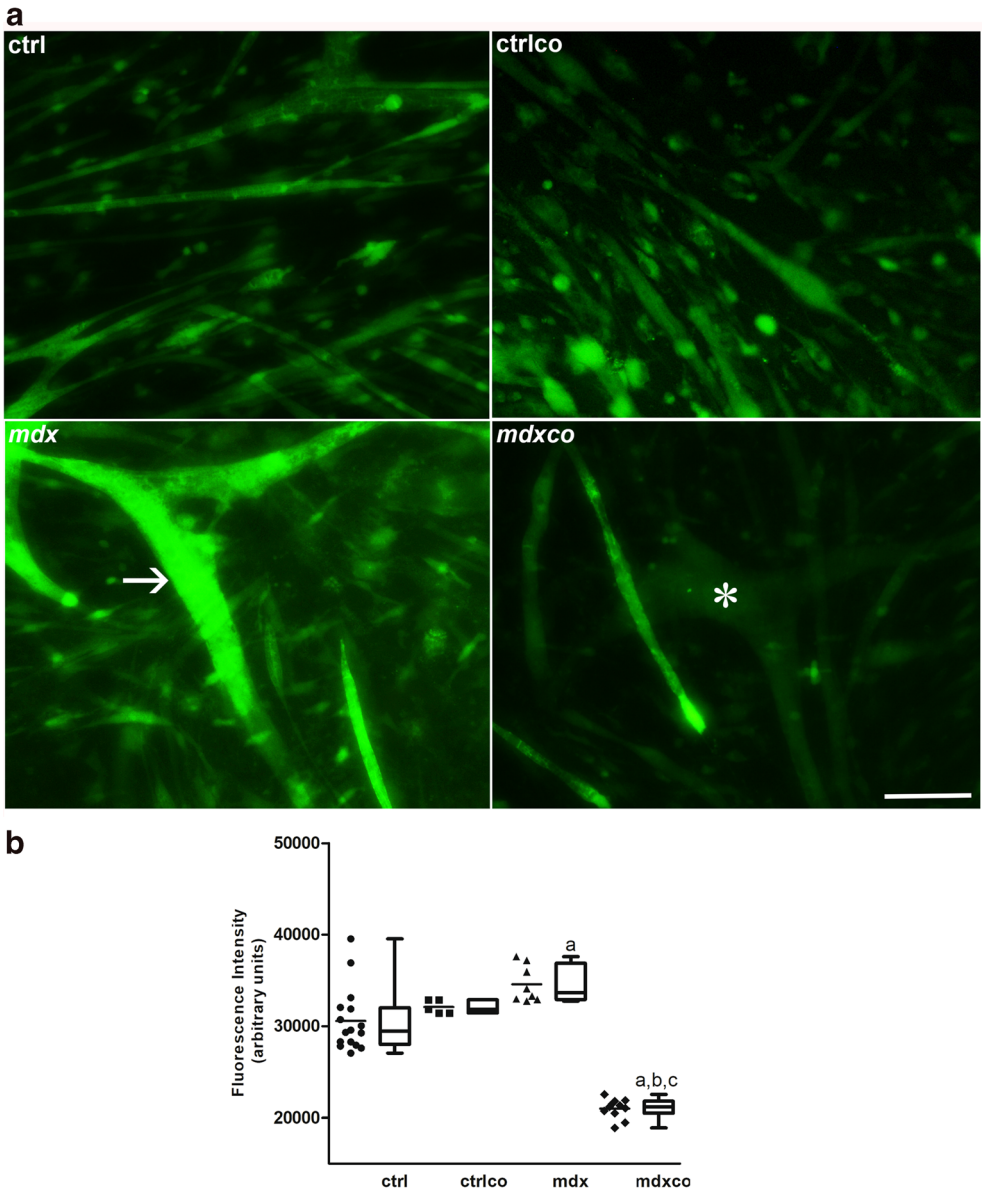

Fig. 3 In (**A**), intracellular calcium [Ca^{2+}] concentrations (20×), assessed by measurement of calcium-sensitive dye Fluo-4 (green) in C57BL/10 muscle cells (ctrl), C57BL/10 muscle cells treated with coenzyme Q10 (ctrlco), untreated *mdx* muscle cells (*mdx*) and *mdx* muscle cells treated with Coenzyme Q10 (mdxco). The arrow shows fluorescence intensity of muscle cell in the mdx group. Asterisk shows fluorescence intensity of muscle cell in mdxco. Scale bar 100 μm. In (**B**), graph showing fluorescence intensity of [Ca^{2+}] in ctrl, ctrlco, mdx, and mdxco groups ($n = 5$). Box-and-whisker plot (median, first, and third percentiles, range) displayed with the scatter plot of raw data. $^{a}P < 0.05$ versus ctrl; $^{b}P < 0.05$ versus ctrlco; $^{c}P < 0.05$ versus *mdx*

CtrlCo and mdx groups (by 36.7% and 26.2%, respectively; Fig. 5E).

Discussion

DMD is one of the most common X-linked genetic disorders in humans; it arises from point mutations, deletions, or duplications in the DMD gene that prevent expression of its encoded protein, dystrophin (Guiraud and Davies 2017). It is a lethal disease for which, so far, there is no cure. Currently, corticosteroids are the main treatment recommended for dystrophic patients (Wu et al. 2016). Although, corticosteroids slow the decline in muscle strength and function and are used to prolong ambulation and stabilize pulmonary function, their prolonged use may cause several side effects such as weight gain, behavioral changes, growth retardation, late puberty, bone demineralization, and gastroesophageal reflux (Adrews and Wahl 2018; Bushby et al. 2010; Balaban et al. 2005). There is a recognized urgent need for a therapy that can alter the fundamental course of the disease (Adrews and Wahl 2018; Ryder et al. 2017).

In the last years, the *in vitro* models have become a potential solution for studying disease pathology, serving as a platform which can be used as phenotypic drug screens to identify compounds capable of alleviating or reversing congenital myopathies, such as DMD (Smith et al. 2016). In this way, this is the first study to evaluate the effects of coenzyme Q10 treatment on dystrophic muscle cells. The main findings suggest that coenzyme Q10 can reduce oxidative stress markers in primary skeletal muscle cell cultures derived from *mdx* mice.

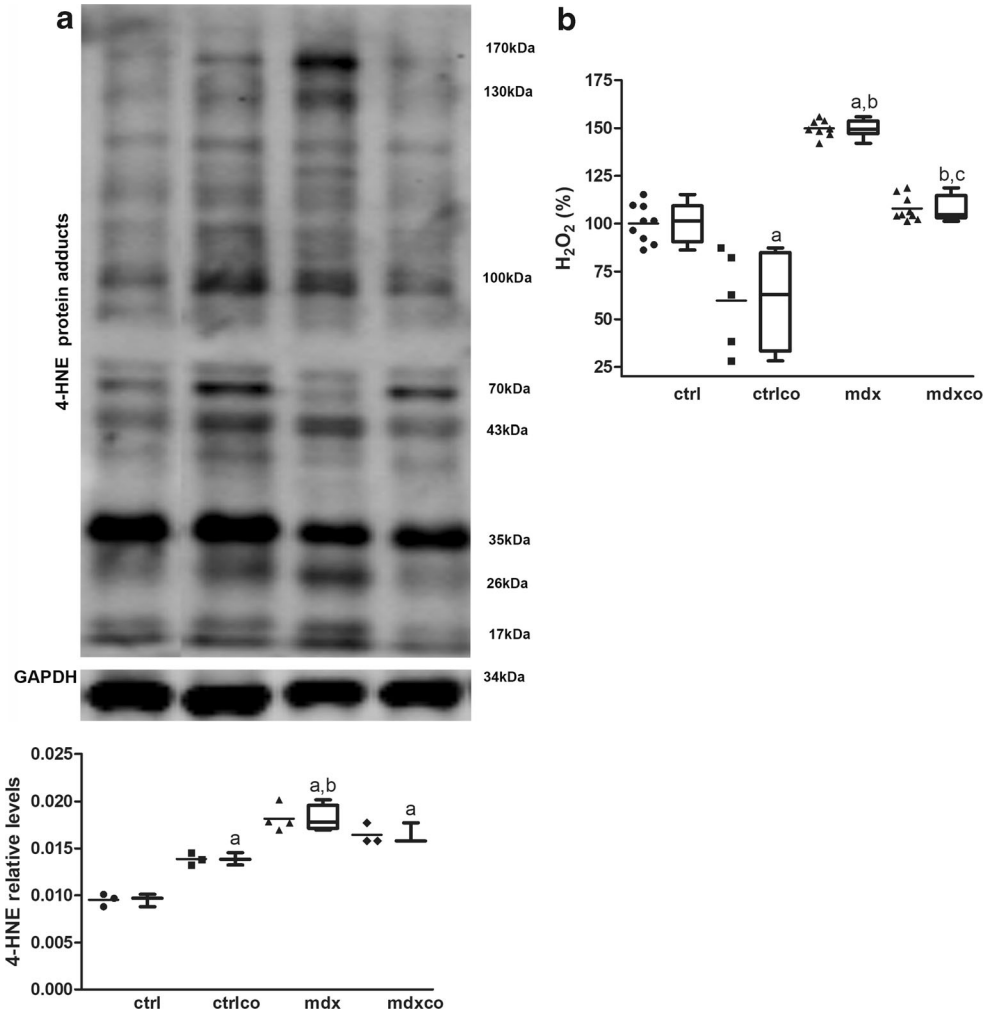

Fig. 4 In (**A**), immunoblot analysis shows several bands of 4-HNE-protein adducts, ranging from 26 to 170 kDa. Box-and-whisker plot (median, first, and third percentiles, range) displayed with the scatter plot of raw data. Box-and-whisker plot (median, first, and third percentiles, range) show protein level in C57BL/10 muscle cells (ctrl), C57BL/10 muscle cells treated with coenzyme Q10 (ctrlco), untreated *mdx* muscle cells (mdx) and *mdx* muscle cells treated with Coenzyme Q10 (mdxco) (n = 3). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. The relative value of the band intensity was quantified and normalized by the corresponding Ctrl. In (**B**), box-and-whisker plot (median, first, and third percentiles, range) displayed with the scatter plot of raw data of quantification of H_2O_2 production in ctrl, ctrlco, mdx, and mdxco groups (n = 5). $^{a}P < 0.05$ versus ctrl; $^{b}P < 0.05$ versus ctrlco; $^{c}P < 0.05$ versus *mdx*

In the dystrophic (*mdx)* mouse, skeletal muscle undergoes cycles of degeneration and regeneration, and myogenic progenitors (satellite cells) show ongoing proliferation and differentiation at a time when counterpart cells in normal healthy muscles enter quiescence (Anderson and Wozniak 2004). At 3 weeks of age, histological changes occur in most muscles of the limbs of both male and female *mdx* mice (Bulfield et al. 1984). Ever since the spontaneous onset of *mdx* mouse in a C57BL/10ScSn colony, many mouse models have arisen over the years to better understand the disease. Because *mdx* manifests only a mild phenotype, many of these models hinge on additive effects of either structural or myogenic gene knockouts that are not present in the human disease, in order to increase the severity of the phenotype. As a result, the *mdx* model, for all its caveats, is still by far the most widely used (Yucel et al. 2018).

Primary cell cultures from normal and dystrophic muscles showed the typical progression of robust proliferation to differentiation and fusion into thick, branching myotubes (Yablonka-Reuveni and Anderson 2006), in agreement with the findings of the present study. In addition, in our experimental conditions, the coenzyme Q10 presented no cytotoxic effect on dystrophic muscle cells.

Sarcolemmal Ca^{2+} influxes are crucial early events in the pathophysiology of DMD, prior to the onset of observable histological muscle damage and inflammation (Loehr et al. 2016). Previous studies have shown that muscular dystrophy is characterized by increased membrane permeability to calcium (Ca^{2+}), resulting in greater Ca^{2+} influx across the

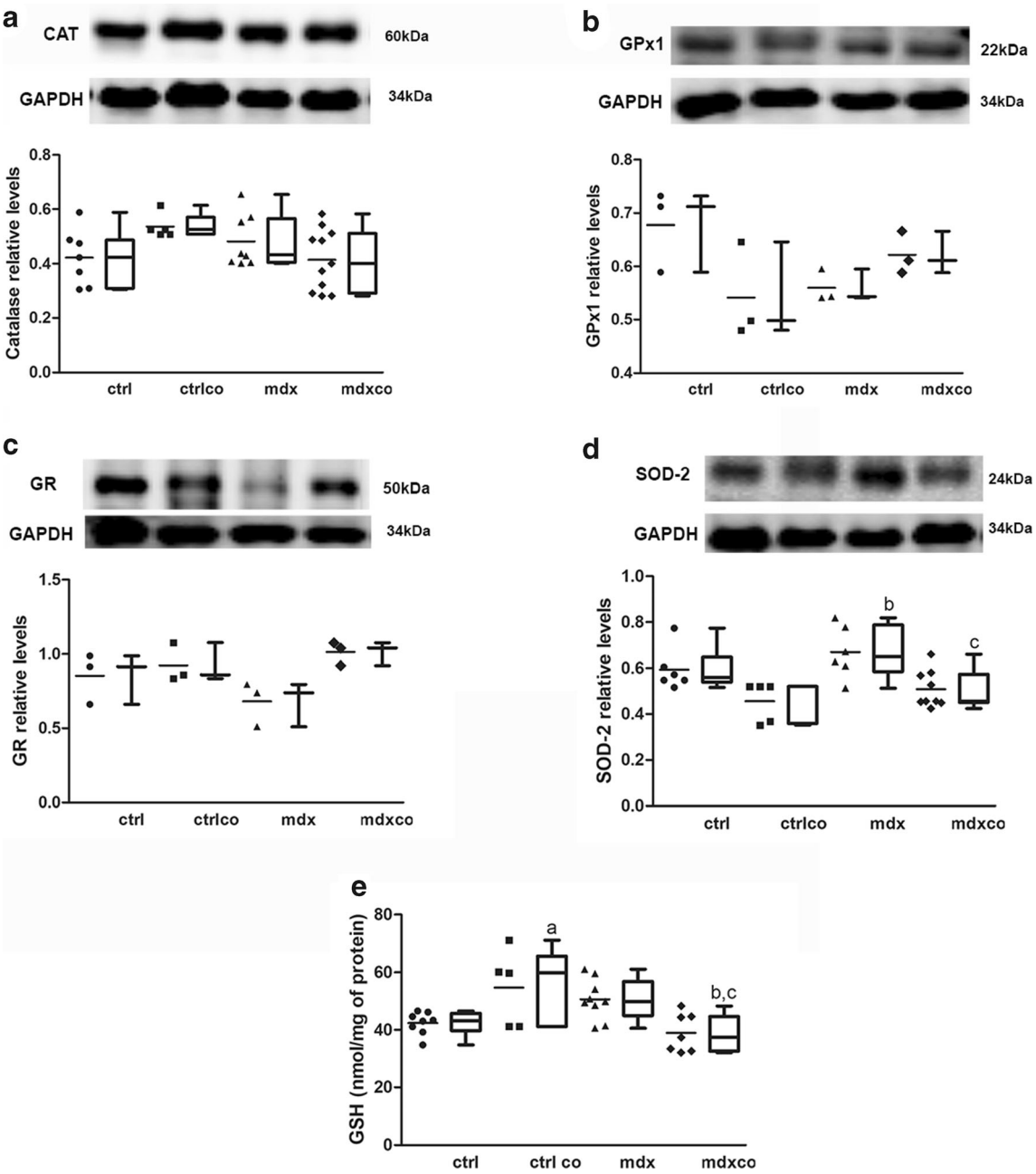

Fig. 5 Analysis of catalase (**A**), GPx (**B**), GR (**C**), SOD-2 (**D**), and GSH levels (**E**). Box-and-whisker plot (median, first, and third percentiles, range) displayed with the scatter plot of raw data. Graphs show protein level in C57BL/10 muscle cells (ctrl), C57BL/10 muscle cells treated with coenzyme Q10 (ctrlco), untreated *mdx* muscle cells (mdx), and *mdx* muscle cells treated with coenzyme Q10 (mdxco). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. The relative value of the band intensity was quantified and normalized by the corresponding Ctrl. Analysis in ctrl, ctrlco, mdx, and mdxco groups. (n = 3–9). [a]$P < 0.05$ versus ctrl; [b]$P < 0.05$ versus ctrlco; [c]$P < 0.05$ versus *mdx*

sarcolemma (Pal et al. 2014; Baker et al. 2002; Tutdibi et al. 1999), and increased NADPH oxidase 2 (Nox2)-derived reactive oxygen species (ROS); including hydrogen peroxidase (H2O2) (Pal et al. 2014; Whitehead et al. 2010). Complementing these studies, Pal et al. (2014) and Altamirano et al. (2013) showed an improvement of dystrophic pathophysiology associated a reduction of intracellular Ca^{2+} by blocking Ca^{2+} channels or downregulating Nox2 ROS production in young *mdx* mice. Still reinforcing the link between the increase of intracellular calcium and ROS, it was demonstrated that eliminating Nox 2 ROS production reduces exacerbated calcium influx and functional deficits in dystrophic muscles (Loehr et al. 2016).

It has been well established that CoQ10 inhibits lipid peroxidation by preventing the production of lipid peroxyl radicals (LOO.) and, moreover, CoQH2 reduces the initial

preferred radical, with concomitant formation of ubisemiquinone and H_2O_2 (Bentinger et al. 2010). In agreement with our findings, pancreatic stellate cells treated with CoQ10 for 72 h had a significant decrease in ROS positive cells and malondialdehyde (MDA) levels (Xue et al. 2017). Another study that evaluated the role of CoQ10 on oxidative stress markers in the serum of multiple sclerosis patients also demonstrated a significant reduction of lipid peroxidation levels (Sanoobar et al. 2013).

CoQ10 is located in the inner mitochondrial membrane, whose main function is to accept electrons for the nicotinamide adenine dinucleotide dehydrogenase (NADH) and succinate dehydrogenase (SDH) complexes of the respiratory chain (Acosta et al. 2016). Exogenous administration of CoQ10 can increase the oxidative capacity of NADH and assist in metabolically supporting the dystrophic muscle through a direct modulation of the mitochondrial permeability transition pore (mPTP) (Guescini et al. 2017). Occupancy of this site can modulate the PTP open-closed transitions, possibly through secondary changes of the Ca^{2+}-binding affinity for the pore (Devun et al. 2010; Fontaine et al. 1998). Failure of cellular Ca^{2+} homeostasis and consequent mitochondrial Ca^{2+} overload is the principal trigger for mitochondrial mPTP opening.

A high-conductance mPTP opening is associated with osmotic swelling, loss of inner mitochondrial membrane potential, uncoupling of oxidative phosphorylation, and metabolic collapse (Briston et al. 2017). As the mPTP opening is a determinant of cell death in an ever-growing list of diseases (Duchen et al. 2008), the preventing calcium accumulation observed in our study may be contributing to the reduction of ROS production. At the same time, positive feedback between ROS and calcium may be occurring in our study. Previous work shows that a combination of high Ca^{2+} in the mitochondria and ROS generation triggers the mitochondrial permeability transition (MPT) (Kowaltowski et al. 2001). Reinforcing this idea, it was recently demonstrated that CoQ10 diet supplementation prevented MPT in the liver mitochondria of pravastatin-treated hypercholesterolemic mice (Marques et al. 2018). Previously, it had also been proposed that the inhibition of muscle mitochondrial respiration by pravastatin leads to oxidative stress that in the presence of calcium opens the mPTP and that this pravastatin toxic effect was protected by the CoQ10 antioxidant (Busanello et al. 2017).

CoQ10 appears to have no expressive effect on the antioxidant pathways of the CAT, GPx, and GR enzymes. It is known that ROS could inactivate GPx and GR (Ishrat et al. 2006; Fouad et al. 2010) which is in agreement with our findings in the mdx group. Furthermore, CoQ10 treatment did not change the GPx activity in the kidney transplant patients (Długosz et al. 2004), in patients with coronary artery disease (Lee et al. 2012) and in the blood samples of sclerosis multiple patients (Sanoobar et al. 2013). To protect themselves from an oxidative attack, cells have developed a wide range of antioxidant systems to inactivate excessive ROS (Sies 2015). The induction of oxidative stress by postnatal protein restriction in rats promoted upregulation of MnSOD, Gpx-1, and GR antioxidant enzymes (Tarry-Adkins et al. 2007). In the same way, impairment of kidney function and increment in blood pressure increased the expression of superoxide dismutase (SOD) isoforms and catalase in the renal cortex from old rats (Gomes et al. 2009). Although catalase reduction was not significant in our experiments, our data suggest that the reduction of ROS production promoted by CoQ10 decreased the need for overexpression of the SOD antioxidant enzyme and GSH, which returned to levels similar to those of the control group.

CoQ10 is useful for the neutralization of reactive oxygen species, due to its redox property (Maraver et al. 2014). CoQ10 is the only endogenously synthesized liposoluble antioxidant that can participate in redox reactions, acting on the prevention of damage to DNA and proteins and on lipid peroxidation, and indirectly stabilizing the calcium channels by preventing calcium overload (Sugiyama et al. 1980). The enzyme acts on lipid peroxidation by either sequestering free radicals or reducing the α-tocopheryl radical to α-tocopherol (Kagan et al. 1990).

This study demonstrated that CoQ10 treatment was able to reduce levels of oxidative stress markers, such as H_2O_2, acting as an antioxidant as well as decreasing abnormal intracellular calcium influx in dystrophic muscles cells. Our findings suggest that the decrease of oxidative stress reduces the need for upregulation of antioxidant pathways, such as SOD and GSH. However, more studies are required to better understand the interactions of CoQ10 with the dystrophic muscles.

Author contributions The authors contributed substantially to conception and design, acquisition of data, analysis and interpretation of data. All authors participated in drafting the article, revised it critically for important intellectual content and gave final approval of the version to be submitted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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