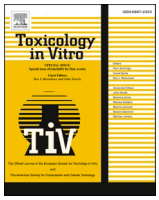
Toxicology in Vitro 106 (2025) 106052

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit



Prolonged exposure to simvastatin affects coenzyme $Q_{9/10}$ status leading to impaired mitochondrial respiratory capacity and reduced viability of cultured cardiac cells

Sinenhlanhla X.H. Mthembu [a,b,*], Sithandiwe E. Mazibuko-Mbeje [b], Sonia Silvestri [c], Patrick Orlando [c], Bongani B. Nkambule [d], Christo J.F. Muller [a,e,f], Luca Tiano [c], Phiwayinkosi V. Dludla [f]

[a] Biomedical Research and Innovation Platform, South African Medical Research Council, Tygerberg 7505, South Africa
[b] Department of Biochemistry, Mafikeng Campus, Northwest University, Mmabatho 2735, South Africa
[c] Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona 60131, Italy
[d] School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban 4000, South Africa
[e] Centre for Cardiometabolic Research Africa (CARMA), Division of Medical Physiology, Stellenbosch University, Tygerberg 7505, South Africa
[f] Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa 3886, South Africa

ARTICLE INFO

Editor: Dr. P Jennings

Keywords:
Statin
Coenzyme $Q_{9/10}$
Mitochondrial respiration
Oxidative stress
Cardiac cells

ABSTRACT

This study investigates the effects of prolonged simvastatin exposure on coenzyme $Q_{9/10}$ ($CoQ_{9/10}$) levels, an essential component of antioxidant defense, in cultured cardiac cells. Statins, commonly used to manage dyslipidemia and reduce cardiovascular risk, may impair mitochondrial function, but their impact on CoQ_{10} depletion and oxidative stress is not well understood. We examined the influence of simvastatin on mitochondrial oxidative capacity, reactive oxygen species (ROS) production, and $CoQ_{9/10}$ status at concentrations of 0.3, 0.6, 1.25, 2.5, 5, 10, and 20 μM, over durations of 24, 48, and 72 h. Using an in vitro model of cultured H9c2 cardiomyoblasts, our results showed that short-term exposure (24 h) at lower concentrations (<5 μM) enhanced cytosolic and mitochondrial ROS levels without affecting mitochondrial function or $CoQ_{9/10}$ status. However, prolonged exposure to higher concentrations (≥10 μM for >48 h) resulted in impaired mitochondrial oxidative capacity, indicated by increased proton leak and elevated ROS levels, which were followed by significantly reduced cell viability. These findings suggest that prolonged, high-dose simvastatin exposure may disrupt the oxidative balance of $CoQ_{9/10}$, leading to myocardial injury. This research addresses a gap in understanding the long-term effects of statins on mitochondrial health and underscores the need for further studies to optimize statin therapy and minimize adverse effects on myocardial function.

1. Introduction

Type 2 diabetes (T2D) and its associated comorbidities exacerbate the burden of cardiovascular diseases (CVDs) (Martín-Timón et al., 2014). Dyslipidemia, a common consequence of T2D, is characterized by elevated levels of triglycerides, low-density lipoprotein (LDL) cholesterol can also be implicated in cardiovascular disease (CVD) events, often due to unhealthy lifestyle choices and hereditary factors (Gonna and Ray, 2019). In conditions of dyslipidemia, there is uncontrolled uptake of free fatty acids within the myocardium, which triggers pathological processes that can lead to accelerated cardiac toxicity and increased risk of CVD events (Hue and Taegtmeyer, 2009; Boudina et al., 2009). Yet, much remains to be understood about the precise pathophysiological mechanisms explaining enhanced cardiac injury within diverse conditions of dyslipidemia or related metabolic stress. However, statins have proven effective in reducing total cholesterol levels,

Abbreviations: ATP, adenosine triphosphate; $CoQ_{9/10}$, coenzyme $Q_{9/10}$; Ctrl, control; CVD, cardiovascular disease; DCFH-DA, 2′, 7′-dichlorodihydrofluorescin diacetate; ECAR, glycolytic energy as measured by extracellular acidification rate; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme-A reductase; MTT, (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide); OCR, oxygen consumption rate; ROS, reactive oxygen species; SEM, standard error mean;; T2D, Type 2 diabetes.

* Corresponding author at: Department of Biochemistry, Mafikeng Campus, Northwest University, Mmabatho 2735, South Africa.
E-mail address: 40624560@mynwu.ac.za (S.X.H. Mthembu).

https://doi.org/10.1016/j.tiv.2025.106052
Received 13 December 2024; Received in revised form 7 March 2025; Accepted 10 March 2025
Available online 13 March 2025

especially lower low-density lipoprotein (LDL) cholesterol thereby protecting against CVD-related complications in people with dyslipidemia or related conditions (Taylor et al., 2013; Force, 2022). However, despite their acknowledged efficacy, the persistent increase in CVD-related deaths alongside the growing prevalence of metabolic diseases (Minja et al., 2022; Roth et al., 2020) warrants further investigation into potential limitations on the therapeutic effects of statins. It has been hypothesized that one of the limitations is that statins may worsen oxidative stress, a condition marked by the overproduction of harmful reactive oxygen species (ROS), which plays a major role in lipid-induced cardiac damage (Ahmadi et al., 2018).

Amidst the ongoing discussion regarding the efficacy of statins in managing dyslipidemia (Lempp, 2021), the growing literature suggests that the long-term benefits of statins may be hindered by their potential interference with the intracellular coenzyme Q_{10} (CoQ_{10}) levels thus causing enhanced oxidative stress and cardiotoxicity (Mthembu et al., 2023; Fitzgerald et al., 2012; Braillon, 2015). This raises concerns about the potential for statin therapy to deplete CoQ_{10} levels, thereby enhancing oxidative stress and cardiotoxicity over time. Therefore, is it crucial to revise an approach to dyslipidemia management, where the delicate balance between statin-induced lipid modulation and the preservation of CoQ_{10} levels is to mitigate these adverse effects (Mthembu et al., 2025). Available evidence supports the vital role of CoQ_{10} within the mitochondrial electron transport chain, while also encoring its role as an essential antioxidant that can neutralize the detrimental effects of oxidative stress (Mthembu et al., 2023; Fitzgerald et al., 2012; Cirilli et al., 2021; Littarru and Tiano, 2007; Marcoff and Thompson, 2007).

The current study examines the concentration- and time-dependent effects of simvastatin on $CoQ_{9/10}$ status, mitochondrial respiratory capacity, and cardiac cell viability. It aims to provide insights into the long-term impact of statins on myocardial health, highlighting the need for strategies to optimize statin therapy while minimizing potential adverse outcomes. By linking the mechanistic understanding of statin-induced oxidative stress with clinical concerns, this study emphasizes the importance of revising therapeutic approaches to better manage cardiovascular risks associated with T2D and dyslipidemia.

2. Methods

2.1. Cell culture conditions for H9c2 cardiomyoblasts

The H9c2 cardiomyoblasts, rat ventricular-derived heart cells, were purchased from the American Type Culture Collection (Manassas, VA, USA; catalog number: CRL-1446). These H9c2 cardiomyoblasts were cultured in Dulbecco's modified Eagle medium (Lonza, MD, USA), (BE12-604F) supplemented with 10 % fetal bovine serum (10493106) purchased from Thermo Fisher Scientific (Waltham, MA, USA) at standard tissue culture conditions (37 °C, in humidified air and 5 % CO_2) (Mthembu et al., 2024; Dludla et al., 2020a). Cells were regularly sub-cultured at a confluency of 80–90 % and seeded in 96-well or 6-well plates at a density of 5×10^4 cells/mL. For the seahorse experiments, cells were seeded in 96-cell culture XF-96 microplate plates at the density of 12,000 cells/80 μL. To test the concentration- and time-dependent effects of simvastatin, ≥97 % high-performance liquid chromatography (HPLC), (S6196-25MG, Sigma–Aldrich St. Louis, MO, USA), H9c2 cardiomyoblasts were exposed to different concentrations of simvastatin (0.3, 0.6, 1.25, 5, 10, and 20 μM) at varied time intervals, including 24, 48 and 72 h. This selection of concentrations and duration of treatment with simvastatin was based on previous/published research (Lusha and Jiang, 2020; Marcheggiani et al., 2019). The rationale for including concentrations beyond therapeutic plasma levels (20 μM) is to simulate the potential effects of prolonged or higher-dose statin therapy, which may be relevant in patients with severe dyslipidemia or those receiving high-dose statin prescriptions. Briefly, simvastatin was dissolved in 100 % dimethyl sulfoxide (DMSO), (D8418, Sigma–Aldrich St. Louis, MO, USA), and the highest working concentration (20 μM simvastatin) contained approximately 0.042 % DMSO. To ensure consistency across all experimental conditions, the control group was also exposed to 0.042 % DMSO.

2.2. Coenzyme $Q_{9/10}$ quantification and assessment of its oxidation status

The concentration- and time-dependent effects of simvastatin on $CoQ_{9/10}$ levels, including both endogenous content and oxidative status, in cultured H9c2 cardiomyoblasts, were quantified using a method that has already been described (Silvestri et al., 2015; Dludla et al., 2019; Sekine et al., 2005). Briefly, cells (~200,000 cells) were extracted using isopropanol (34,863, Sigma–Aldrich St. Louis, MO, USA). Thereafter, the cell suspension was injected into an HPLC with an electrochemical detector (Shiseido Co. Ltd.; Tokyo, Japan). Post-separation reducing column (Shiseido CQR) was used, to elute ubiquinone from the cell suspension referencing the $CoQ_{9/10}$ standards. To further minimize the risk of CoQ9/10 oxidation, all samples were processed on ice to maintain stability. Additionally, all solutions and equipment were thoroughly prepared to avoid any potential sources of oxidative stress. Protein content, to normalize the $CoQ_{9/10}$ content, was quantified using Bradford Assay (#5000201, Bio-Rad Laboratories, CA, USA). Results for $CoQ_{9/10}$ content are expressed as $CoQ_{9/10}$ levels/ protein content (μg/mg). Whilst $CoQ_{9/10}$ oxidation was expressed as a percentage of the ubiquinone/total $CoQ_{9/10}$ in H9c2 cardiomyoblasts, as previously described (Dludla et al., 2019).

2.3. Determination of mitochondrial respiration status

Mitochondrial respiration was measured using the Mito Stress Kit and XF-96 Extracellular Flux Analyser (103015–100, Seahorse, Bioscience, MA, USA), following a method that has already been described (Mazibuko-Mbeje et al., 2021). This encompassed measurements for both oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Briefly, after the addition of 10 mM glucose and 1 mM pyruvate substrates, OCR (pmol/min) and ECAR were simultaneously measured by injecting 1 μM oligomycin (ATP-synthase inhibitor), 0.75 μM carbonyl cyanide-p-trifluoromethoxy phenylhydrazone (FCCP, a mitochondrial uncoupler), and 5 μM rotenone (complex I inhibitor) plus antimycin A (complex III inhibitor) at specified time points. Protein content was quantified using Bradford Assay (Bio-Rad Laboratories, CA, USA), to normalize the OCR data, as reported in absolute rates (pmol/min/mg protein). All Seahorse reagents were bought from Agilent Technologies (Santa Clara, CA, USA).

2.4. Evaluation of mitochondrial and cytosolic ROS production

Mitochondrial and cytosolic ROS production were assessed by staining cells with fluorescent dyes; MitoSOX Red (mitochondrial superoxide indicators) from Thermo Fisher Scientific (M36008, MA, USA), and 2′, 7′-dichlorodihydrofluorescin diacetate (DCFH-DA) from Cell biolab (STA-342, San Diego, USA), respectively, following the manufacturer's instructions. Fluorescence was measured using BD Accuri® C6 flow cytometer (Becton Dickinson, NJ, USA). Most importantly, the analysis of mitochondrial and cytosolic ROS production was done together with cell viability assay using Via Count and this was based on single-cell analysis for optimal estimation of ROS in viable cells. This assay was performed using a method previously described (Dludla et al., 2019).

2.5. Measurement of metabolic activity using MTT assay

To measure the cell viability, a colorimetric assay that measures the reduction of tetrazolium dye (MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to its insoluble formazan product was used as previously described (Mosmann, 1983). Briefly, H9c2

cardiomyoblasts were exposed to different concentrations of simvastatin before the addition of 2 mg/ml of MTT solution (475,989, Sigma–Aldrich St. Louis, MO, USA) in Dulbecco's Phosphate-Buffered Saline (DPBS), (BE17-512F) from Lonza BioWhittaker, Walkersville, MD, USA) and incubation for 30 min at 37 °C in 5 % CO_2. Subsequently, 200 μM DMSO (Sigma–Aldrich St. Louis, MO, USA) and 25 μM Sorenson's glycine buffer (glycine 0.1 M, NaCl 0.1 M, pH:10.5 with 0.1 NaOH), ingredient purchased from Sigma–Aldrich (St. Louis, MO, USA) were added to dissolve the formazan, and then absorbance was read at 570 nm, with a SpectraMax i3x multi-mode microplate reader (Molecular Devices, CA, USA).

2.6. Statistical analysis

Data was expressed as the mean ± standard error of the mean (SEM). Results for all experiments are of at least three independent experimental repeats, with each having at least three technical replicates. Statistical analysis was performed using GraphPad Prism software version 8.0.1 (GraphPad Software, Inc., La Jolla, CA, USA). Comparisons between groups were performed using one-way multivariate ANOVA, followed by Tukey post-hoc test, or two-way multivariate ANOVA, followed by Dunnett's multiple comparison tests with $p < 0.05$ considered significant.

3. Results

3.1. Prolonged exposure to simvastatin promotes the oxidation status of $CoQ_{9/10}$ in cardiomyoblasts

Initially, we assessed the effect of simvastatin on the endogenous levels and oxidative status of $CoQ_{9/10}$ (Fig. 1A-D). Our data revealed that within just 24 h of exposure to simvastatin in H9c2 cardiomyoblasts, the endogenous levels of $CoQ_{9/10}$ significantly decreased from a simvastatin concentration of ≥0.3 μM ($p < 0.05$) when compared to the experimental control (Fig. 1A-B). These results may signal an effective therapeutic response by statins to combat the induced stress, as this decrease persisted even at 48 and 72 h in all tested concentrations of simvastatin ($p < 0.01$ and $p < 0.001$), respectively. However, inconclusive outcomes were noted for the concentration of 10 μM, which did not display a statistically significant reduction in $CoQ_{9/10}$ levels at 48 h (Fig. 1A-B). The oxidation status of CoQ_9 was affected by time, with increased oxidation of this cofactor seen from 0.6 μM at 24 h ($p < 0.001$), 48 h ($p < 0.05$), and 72 h ($p < 0.01$), respectively (Fig. 1C). This may signal that the oxidation of this co-factor may be a strong signal to indicate the therapeutic effect of drugs compared to measuring its endogenous levels. Notably, a similar effect was observed where all tested concentrations of simvastatin completely oxidized the CoQ_{10} status, especially for the time points of 48 and 72 h ($p < 0.001$) (Fig. 1D).

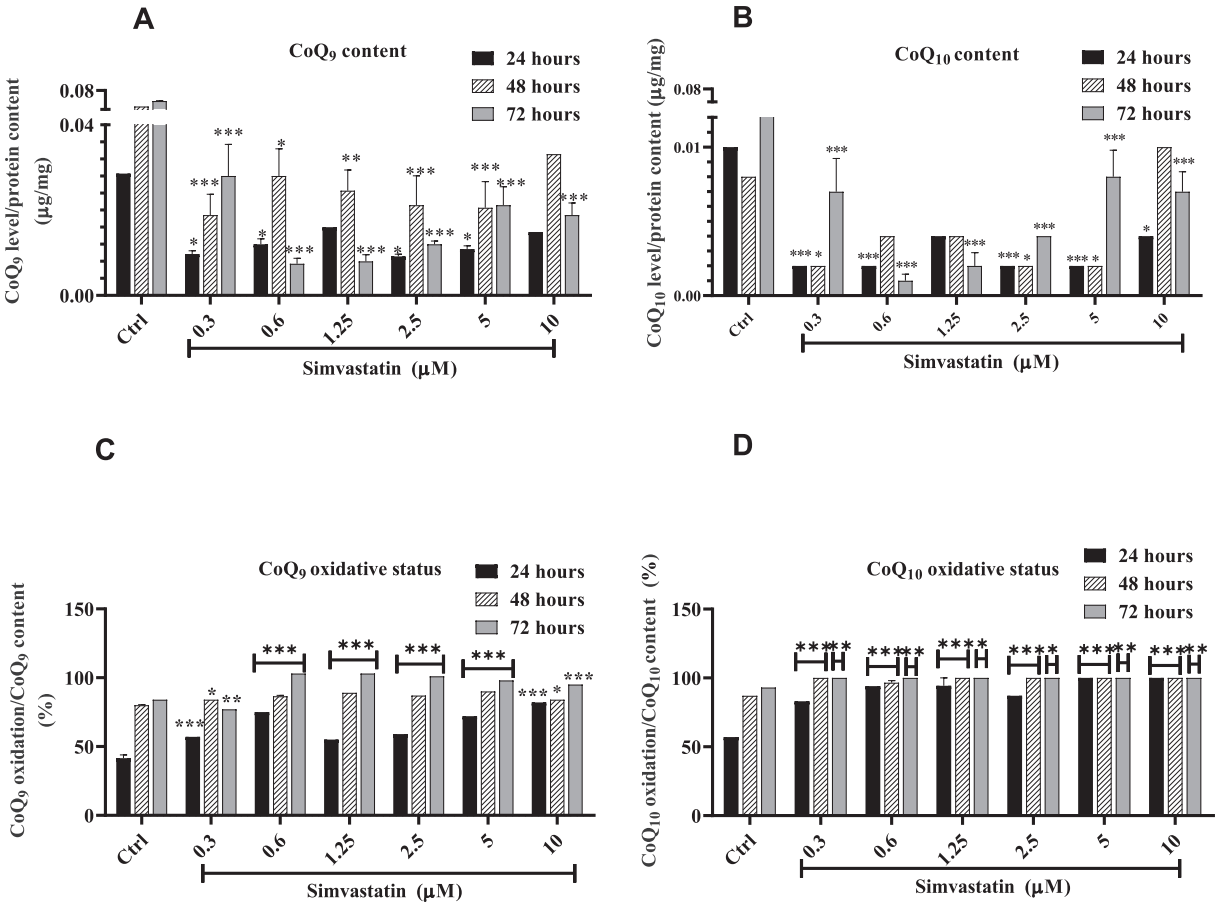


Fig. 1. Concentration and time-dependent effects of simvastatin on endogenous coenzyme $Q_{9/10}$ ($CoQ_{9/10}$) levels and its oxidation status. Briefly, H9c2 cardiomyoblasts were exposed to different concentrations of simvastatin (0.3, 0.6, 1.25, 5, 10, and 20 μM) for various time intervals, 24, 48 and 72 h. Subsequently, endogenous $CoQ_{9/10}$ levels were quantified (A and B), as well as the oxidation status (C and D). Results are expressed as the mean ± standard error of the mean (SEM) of three independent experiments, relative to the experimental control. Comparisons between groups were performed using two-way multivariate ANOVA, followed by Dunnett's multiple comparison test, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ versus the experimental control (Ctrl).

3.2. Simvastatin does not affect mitochondrial respiration and glycolytic energy levels of cardiomyoblasts after 24 h of exposure

To evaluate the effect of simvastatin on mitochondrial respiration, we have measured the mitochondrial oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), following the treatment with various concentrations of simvastatin at different time points for 24 h (Fig. 2A-B). Our data demonstrated that 24 h-exposure to simvastatin did not have any significant effect on basal respiration, maximal respiration, and ATP production, respectively (Fig. 2C-E). This is likely to reflect the therapeutic effect of simvastatin within a short-term treatment period. However, simvastatin increased the mitochondrial proton leak from 1.25 μM ($p < 0.05$), 2.5 μM ($p < 0.05$), 5 μM ($p < 0.001$), 10 μM ($p < 0.01$), and 20 μM ($p < 0.001$) when compared to the experimental control (Fig. 2F). Furthermore, simvastatin did not affect non-mitochondrial respiration and spare capacity after 24 h of exposure (Fig. 2G-H).

3.3. Simvastatin has a marginal effect on mitochondrial respiration and glycolytic energy levels of cardiomyoblasts after 48 h of exposure

Next, we evaluated the effect of simvastatin on mitochondrial respiration by measuring the OCR and ECAR parameters, following the treatment with various concentrations of simvastatin for 48 h (Fig. 3A-B). The results showed that all concentrations of simvastatin did not have any effect on basal respiration but suppressed the maximal respiration after 48-h exposure at concentrations starting from 2.5 μM ($p < 0.05$), to 5 μM ($p < 0.01$), 10 μM ($p < 0.01$) and 20 μM ($p < 0.05$) when compared to the experimental control (Fig. 3D). A similar effect was observed, where the highest simvastatin concentration of 20 μM ($p < 0.05$) significantly reduced ATP production in comparison to the experimental control (Fig. 3E). Proton leak and nonmitochondrial respiration parameters were not significantly affected in response to simvastatin exposure (Fig. 3F-G). However, simvastatin reduced spare respiratory capacity, for the concentrations 2.5 μM ($p < 0.05$), 5 μM (p

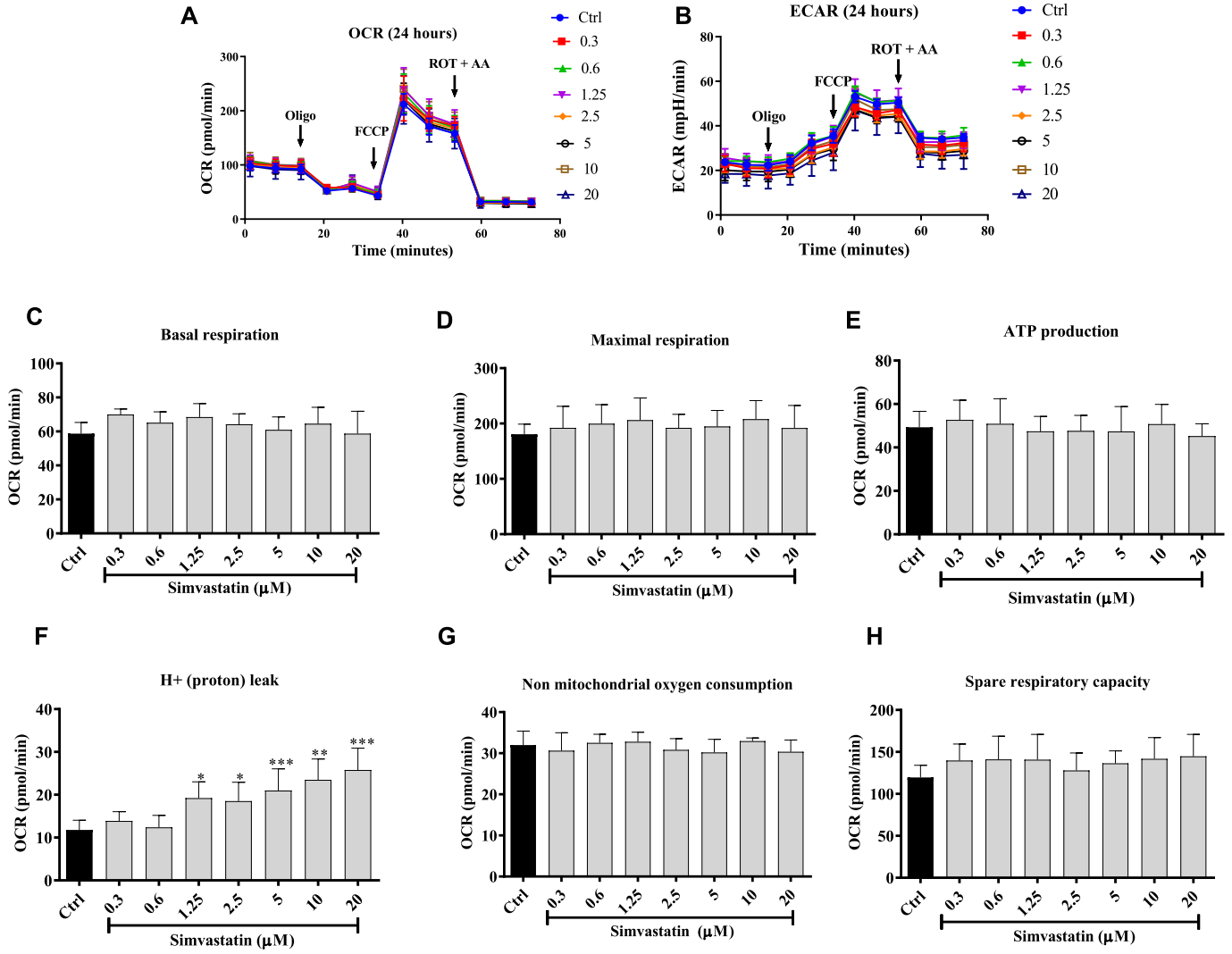


Fig. 2. Concentration- and time-dependent effects of simvastatin on mitochondrial respiration and glycolytic energy levels in cultured H9c2 cardiomyoblasts after 24 h. Briefly, H9c2 cardiomyoblasts were exposed to different concentrations of simvastatin (0.3, 0.6, 1.25, 5, 10, and 20 μM) for 24 h. Specifically, 10 mM glucose and 1 mM pyruvate were utilized as substrates. Inhibitors, including 1 μM oligomycin (Oligo), 0.75 μM carbonyl cyanide-p-trifluoromethoxy phenylhydrazone (FCCP), and a combination of 5 μM rotenone (ROT) with antimycin A (AA), were added sequentially at specified time points to assess different aspects of mitochondrial respiration. The graphs show the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) for all treatments (A and B). With others depicting basal respiration (B), maximal respiration (C), ATP production (E), proton leak (F), nonmitochondrial respiration (G), and spare respiratory capacity (H), respectively. Results are expressed as the mean ± standard error of the mean (SEM) of at least three independent experiments, with at least six repeats, relative to the experimental control. Comparisons between groups were performed using one-way multivariate ANOVA, followed by a Tukey post-hoc test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the experimental control (Ctrl).

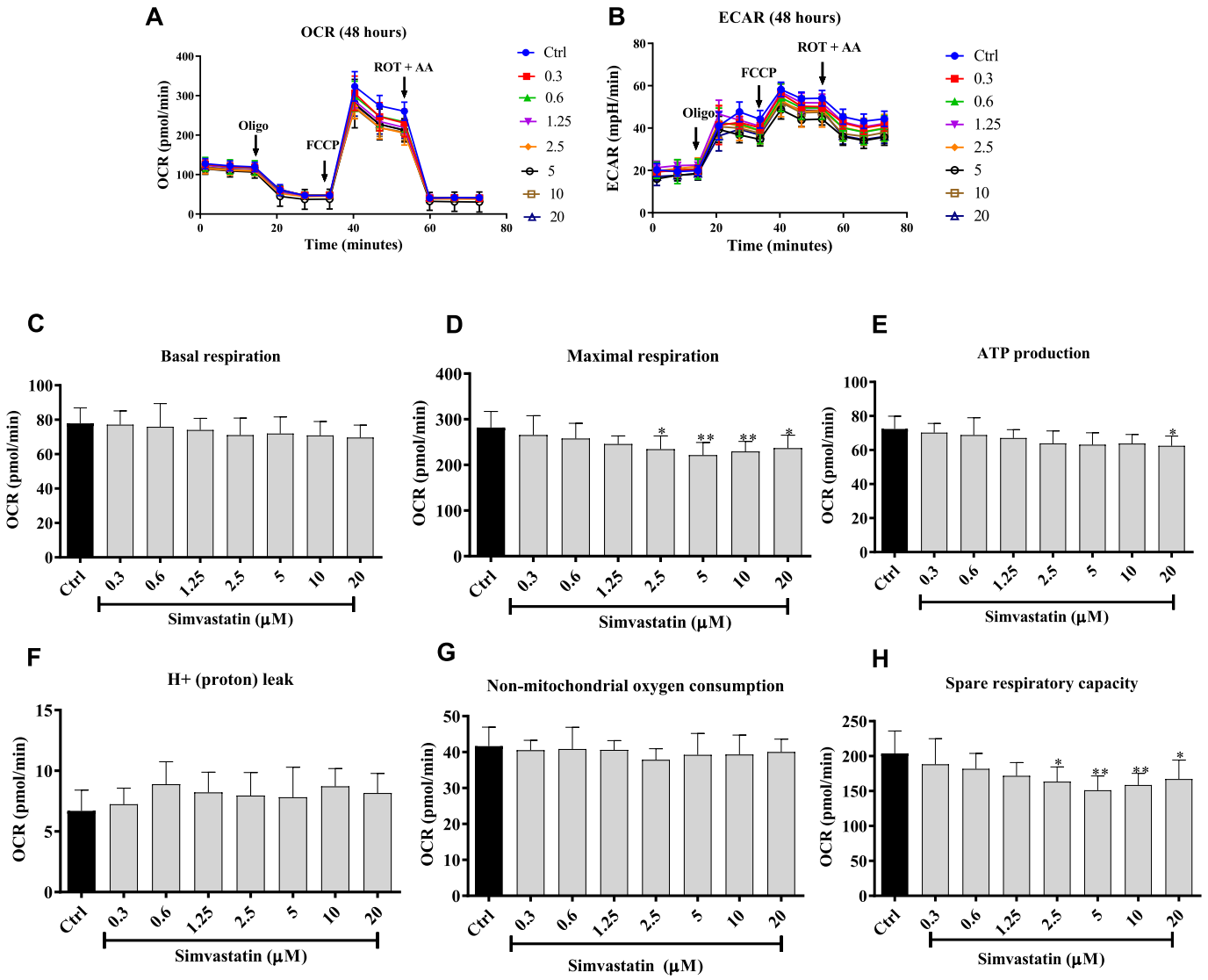


Fig. 3. Concentration- and time-dependent effects of simvastatin on mitochondrial respiration and glycolytic energy levels in cultured H9c2 cardiomyoblasts after 48 h. Briefly, H9c2 cardiomyoblasts were exposed to different concentrations of simvastatin (0.3, 0.6, 1.25, 5, 10, and 20 μM) for 48 h. Specifically, 10 mM glucose and 1 mM pyruvate were utilized as substrates. Inhibitors, including 1 μM oligomycin (Oligo), 0.75 μM carbonyl cyanide-p-trifluoromethoxy phenylhydrazone (FCCP), and a combination of 5 μM rotenone (ROT) with antimycin A (AA) were added sequentially at specified time points to assess different aspects of mitochondrial respiration. The graphs show the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) for all treatments (A and B). With others depicting basal respiration (B), maximal respiration (C), ATP production (E), proton leak (F), non-mitochondrial respiration (G), and spare respiratory capacity (H), respectively. Results are expressed as the mean ± standard error of the mean (SEM) of at least three independent experiments, with at least six repeats, relative to the experimental control. Comparisons between groups were performed using one-way multivariate ANOVA, followed by a Tukey post-hoc test, $*p < 0.05$, $**p < 0.01$ versus the experimental control (Ctrl).

< 0.01), 10 μM ($p < 0.01$), and 20 μM ($p < 0.05$) (Fig. 3H), and this was accompanied by reduced glycolytic energy levels (Fig. 3B). Reduced glycolytic energy levels may lead to various cellular dysfunctions, including impaired energy production.

3.4. High concentrations of simvastatin affect mitochondrial respiration and glycolytic energy levels of cardiomyoblasts after 72 h

Prolonged exposure to simvastatin (72 h), especially high concentrations (≥ 10 μM), resulted in reduced OCR (Fig. 4A) and ECAR (Fig. 4B). These results were corroborated by suppressed basal respiration (Fig. 4C) and maximal respiration (Fig. 4D) at higher concentrations ≥10 μM ($p < 0.05$). While only 20 μM ($p < 0.001$) was able to reduce ATP production (Fig. 4E). Interestingly, after 72 h, simvastatin reduced the proton leak (when compared to the experimental control) at concentrations of 5 μM ($p < 0.05$) and 20 μM ($p < 0.05$) (Fig. 4F). No significant effects were observed with nonmitochondrial respiration (Fig. 4G), but higher concentrations, starting from ≥10 μM ($p < 0.05$) and 20 μM ($p < 0.01$) of simvastatin, were able to reduce the spare respiratory capacity (Fig. 4H). Consistent results were seen with glycolytic energy levels, which were suppressed as indicated by the reduced ECAR when compared to the experimental control (Fig. 4B). These results are consistent with enhanced oxidation status of $CoQ_{9/10}$ in cardiomyoblasts seen after prolonged exposure to simvastatin (Figure 1).

3.5. Simvastatin promotes mitochondrial reactive oxygen species (ROS) production in cardiomyoblasts

We further evaluated the concentration- and time-dependent effects of simvastatin on mitochondrial ROS production after 24, 48, and 72 h in H9c2 cardiomyoblasts (Fig. 5A-C). Our data showed that all tested concentrations of simvastatin (0.3 μM ($p < 0.05$), 0.6 μM ($p < 0.05$),

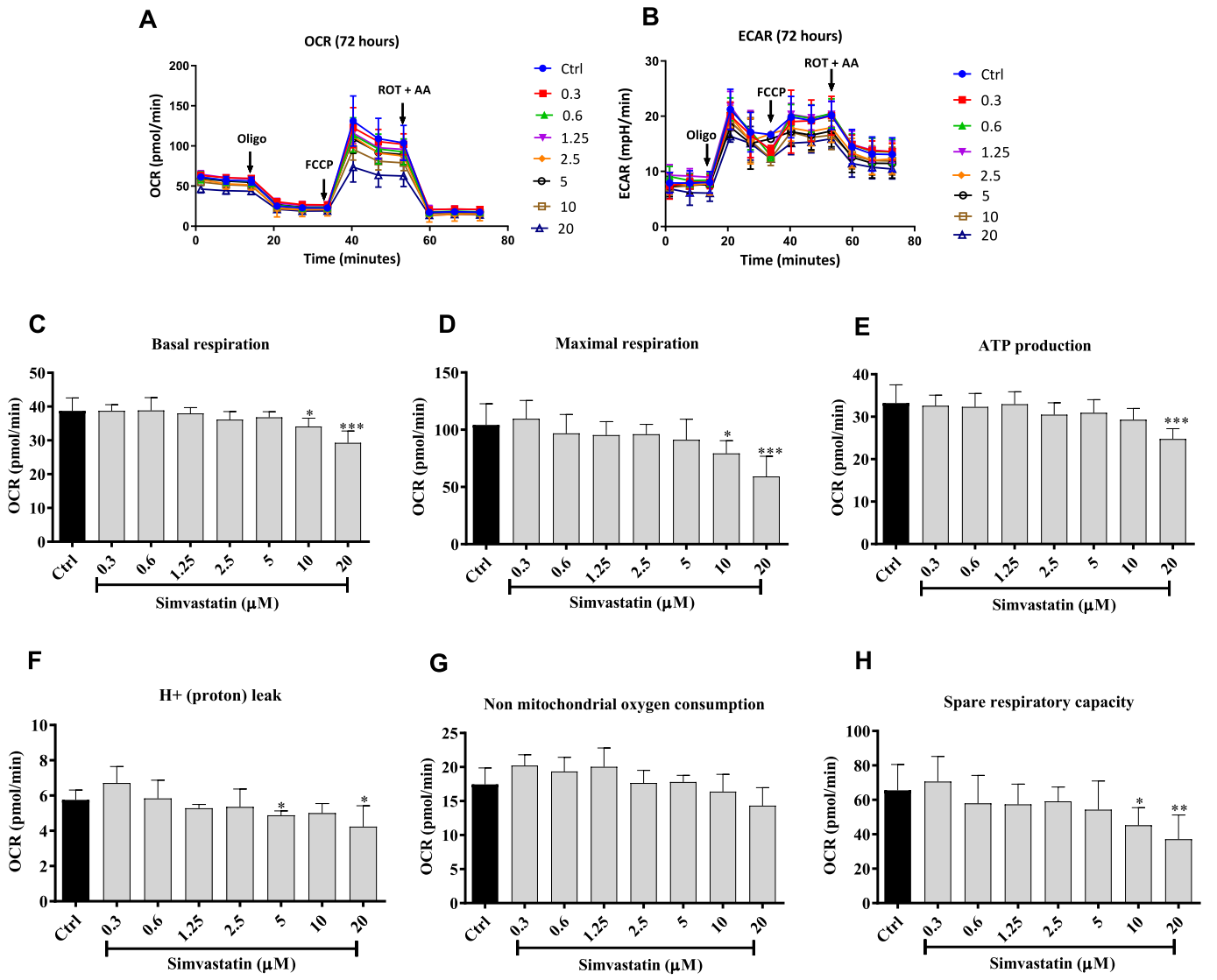


Fig. 4. Concentration- and time-dependent effects of simvastatin on mitochondrial respiration and glycolytic energy levels in cultured H9c2 cardiomyoblasts after 72 h. Briefly, H9c2 cardiomyoblasts were exposed to different concentrations of simvastatin (0.3, 0.6, 1.25, 5, 10, and 20 μM) for 72 h. Specifically, 10 mM glucose and 1 mM pyruvate were utilized as substrates. Inhibitors, including 1 μM oligomycin (Oligo), 0.75 μM carbonyl cyanide-p-trifluoromethoxy phenylhydrazone (FCCP), and a combination of 5 μM rotenone (ROT) with antimycin A (AA), were added sequentially at specified time points to assess different aspects of mitochondrial respiration. The graphs show the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) for all treatments (A and B). With others depicting basal respiration (B), maximal respiration (C), ATP production (E), proton leak (F), non-mitochondrial respiration (G), and spare respiratory capacity (H), respectively. Results are expressed as the mean ± standard error of the mean (SEM) of at least three independent experiments, with at least six repeats, relative to the experimental control. Comparisons between groups were performed using one-way multivariate ANOVA, followed by a Tukey post-hoc test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, versus the experimental control (Ctrl).

1.25 μM ($p < 0.001$), 2,5 μM ($p < 0.001$), 5 μM ($p < 0.001$), 10 μM ($p < 0.001$) to 20 μM ($p < 0.001$), respectively) significantly increased ROS production at 24 h when compared to the experimental control (Fig. 5A). This effect persisted after 48 h, with simvastatin concentrations from 0.3 μM ($p < 0.05$), 0.6 μM ($p < 0.01$), 1.25 μM ($p < 0.001$), 2,5 μM ($p < 0.001$), 5 μM ($p < 0.001$), 10 μM ($p < 0.001$) to 20 μM ($p < 0.001$) showing elevated mitochondrial ROS production compared to experimental control (Fig. 5B). Similarly, exposure to simvastatin for 72 h also resulted in a significant increase in ROS production at concentrations from 0.3 μM ($p < 0.01$), 0.6 μM ($p < 0.001$), 1.25 μM ($p < 0.01$), 2,5 μM ($p < 0.05$), 5 μM ($p < 0.05$), 10 μM ($p < 0.05$) and 20 μM ($p < 0.001$) (Fig. 5C). The prolonged exposure to simvastatin at 72 h proved too toxic to the cells, as seen with a low number of viable cells at concentrations >0.6 μM. Again, these results were consistent with enhanced oxidation status of $CoQ_{9/10}$ in cardiomyoblasts seen after prolonged exposure to simvastatin (Figure 1).

3.6. Simvastatin promotes cytosolic reactive oxygen species (ROS) production in cardiomyoblasts

To evaluate the effects of simvastatin on cytosolic ROS production, cells were stained with DCFH-DA fluorescent dye (Fig. 6). We observed that all tested concentrations of simvastatin (0.3, 0.6, 1.25, 5, 10, and 20 μM) significantly decreased the number of cells producing low ROS when compared to the experimental control, which occurred in a concentration-dependent manner at all time points (24, 48 and 72 h), ($p < 0.001$, $p < 0.001$ and $p < 0.01$) respectively, as shown in Fig. 6A-C. This was complementary with a significant increase in the number of cells producing high ROS in all time points, 24, 48, and 72 h ($p < 0.001$, $p < 0.001$, and $p < 0.01$), which also occurred in a concentration-dependent manner (Fig. 6A-C). The time-dependent effect in increasing the number of cells with high ROS was higher for 72 h compared to other time points.

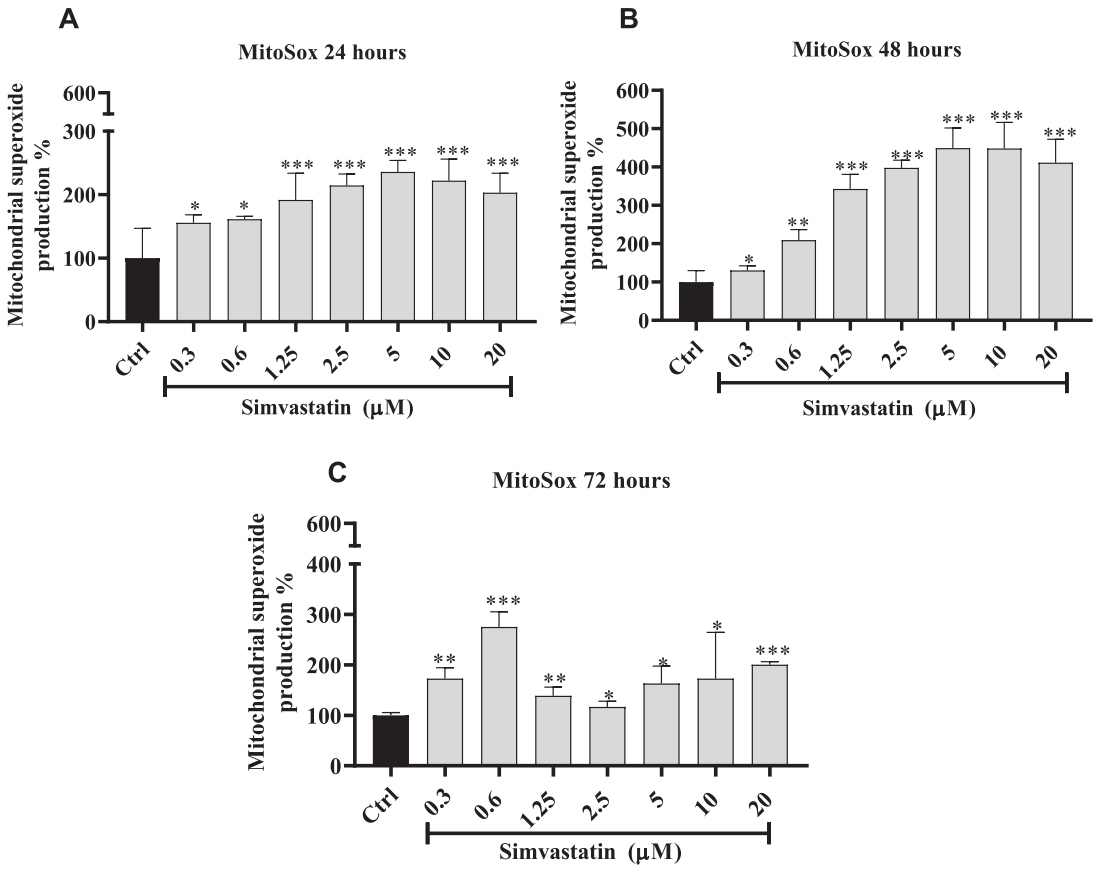


Fig. 5. Concentration- and time-dependent effects of simvastatin on mitochondrial reactive oxygen species (ROS) production in cultured H9c2 cardiomyoblasts. Briefly, H9c2 cardiomyoblasts were exposed to different concentrations of simvastatin (0.3, 0.6, 1.25, 5, 10, and 20 μM) for 24, 48, and 72 h. MitoSox (mitochondrial superoxide indicators) fluorescent stain was used for the detection of mitochondrial superoxide production. Results are expressed as the mean ± standard error of the mean (SEM) of three independent experiments, relative to the experimental control. Comparisons between groups were performed using one-way multivariate ANOVA, followed by a Tukey post-hoc test. *$p < 0.01$, **$p < 0.01$, ***$p < 0.001$ versus experimental control (Ctrl).

3.7. Concentration- and time-dependent effects of simvastatin on the viability of cardiomyoblasts

Cell viability was measured using an MTT assay. H9c2 cardiomyoblasts were exposed to different concentrations of simvastatin (0.3, 0.6, 1.25, 5, 10, and 20 μM) for 24, 48, and 72 h. Results showed a concentration-dependent reduction of cell viability by simvastatin in all time points (Fig. 7A). However, at 24 and 48 h, only 20 μM showed statistical significance ($p < 0.01$). Whereas 72 h of exposure to simvastatin at concentrations ≥2.5 μM significantly reduced cell viability by $p < 0.05$ (0.25 μM) and $p < 0.001$ (5–20 μM), respectively.

4. Discussion

Statins are widely known for their ability to reduce cardiovascular events and mortality among individuals with dyslipidemia at high risk of CVDs (Lim, 2013). These therapies act by inhibiting an enzyme called 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase, which reduces the endogenous production of cholesterol (Stancu and Sima, 2001; Liao and Laufs, 2005). However, apart from lowering cholesterol levels, the inhibition of HMG-CoA reductase can lead to the depletion of several cholesterol-based compounds, such as sex hormones, bile acid, cholecalciferol, and CoQ_{10} (Fitzgerald et al., 2012; Hussain et al., 2023; Csengo et al., 2025). Importantly, research from the Multi-Ethnic Study of Atherosclerosis (MESA) highlights a concerning trend: statin users show significantly reduced levels of sex hormone-binding globulin (SHBG) and dehydroepiandrosterone (DHEA), this underscores the complex impact of statins on our hormonal health (Oluleye et al., 2019). In fibroblast-like synoviocytes, 5 to 10 μM lovastatin was found to cause cell death, impair cellular proliferation, and decrease the cellular viability (Wu et al., 2024). CoQ_{10}, a critical component of the mitochondrial electron transport chain and a potent antioxidant, is often depleted by statin use, potentially contributing to myopathy and cardiotoxicity (Vinci et al., 2021; Chaulin, 2023). As a result, CoQ_{10} supplementation has been proposed as a therapeutic strategy to counteract statin-induced myopathy and cardiovascular side effects (Hashim et al., 2025). The reviewed literature has shown that CoQ_{10} can restore mitochondrial function and reduce oxidative stress, suggesting its potential to improve patient outcomes in those receiving statin therapy (Mthembu et al., 2023; Górski et al., 2025). However, the efficacy of CoQ_{10} supplementation, particularly in the context of long-term statin use, to determine whether it can reverse or alleviate the proposed pathological consequences remains a point of interest. Considering the high concentration of mitochondria in cardiac cells and the crucial role of CoQ_{10} in mitochondrial energy production, it is imperative to investigate the potential impact of prolonged statin use.

Our study aimed to explore the impact of prolonged exposure to simvastatin on $CoQ_{9/10}$ levels and mitochondrial function in H9c2 cardiomyoblasts, a well-established experimental model for studying cardiac physiology and pathology (Hescheler et al., 1991). Previously, we established that exposing cultured cardiac cells, H9c2 cardiomyoblasts, to different concentrations of palmitate negatively affects cellular responses and molecular mechanisms associated with $CoQ_{9/10}$ dysregulation (Mthembu et al., 2024; Dludla et al., 2020b). For example, low levels of palmitic acid can improve cardiac cellular responses by improving mitochondrial respiratory function and attenuating oxidative

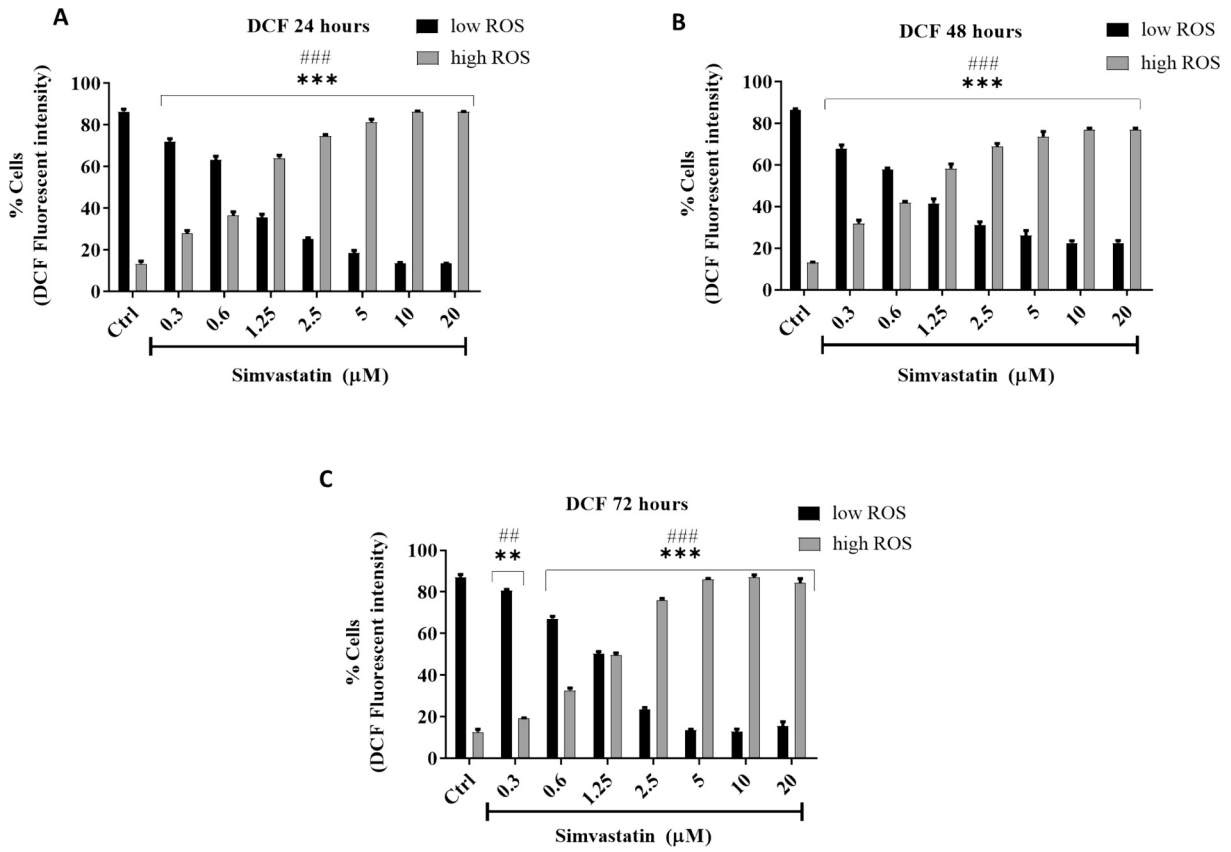


Fig. 6. Concentration- and time-dependent effects of simvastatin on cytosolic reactive oxygen species (ROS) production in cultured H9c2 cardiomyoblasts. Briefly, H9c2 cardiomyoblasts were exposed to different concentrations of simvastatin (0.3, 0.6, 1.25, 5, 10, and 20 μM) for 24, 48, and 72 h. 2′, 7′-dichlorodihydrofluorescin diacetate (DCFH-DA) fluorescent stain was used for the detection of cytosolic low (minimal oxidative stress) and high (elevated oxidative stress) ROS production. Results are expressed as the mean ± standard error of mean (SEM) of three independent experiments, relative to the experimental control. Comparisons between groups were performed using one-way multivariate ANOVA, followed by a Tukey post-hoc test, $^{**}p < 0.01$, $^{***}p < 0.001$ versus experimental control (Ctrl) for low ROS and $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus experimental Ctrl for high ROS.

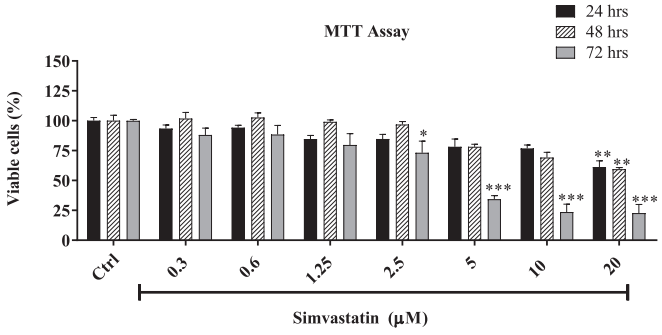


Fig. 7. Concentration- and time-dependent effects of simvastatin on the cell viability in H9c2 cardiomyoblasts. Briefly, H9c2 cardiomyoblasts were exposed to different concentrations of simvastatin (0.3, 0.6, 1.25, 5, 10, and 20 μM) for 24, 48, and 72 h, followed by cell viability evaluation using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay. Results are represented as the mean ± standard error of the mean (SEM) of three independent experiments, with at least six technical repeats per experiment relative to the experimental control (Ctrl). Comparisons between groups were performed using two-way multivariate ANOVA, followed by Dunnett's multiple comparison test, *p < 0.05, **p < 0.01, ***p < 0.001 versus the experimental control.

stress-induced cellular damage (Mthembu et al., 2024), However, prolonged exposure to this saturated fatty acid is consistent with the suppression of endogenous levels of $CoQ_{9/10}$ and the accelerated generation of damaging free radical species (Dludla et al., 2020b). In fact, beyond the detrimental effects of palmitic acid, it has remained significant to understand whether prolonged exposure to simvastatin has any impact on $CoQ_{9/10}$ levels and whether this has any relationship with impaired activity of the mitochondrial electron transport chain or oxidative stress-induced cellular damage. Although the direct link between palmitate metabolism and simvastatin toxicity is not yet well-defined, both factors can significantly impact CoQ_9 and CoQ_{10} regulation, as well as mitochondrial health (Dludla et al., 2020b; Deichmann et al., 2010). Investigating these connections will be relevant to identifying potential therapeutic targets and interventions to alleviate cardiac dysfunction in the future.

Studies in both human and animal models have demonstrated the pleiotropic effects of statins across a concentration range of 1 to 50 μmol l^{-1} (Björkhem-Bergman et al., 2011; Bu et al., 2010). Our research similarly found that exposure to low concentrations of simvastatin (<0.125 μM) did not significantly affect mitochondrial respiration and cellular viability at 24 and 48 h. However, exposure to higher concentrations of simvastatin (>0.3 μM) caused a significant decrease in $CoQ_{9/10}$ levels as early as 24 h, persisting at 48 and 72 h. This decrease was accompanied by increased oxidation of $CoQ_{9/10}$, likely indicating an impaired mitochondrial respiratory process, prominently seen after 72 h of exposure to simvastatin. Interestingly, at 72 h, elevated levels of simvastatin (>0.5 μM) suppressed H^+ (proton) leak below the baseline level of normal cells, suggesting that while the mitochondrial membrane may be tighter, the overall mitochondrial function could still be compromised. This impact was linked with decreased maximal respiration and spare capacity after 48 h, with a more pronounced effect

observed at 72 h, particularly with high concentrations of simvastatin (>0.5 μM), coinciding with decreased basal respiration and ATP production. Reviewed evidence by discussing the similar effects, exposure to statins resulted in mitochondrial impairments, which were correlated with reduced CoQ_{10} levels (Somers et al., 2023; Kucharská et al., 2024). Notably, most studies focused primarily on muscle tissues (Ryan et al., 2024), however, our study extends this observation to cardiac cells, revealing a more pronounced effect on mitochondrial respiration and ATP production in the heart after long-term statin exposure. In fact, the measurement of mitochondrial ATP production and oxygen consumption in response to energy demands serves as a reliable hallmark of its functional state, reflecting cell viability (Smolina et al., 2017). Indeed, decreased ATP production and restricted spare respiratory capacity in cardiac cells reflect overall mitochondrial dysfunction often associated with cellular damage, which subsequently leads to heart failure (Sharov et al., 2000).

Moreover, maintaining proton leak at optimal levels is crucial for reducing excessive ROS generation and enhancing cellular function, including that of cultured cardiac cells (Cadenas, 2018). Improved mitochondrial oxidative capacity is essential to control or even limit the excessive production of toxic ROS within many cellular experiments (Nanayakkara et al., 2019; Qi et al., 2023). In our experimental model, it was obvious that all time points assessed could elevate ROS production for all concentrations of simvastatin. This is expected because all cells require ROS production for normal cellular function (Sies et al., 2022). However, it is the abnormal elevation of these toxic molecules that is a cause for concern (Panth et al., 2016; Scribano et al., 2014). Here, we saw that a simvastatin concentration $\geq$ 1.25 μM was sufficient to promote abnormal production of ROS in these cells, which was even escalated for 72 h. Our results are consistent with research indicating that statins, depending on the concentration used and even the treatment time, may interfere with mitochondrial function and oxidative imbalance, which may lead to enhanced cellular toxicity (Marcheggiani et al., 2019; Bell et al., 2024). Interestingly, recent evidence has indicated that prolonged exposure to simvastatin can activate pro-apoptotic mechanisms that promote cell death, and this is even considered in cancer research (Borahay et al., 2014; Kim et al., 2014; Kafka et al., 2022). However, there is still an evidence gap in understanding the pathological consequences of exposing cardiac cells to elevated concentrations of simvastatin, especially over the long term.

Nonetheless, we have found that higher concentrations of simvastatin (>5 μM) resulted in reduced viability of cardiomyoblasts, predominantly seen after 72-h exposure. These results validate the potential detrimental effects of prolonged exposure of the myocardium to elevated concentrations of statins. These results are consistent with some clinical findings (Zhou et al., 2020) and are increasingly reviewed elsewhere (Ward et al., 2019). Importantly, these results corroborate our hypothesis that preserving the oxidative status of CoQ_{10} is vital to strengthening intracellular responses and improving cellular function, as this was significantly affected in our H9c2 experimental model. These findings are aligned with the literature, it has been reported that exposing H9c2 cells to 10 μM simvastatin for 24 h could interfere with normal cellular function by impairing mitochondrial oxidative capacity, likely through the depletion of intracellular CoQ_{10} content (Bonifacio et al., 2016).

Our findings highlight the critical role of $CoQ_{9/10}$ in mitochondrial oxidative phosphorylation, in agreement with previously published evidence (Crane, 2001). These results suggest that $CoQ_{9/10}$ deficiency contributes to statin-induced mitochondrial dysfunction. These discoveries highlight the vital function of $CoQ_{9/10}$ in cellular viability, acting as a key antioxidant in protecting cell membranes from oxidative damage (López-Lluch, 2019). Furthermore, these findings highlight that high concentrations of simvastatin are likely to provoke myocardial injury and cardiotoxicity through pathways involving oxidative stress, as increasingly hypothesized (Mthembu et al., 2023; Cheng et al., 2017; Marcheggiani et al., 2021; Zhang et al., 2022). However, additional evidence is necessary to comprehensively elucidate the precise molecular mechanisms underlying statin-induced impairment of the myocardium. The current study provides an important foundation to determine the potential role of statins in driving oxidative stress-induced cardiac damage through reduced endogenous levels of $CoQ_{9/10}$. While many studies have suggested that CoQ_{10} supplementation may help mitigate these effects (Fogacci et al., 2024; Heshmat-Ghahdarijani et al., 2024), the current study presents the need for alternative or complementary therapies, as we found that high concentrations of simvastatin (>5 μM) led to cellular toxicity and reduced cell viability. Importantly, the study is not without limitations, as such evidence needs to be confirmed in well-organized in vivo and clinical evidence of dyslipidemia. The establishment of implicated molecular mechanisms, especially those targeting the mevalonate pathway, may add another dimension to understanding the consequences of exposing cardiac cells to prolonged and elevated concentrations of simvastatin and possibly identify novel therapeutic targets.

5. Conclusion and future perspectives

While statins remain beneficial in reducing cardiovascular risk, our findings suggest that prolonged exposure ($\geq$ 72 h) to high concentrations of statins (>0.5 μM) may exacerbate myocardial injury by compromising the oxidative status of $CoQ_{9/10}$ and impairing mitochondrial function. This could potentially explain the persistent burden of CVD despite the administration of lipid-lowering drugs like statins. Given this, the potential role of CoQ_{10} supplementation or the exploration of alternative lipid-lowering strategies may be crucial to enhancing the efficacy of statins, particularly in individuals with dyslipidemia and those at high risk for cardiovascular diseases. However, this study has limitations future investigations should focus on exploring the lower concentrations of simvastatin (<0.3 μM) and the connection between increased ROS and cellular apoptosis. These investigations will help further elucidate the impact of statins on myocardial health and their broader implications for heart disease. While the pathological consequences of statin-induced cardiotoxicity remain an area of ongoing exploration, the depletion of CoQ_{10} observed in our study underscores the need for continued investigation into the potential benefits of CoQ_{10} supplementation. Further studies will be essential to validate these findings and determine the most effective strategies for mitigating statin-induced oxidative damage.

Funding statement

The work reported herein was made possible through the research funding received from the National Research Foundation (NRF) (Grant numbers: 117829 and 141929), awarded to Phiwayinkosi V. Dludla. The authors also acknowledge the Research of Capacity Development under the Early Investigators Program from the South African National Treasury (funding number: HDID8682/MB2022/EIP052) and Baseline funding from the Biomedical Research and Innovation Platform of the South African Medical Research Council (SAMRC) as well as Northwest University. The content hereof is the sole responsibility of the authors and do not necessarily represent the official views of the NRF or the funders.

CRediT authorship contribution statement

Sinenhlanhla X.H. Mthembu: Conceptualization, Writing – original draft, Writing – review & editing. **Sithandiwe E. Mazibuko-Mbeje:** Conceptualization, Writing – original draft, Writing – review & editing. **Sonia Silvestri:** Writing – review & editing. **Patrick Orlando:** Writing – review & editing. **Bongani B. Nkambule:** Writing – review & editing. **Christo J.F. Muller:** Writing – review & editing. **Luca Tiano:** Writing – review & editing. **Phiwayinkosi V. Dludla:** Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The work reported herein was made possible through funding by the South African Medical Research Council through its Division of Research Capacity Development under the SAMRC Postgraduate Research Associate Programme. The content hereof is the sole responsibility of the authors and does not necessarily represent the official views of the SAMRC.

Data availability

All data used to support the findings of this study are included in the article. Raw data can be available on request after publication.

References

Ahmadi, Y., et al., 2018. Oxidative stress as a possible mechanism of statin-induced myopathy. Inflammopharmacology 26 (3), 667–674. https://doi.org/10.1007/s10787-018-0469-x.

Bell, G., et al., 2024. The role of mitochondria in statin-induced myopathy. Drug Saf. 47 (7), 643–653. https://doi.org/10.1007/s40264-024-01413-9.

Björkhem-Bergman, L., Lindh, J.D., Bergman, P., 2011. What is a relevant statin concentration in cell experiments claiming pleiotropic effects? Br. J. Clin. Pharmacol. 72 (1), 164–165. https://doi.org/10.1111/j.1365-2125.2011.03907.x.

Bonifacio, A., et al., 2016. Simvastatin induces mitochondrial dysfunction and increased atrogin-1 expression in H9c2 cardiomyocytes and mice in vivo. Arch. Toxicol. 90 (1), 203–215. https://doi.org/10.1007/s00204-014-1378-4.

Borahay, M.A., et al., 2014. Simvastatin potently induces calcium-dependent apoptosis of human leiomyoma cells*. J. Biol. Chem. 289 (51), 35075–35086. https://doi.org/10.1074/jbc.M114.583575.

Boudina, S., et al., 2009. Contribution of impaired myocardial insulin signaling to mitochondrial dysfunction and oxidative stress in the heart. Circulation 119 (9), 1272–1283. https://doi.org/10.1161/CIRCULATIONAHA.108.792101.

Braillon, A., 2015. Coenzyme Q10 and statin-induced myopathy—II. Mayo Clin. Proc. 90 (3), 420. https://doi.org/10.1016/j.mayocp.2015.01.005.

Bu, D.-X., et al., 2010. Statin-induced Kruppel-like factor 2 expression in human and mouse T cells reduces inflammatory and pathogenic responses. J. Clin. Invest. 120 (6), 1961–1970. https://doi.org/10.1172/jci41384.

Cadenas, S., 2018. Mitochondrial uncoupling, ROS generation and cardioprotection. Biochim. Biophys. Acta Bioenerg. 1859 (9), 940–950. https://doi.org/10.1016/j.bbabio.2018.05.019.

Chaulin, A., 2023. Cardiotoxicity as a possible side effect of statins. RCM 24 (1). https://doi.org/10.31083/j.rcm2401022.

Cheng, J., et al., 2017. Mitochondrial proton leak plays a critical role in pathogenesis of cardiovascular diseases. Adv. Exp. Med. Biol. 982, 359–370. https://doi.org/10.1007/978-3-319-55330-6_20.

Cirilli, I., et al., 2021. Role of coenzyme Q(10) in health and disease: an update on the last 10 years (2010−2020). Antioxidants (Basel) 10 (8). https://doi.org/10.3390/antiox10081325.

Crane, F.L., 2001. Biochemical functions of coenzyme Q10. J. Am. Coll. Nutr. 20 (6), 591–598. https://doi.org/10.1080/07315724.2001.10719063.

Csengo, E., et al., 2025. Newly initiated statin treatment is associated with decreased plasma coenzyme Q10 level after acute ST-elevation myocardial infarction. Int. J. Mol. Sci. 26. https://doi.org/10.3390/ijms26010106.

Deichmann, R., Lavie, C., Andrews, S., 2010. Coenzyme q10 and statin-induced mitochondrial dysfunction. Ochsner J. 10 (1), 16–21.

Dludla, P.V., et al., 2019. N-acetyl cysteine ameliorates hyperglycemia-induced cardiomyocyte toxicity by improving mitochondrial energetics and enhancing endogenous coenzyme Q(9/10) levels. Toxicol. Rep. 6, 1240–1245. https://doi.org/10.1016/j.toxrep.2019.11.004.

Dludla, P.V., et al., 2020a. Palmitate-induced toxicity is associated with impaired mitochondrial respiration and accelerated oxidative stress in cultured cardiomyocytes: the critical role of coenzyme Q(9/10). Toxicol. in Vitro 68, 104948. https://doi.org/10.1016/j.tiv.2020.104948.

Dludla, P.V., et al., 2020b. Palmitate-induced toxicity is associated with impaired mitochondrial respiration and accelerated oxidative stress in cultured cardiomyocytes: the critical role of coenzyme Q9/10. Toxicol. in Vitro 68, 104948. https://doi.org/10.1016/j.tiv.2020.104948.

Fitzgerald, K., Redmond, E., Harbor, C., 2012. Statin-induced myopathy. Glob. Adv. Health Med. 1 (2), 32–36. https://doi.org/10.7453/gahmj.2012.1.2.008.

Fogacci, F., et al., 2024. Effect of coenzyme Q10 on physical performance in older adults with statin-associated asthenia: a double-blind, randomized, placebo-controlled clinical trial. J. Clin. Med. 13 (13), 3741. https://doi.org/10.3390/jcm13133741.

Force, U.P.S.T., 2022. Statin use for the primary prevention of cardiovascular disease in adults: US preventive services task force recommendation statement. JAMA 328 (8), 746–753. https://doi.org/10.1001/jama.2022.13044.

Gonna, H., Ray, K.K., 2019. The importance of dyslipidaemia in the pathogenesis of cardiovascular disease in people with diabetes. Diabetes Obes. Metab. 21 (Suppl. 1), 6–16. https://doi.org/10.1111/dom.13691.

Górski, M., et al., 2025. The role of coenzyme Q10 in modern medicine: insights into energy metabolism and antioxidant therapy. Qual. Sport 37, 57174. https://doi.org/10.12775/QS.2025.37.57174.

Hashim, O.A., Numan, I.T., Mohammed, N.H., 2025. The protective effect of coadministration of coenzyme Q10 and vitamin E on myopathy induced by simvastatin in rats. Toxicol. Rep. 14, 101942. https://doi.org/10.1016/j.toxrep.2025.101942. http://www.sciencedirect.com/science/article/pii/S2214750025000605.

Hescheler, J., et al., 1991. Morphological, biochemical, and electrophysiological characterization of a clonal cell (H9c2) line from rat heart. Circ. Res. 69 (6), 1476–1486. https://doi.org/10.1161/01.res.69.6.1476.

Heshmat-Ghahdarijani, K., et al., 2024. The role of coenzyme Q10 supplementation in the treatment of diabetes, hyperlipidemia, and metabolic-associated liver disease: an updated overview of clinical implications. Curr. Treat. Options Cardiovasc. Med. 26 (11), 331–337. https://doi.org/10.1007/s11936-024-01053-3.

Hue, L., Taegtmeyer, H., 2009. The Randle cycle revisited: a new head for an old hat. Am. J. Physiol. Endocrinol. Metab. 297 (3), E578–E591. https://doi.org/10.1152/ajpendo.00093.2009.

Hussain, A., Kaler, J., Ray, S.D., 2023. The benefits outweigh the risks of treating hypercholesterolemia: the statin dilemma. Cureus 15 (1), e33648. https://doi.org/10.7759/cureus.33648.

Kafka, M., et al., 2022. Long-term treatment with simvastatin leads to reduced migration capacity of prostate cancer cells. Biomedicines 11 (1). https://doi.org/10.3390/biomedicines11010029.

Kim, J.H., Cox, M.E., Wasan, K.M., 2014. Effect of simvastatin on castration-resistant prostate cancer cells. Lipids Health Dis. 13 (1), 56. https://doi.org/10.1186/1476-511X-13-56.

Kucharská, J., Langsjoen, A., Langsjoen, P., 2024. Statin-induced mitochondrial damage. In: Gvozdjáková, A., Lluch, G. López (Eds.), Translational Mitochondrial Medicine. Springer Nature Switzerland, Cham, pp. 263–283. https://doi.org/10.1007/978-3-031-75741-9_16.

Lempp, D., 2021. An evidence-based guideline for treating dyslipidemia in statin-intolerant patients. J. Nurse Pract. 17 (8), 910–915. https://doi.org/10.1016/j.nurpra.2021.06.005.

Liao, J.K., Laufs, U., 2005. Pleiotropic effects of statins. Annu. Rev. Pharmacol. Toxicol. 45, 89–118. https://doi.org/10.1146/annurev.pharmtox.45.120403.095748.

Lim, S.Y., 2013. Role of statins in coronary artery disease. Chonnam Med. J. 49 (1), 1–6. https://doi.org/10.4068/cmj.2013.49.1.1.

Littarru, G.P., Tiano, L., 2007. Bioenergetic and antioxidant properties of coenzyme Q10: recent developments. Mol. Biotechnol. 37 (1), 31–37. https://doi.org/10.1007/s12033-007-0052-y.

López-Lluch, G., 2019. The important role of CoQ(10) in aging. Antioxidants (Basel) 8 (12). https://doi.org/10.3390/antiox8120570.

Lusha, E., Jiang, H., 2020. Simvastatin protects high glucose-induced H9c2 cells from injury by inducing autophagy. Pharm. Biol. 58 (1), 1077–1084. https://doi.org/10.1080/13880209.2020.1839512.

Marcheggiani, F., et al., 2019. Modulation of coenzyme Q(10) content and oxidative status in human dermal fibroblasts using HMG-CoA reductase inhibitor over a broad range of concentrations. From mitohormesis to mitochondrial dysfunction and accelerated aging. Aging (Albany NY) 11 (9), 2565–2582. https://doi.org/10.18632/aging.101926.

Marcheggiani, F., et al., 2021. Anti-ageing effects of ubiquinone and ubiquinol in a senescence model of human dermal fibroblasts. Free Radic. Biol. Med. 165, 282–288. https://doi.org/10.1016/j.freeradbiomed.2021.01.032.

Marcoff, L., Thompson, P.D., 2007. The role of coenzyme Q10 in statin-associated myopathy: a systematic review. J. Am. Coll. Cardiol. 49 (23), 2231–2237. https://doi.org/10.1016/j.jacc.2007.02.049.

Martín-Timón, I., et al., 2014. Type 2 diabetes and cardiovascular disease: have all risk factors the same strength? World J. Diabetes 5 (4), 444–470. https://doi.org/10.4239/wjd.v5.i4.444.

Mazibuko-Mbeje, S.E., et al., 2021. Antimycin A-induced mitochondrial dysfunction is consistent with impaired insulin signaling in cultured skeletal muscle cells. Toxicol. in Vitro 76, 105224. https://doi.org/10.1016/j.tiv.2021.105224.

Minja, N.W., et al., 2022. Cardiovascular diseases in Africa in the twenty-first century: gaps and priorities going forward. Front. Cardiovasc. Med. 9, 1008335. https://doi.org/10.3389/fcvm.2022.1008335.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65 (1), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4.

Mthembu, S.X.H., et al., 2023. Impact of dyslipidemia in the development of cardiovascular complications: delineating the potential therapeutic role of coenzyme Q(10). Biochimie 204, 33–40. https://doi.org/10.1016/j.biochi.2022.08.018.

Mthembu, S.X.H., et al., 2024. Low levels and partial exposure to palmitic acid improves mitochondrial function and the oxidative status of cultured cardiomyoblasts. Toxicol. Rep. 12, 234–243. https://doi.org/10.1016/j.toxrep.2024.01.014.

Mthembu, S.X.H., et al., 2025. Supplementation with aspalathin and sulforaphane protects cultured cardiac cells against dyslipidemia-associated oxidative damage. Metabol. Open 25, 100346. https://doi.org/10.1016/j.metop.2025.100346.

Nanayakkara, G.K., Wang, H., Yang, X., 2019. Proton leak regulates mitochondrial reactive oxygen species generation in endothelial cell activation and inflammation -

a novel concept. Arch. Biochem. Biophys. 662, 68–74. https://doi.org/10.1016/j.abb.2018.12.002.
Oluleye, O.W., et al., 2019. Association between statin use and sex hormone in the multi-ethnic study of atherosclerosis cohort. J. Clin. Endocrinol. Metab. 104 (10), 4600–4606. https://doi.org/10.1210/jc.2019-00530.
Panth, N., Paudel, K.R., Parajuli, K., 2016. Reactive oxygen species: a key hallmark of cardiovascular disease. Adv. Med. 2016, 9152732. https://doi.org/10.1155/2016/9152732.
Qi, X., et al., 2023. Mitochondrial proton leak in cardiac aging. Geroscience 45 (4), 2135–2143. https://doi.org/10.1007/s11357-023-00757-x.
Roth, G.A., et al., 2020. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. J. Am. Coll. Cardiol. 76 (25), 2982–3021. https://doi.org/10.1016/j.jacc.2020.11.010.
Ryan, T.E., et al., 2024. High-dose atorvastatin therapy progressively decreases skeletal muscle mitochondrial respiratory capacity in humans. JCI Insight 9 (4). https://doi.org/10.1172/jci.insight.174125.
Scribano, M.D.L.P., et al., 2014. Effects of atorvastatin on oxidative stress biomarkers and mitochondrial morphofunctionality in hyperfibrinogenemia-induced atherogenesis. Adv. Med. 2014, 947258. https://doi.org/10.1155/2014/947258.
Sekine, K., et al., 2005. Estimation of plasma and saliva levels of coenzyme Q10 and influence of oral supplementation. BioFactors 25 (1–4), 205–211. https://doi.org/10.1002/biof.5520250125.
Sharov, V.G., et al., 2000. Abnormal mitochondrial respiration in failed human myocardium. J. Mol. Cell. Cardiol. 32 (12), 2361–2367. https://doi.org/10.1006/jmcc.2000.1266.
Sies, H., et al., 2022. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. Nat. Rev. Mol. Cell Biol. 23 (7), 499–515. https://doi.org/10.1038/s41580-022-00456-z.
Silvestri, S., et al., 2015. Coenzyme Q_{10} and α-lipoic acid: antioxidant and pro-oxidant effects in plasma and peripheral blood lymphocytes of supplemented subjects. J. Clin. Biochem. Nutr. 57 (1), 21–26. https://doi.org/10.3164/jcbn.14-130.
Smolina, N., et al., 2017. Assaying mitochondrial respiration as an indicator of cellular metabolism and fitness. Methods Mol. Biol. 1601, 79–87. https://doi.org/10.1007/978-1-4939-6960-9_7.
Somers, T., et al., 2023. Statins and cardiomyocyte metabolism, friend or foe? J. Cardiovasc. Dev. Dis. 10 (10), 417. https://doi.org/10.3390/jcdd10100417.
Stancu, C., Sima, A., 2001. Statins: mechanism of action and effects. J. Cell. Mol. Med. 5 (4), 378–387. https://doi.org/10.1111/j.1582-4934.2001.tb00172.x.
Taylor, F., et al., 2013. Statins for the primary prevention of cardiovascular disease. Cochrane Database Syst. Rev. 2013 (1), Cd004816. https://doi.org/10.1002/14651858.CD004816.pub5.
Vinci, P., et al., 2021. Statin-associated myopathy: emphasis on mechanisms and targeted therapy. Int. J. Mol. Sci. 22 (21). https://doi.org/10.3390/ijms222111687.
Ward, N.C., Watts, G.F., Eckel, R.H., 2019. Statin toxicity. Circ. Res. 124 (2), 328–350. https://doi.org/10.1161/CIRCRESAHA.118.312782.
Wu, W.-T., et al., 2024. Lovastatin impairs cellular proliferation and enhances hyaluronic acid production in fibroblast-like synoviocytes. Toxicol. in Vitro 97, 105806. https://doi.org/10.1016/j.tiv.2024.105806.
Zhang, Q., et al., 2022. Atorvastatin induces mitochondria-dependent ferroptosis via the modulation of Nrf2-xCT/GPx4 axis. Front. Cell Dev. Biol. 10. https://doi.org/10.3389/fcell.2022.806081.
Zhou, X.H., et al., 2020. Impact of plasma exposure of statins and their metabolites with major adverse cardiovascular events in Chinese patients with coronary artery disease. Front. Pharmacol. 11, 675. https://doi.org/10.3389/fphar.2020.00675.