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# Coenzyme Q10 Alleviates Chronic Nucleoside Reverse Transcriptase Inhibitor-Induced Premature Endothelial Senescence

**Yi-Fan Chen[1] · Valeria Y. Hebert[2] · Krisztian Stadler[3] · Stephen Y. Xue[2] · Kate Slaybaugh[2] · Elliot Luttrell-Williams[2] · Mitzi C. Glover[4] · David M. Krzywanski[5] · Tammy R. Dugas[1]**

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## Abstract

Human immunodeficiency virus (HIV)-infected patients undergoing antiretroviral therapy are afforded an increased lifespan but also exhibit an elevated incidence of cardiovascular disease. HIV therapy uses a combination drug approach, and nucleoside reverse transcriptase inhibitors (NRTI) are a backbone of this therapy. Endothelial dysfunction is an initiating event in cardiovascular disease etiology, and in our prior studies, NRTIs induced an endothelial dysfunction that was dependent upon mitochondrial oxidative stress. Moreover, short-term NRTI administration induced a mitophagy-associated endothelial toxicity and increased reactive oxygen species (ROS) production that was rescued by coenzyme Q10 (Q10) or overexpression of a mitochondrial antioxidant enzyme. Thus, our objective was to examine mitochondrial toxicity in endothelial cells after chronic NRTI treatment and evaluate Q10 as a potential adjunct therapy for preventing NRTI-induced mitochondrial toxicity. Human aortic endothelial cells (HAEC) were exposed to chronic NRTI treatment, with or without Q10. ROS production, cell proliferation rate, levels of senescence, and mitochondrial bioenergetic function were determined. Chronic NRTI increased ROS production but decreased population doubling. In addition, NRTI increased the accumulation of β-galactosidase, indicative of an accelerated rate of senescence. Moreover, ATP-linked respiration was diminished. Co-treatment with Q10 delayed the onset of NRTI-induced senescence, decreased ROS production and rescued the cells' mitochondrial respiration rate. Thus, our findings may suggest antioxidant enrichment approaches for reducing the cardiovascular side effects of NRTI therapy.

**Keywords** Nucleoside reverse transcriptase inhibitors (NRTI) · Endothelial cells · Senescence · Mitochondria · Coenzyme Q10 · Human immunodeficiency virus (HIV)

## Introduction

Nucleoside reverse transcriptase inhibitors (NRTIs) have long been the backbone of therapy for HIV infection. Though it is well established that NRTI treatment results in cardiovascular complications, the precise mechanism of toxicity has remained elusive. We reported that NRTI treatment induces endothelial dysfunction in a murine model [13, 19, 20], and endothelial dysfunction is a hallmark event in the pathogenesis of atherosclerosis [36]. In cultured human endothelial cells, we demonstrated that NRTI induced an endothelial injury resulting from oxidative stress [13, 18, 20, 21, 38]. This injury was coupled to an increased release of endothelin-1 (ET-1) but decreased nitric oxide (NO) levels, suggesting a disrupted ET-1/NO balance, an important marker for endothelial homeostasis [13]. In addition, we showed that acute NRTI treatment decreased mitochondrial electron transport chain (ETC) activity and ATP

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✉ Tammy R. Dugas
tammydugas@lsu.edu

[1] Comparative Biomedical Sciences, LSU School of Veterinary Medicine, Skip Bertman Drive, Baton Rouge, LA 70808, USA

[2] Pharmacology, Toxicology and Neuroscience, LSU Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71103, USA

[3] Oxidative Stress and Disease Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA

[4] Clinical Laboratory Sciences, School of Allied Health Professions, LSU Health Sciences Center, 1900 Gravier Street, New Orleans, LA 70112, USA

[5] Cellular Biology and Anatomy, LSU Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71103, USA

levels while increasing reactive oxygen species (ROS) production [14, 38].

Oxidative stress is known to accelerate the onset of a senescent phenotype in endothelial cells [23]. Moreover, NRTI-induced oxidative stress and mitochondrial damage were shown associated with the onset of premature senescence in fibroblasts [2]. Intriguingly, cells with a prematurely senescent phenotype exhibit a substantially increased lactate production [33], and we reported that in endothelial cells, the ratio of lactate to pyruvate is increased in NRTI-induced mitochondrial dysfunction [38]. Because the lactate-to-pyruvate ratio, a glycolytic index, is an important marker for oxidative phosphorylation capacity [4] and thus, mitochondrial bioenergetic function, increased glycolysis in senescent cells suggests a link between senescence and mitochondrial dysfunction. In addition, we reported that NRTI-induced oxidant injury leads to the autophagic degradation of mitochondria, termed mitophagy, and a subsequently decompensated mitochondrial biogenesis [38]. This suggested that a prematurely senescent phenotype may play a role in NRTI-induced endothelial dysfunction in vivo. We thus set out to investigate whether chronic NRTI treatment induced endothelial senescence.

The excess production of mitochondrial-derived ROS has been considered a basis for NRTI-induced endothelial dysfunction [18]. The overexpression of mitochondrial antioxidant manganese superoxide dismutase (MnSOD) was shown to rescue short-term NRTI-induced endothelial dysfunction [13]. However, chronic, excess production of ROS may overwhelm endogenous antioxidant capacity, inducing a sustained superoxide production that gives rise to endothelial dysfunction. Q10 is well known as an essential cofactor for the ETC and is a free radical scavenger [1]. It can potentially preserve mitochondrial respiration by removing excess mitochondrial ROS. Moreover, we showed that the acute toxicity of NRTI could be reversed by treatment with Q10 [38]. Thus, we hypothesized that the co-administration of Q10 would alleviate NRTI-induced ROS production to prevent the premature senescence of endothelial cells. The NRTIs manifest distinct cellular toxicities in a number of tissues, but the data suggest that the mechanisms for their off-target toxicities may vary among the individual NRTI compounds [25, 27]. Therefore, we chose to compare NRTI from three subclasses for this study: zidovudine (AZT), a thymidine analogue, lamivudine (3TC), a cytidine analogue and tenofovir (TEN), an acyclic nucleotide analogue of adenosine.

## Materials and Methods

### Drugs and Reagents

AZT and 3TC were obtained from Sigma-Aldrich (St. Louis, MO), and TEN was purchased from Moravek Biochemicals (Brea, CA). Stock solutions containing NRTI were prepared in deionized, distilled water and were diluted 1:1000 in cell culture media, such that the final concentration of each NRTI was 10 μM. The peak plasma concentration of these NRTIs in HIV patients ranges from 0.7 to 9 μM [3, 5, 34]. In addition, previous studies in our laboratory using AZT, likely one of the most toxic of the NRTIs, showed dose-dependent increases in endothelial ROS production at doses between 0.5 and 10 μM [18]. Therefore, the final concentration of the each NRTI was rounded up to 10 μM, such that a direct comparison could be made between the differing drugs. Q10 was purchased from Sigma-Aldrich, and stock solutions prepared in ethanol were likewise diluted 1:1000 in cell culture media, such that the final concentration was 5 μM.

### Cell Culture

Human aortic endothelial cells (HAEC) were obtained from Cell Applications (San Diego, CA) and were cultured in MCDB 131 media containing 10% fetal bovine serum on 0.2% gelatin-coated tissue culture plates. Six different isolates of HAEC were used for experiments, with the isolates selected from non-smoking males and females having no known complications from cardiovascular disease and of ages ranging from 41 to 65 years. HAEC were incubated with 10 μM NRTI ± 5 μM Q10 continuously applied across passages and were split every 3 to 4 days at a ratio < 1:4 (v:v).

### Population Doubling

To determine the impact of NRTI on the cumulative number of population doublings, HAEC were subjected to NRTI treatment across consecutive cell passages. Cells were cultured until confluent for 2–12 passages, and at each sub-culture, cells were counted using a Vi-CELL XR Cell Viability Analyzer from Beckman Coulter (Indianapolis, IN). Cumulative population doubling at each passage was calculated from the cell count, as described elsewhere [6].

### Senescence-Associated β-Galactosidase Cell Staining

At each consecutive passage, HAEC were fixed and stained using a Senescence-β-Gal Staining Kit (Cell Signaling Technology, Billerica, MA), following the manufacturer's recommended protocol. Cells staining blue were counted manually under light microscopy, with cells exhibiting significant blue staining encompassing the majority of the cell body counted as senescent. Cells with low intensity blue staining or with non-uniform staining distributed in only one region of the cell were not considered senescent. Eight fields of view were averaged per cell culture well.

### ROS Production Measurement

The mitochondria-selective fluorescent probe MitoSOX (Thermo Fisher, Waltham, MA) was used to assess ROS production. Cells were treated as indicated, then incubated with 5 μM MitoSOX for 30 min and washed twice with warm phosphate-buffered saline. MitoSOX fluorescence was assessed at Ex 510/Em 580 nm. Fluorescence measures in each well were normalized to relative DNA content, determined using Hoechst 33342 dye.

### Analysis of Mitochondrial Respiration

An XF24 Extracellular Flux Analyzer (Seahorse Bioscience, Agilent Technologies, Santa Clara, CA) was used to determine mitochondrial function. 1 day before measurements, HAECs were seeded with 50,000 cells per well in MCDB131 Complete Medium in an XF24 cell culture microplate, except that control wells A1, B4, C3 and D6 contained no cells. The plate was incubated at 37 °C in a humidified $CO_2$ incubator overnight. A sensor cartridge was hydrated with XF Calibrant in a utility plate at 37 °C in a $CO_2$-free incubator overnight. On the day of measurements, the MCDB131 medium was replaced with Seahorse assay medium containing 25 mM glucose. The cell culture plate was equilibrated at 37 °C in a $CO_2$-free incubator for 1 h. Oligomycin, FCCP and rotenone + antimycin A were loaded into ports A, B and C of the cartridge, respectively. The final concentration of each reagent was 1 μM, 1 μM and 0.5 μM, respectively. The sensor cartridge was then loaded into the XF24 Analyzer for calibration. After calibration, the utility plate was exchanged for the cell culture plate. The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) for basal respiration were recorded before reagent injection. OCR of proton leak, maximum OCR and non-mitochondrial oxygen consumption were measured as the oxygen consumption rate over time for treatments with oligomycin, FCCP and rotenone + antimycin A injection, respectively [9].

### Statistical Analysis

Data were analyzed using GraphPad Prism software. All data were presented as group mean ± SEM and were compared using one- or two-way ANOVA, as appropriate. For measures of population doubling (Fig. 1) or percent senescent cells (Fig. 3) assessed over multiple passages from the same cell isolates, we utilized a repeated measures ANOVA. In all cases, a value of $p < 0.05$ was considered statistically significant.

## Results

### Q10 Co-treatment Rescued the Decreased Population Doubling Rate Induced by NRTI

To test our hypothesis that chronic NRTI treatment promotes premature endothelial senescence, we first evaluated population doubling (PD) in HAEC. The nature of the finite replication capacity of primary HAEC provides a useful model for studying senescence. In all three NRTI-treated groups, a significant decrease in PD was noted, beginning at passage 6 and continuing through passage 11 (Fig. 1). Q10 is a component of the mitochondrial electron transport chain and contains the iron-sulfur clusters that serve both as electron carriers and as a scavenging antioxidant. Co-treatment with Q10 rescued the decrease in PD induced by chronic NRTI treatment. These results indicate that chronic NRTI treatment induced early onset of decelerating population doubling that could be alleviated by co-administering Q10.

### Q10 Reduced the Accumulation of Senescent Endothelial Cells After Chronic NRTI Treatment

To confirm that chronic NRTI treatment results in premature endothelial senescence, the accumulation of senescence-associated β-galactosidase (SABG) was determined in HAEC chronically treated with AZT, 3TC, or TDF. SAGB is one of the most widely used senescence marker for in situ and in vitro analyses. The activity of SABG was detected at pH 6.0, the pH at which X-gal is hydrolyzed by β-galactosidase to generate a blue product within senescent cells (Figs. 2, 3). HAEC exhibited a significantly higher percentage of senescent cells starting from passage 3 after treatment with AZT, from passage 4 with 3TC, and at passage 8 with TEN treatments compared to passage-equivalent control cells (Fig. 3a). Co-treatment with Q10 abolished NRTI-induced accumulation of SABG, in that SABG accumulation in NRTI treated cells was not different from passage-equivalent control cells. However, cells treated with Q10 exhibited a lower SABG accumulation compared to control cells at passages 7 and 8 (Fig. 3b). These results indicate that chronic NRTI treatment increases senescence markers and that Q10 co-treatment attenuates this accelerated senescence.

### Q10 Attenuated NRTI-Mediated Increase in Mitochondrial ROS Production

In our prior studies, short-term NRTI treatment increased mitochondrial ROS production in endothelial cells. To investigate the cause of chronic NRTI-induced premature senescence, ROS production was measured in HAEC treated

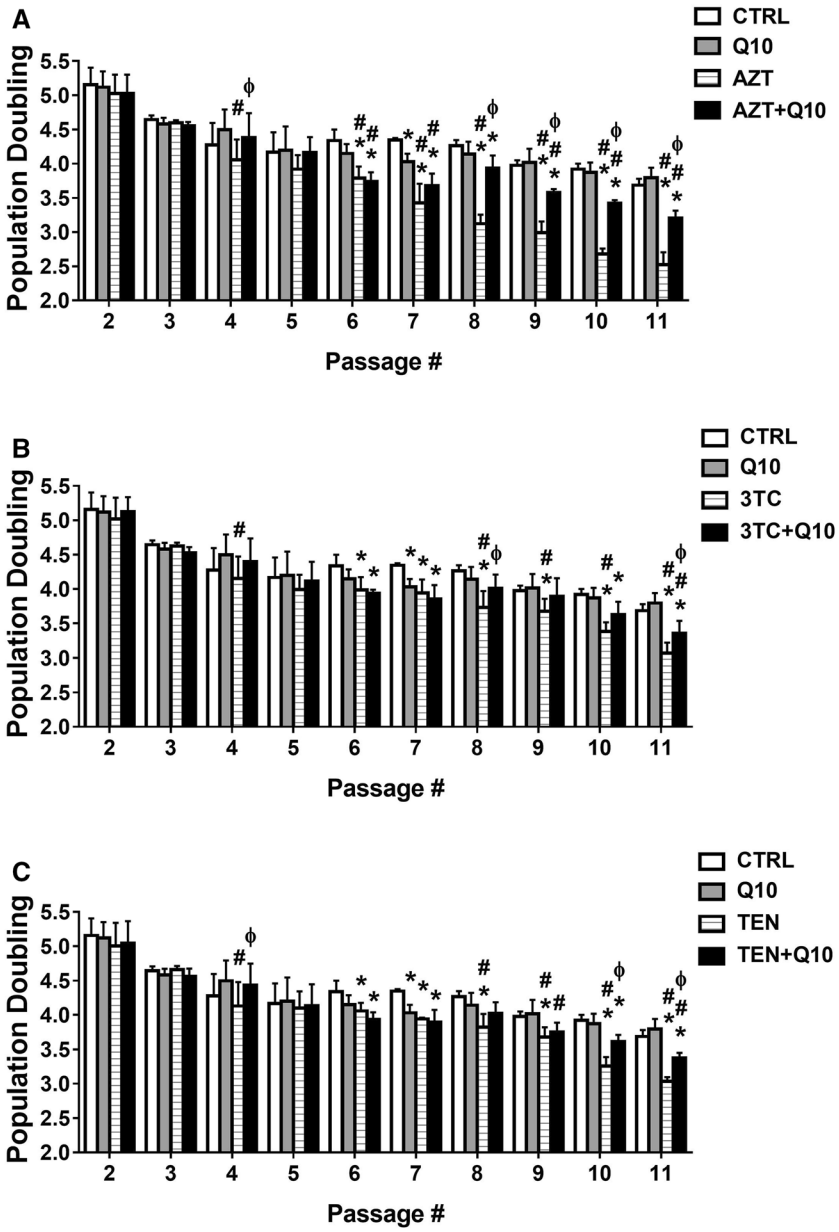


**Fig. 1** Q10 rescued NRTI-mediated decrease in population doubling observed after chronic treatment. HAEC were treated with 10 µM AZT (**a**), 3TC (**b**), or TEN (**c**), with or without 5 µM Q10 for 11 consecutive passages and cell numbers were counted. Population doubling was calculated as: PD = ln(Total Cells seeded/Viable Cells)/ln(2). $^{*}p < 0.05$, compared to equivalent passage control cells; $^{\#}p < 0.05$, compared to Q10-treated cells; $^{\phi}p < 0.05$, compared to NRTI-treated cells; repeated measures two-way ANOVA and Tukey post hoc were applied, $n = 3$. *Q10* Coenzyme Q10, *HAEC* human aortic endothelial cells, *NRTI* nucleoside reverse transcriptase inhibitors, *AZT* azidothymidine, *3TC* lamivudine, *TEN* tenofovir

chronically with NRTI. Chronic AZT, 3TC, or TEN treatment increased mitochondrial ROS production from passage 6 to passage 10 (Fig. 4a). However, co-treatment with Q10 reduced NRTI-mediated increase in mtROS (Fig. 4b). These results suggest that co-treatment of cells with Q10 can potentially moderate excess mtROS production induced by chronic NRTI treatment.

## Q10 Rescued the Suppressed ATP-linked Respiration and Glycolytic Flux Induced by Chronic NRTI

Next, we aimed to investigate whether the increase in mitochondrial ROS production observed after chronic NRTI treatment is associated with an alteration in mitochondrial function. An XF24 Extracellular Flux Analyzer was used to access mitochondrial respiratory function in HAEC treated chronically with AZT, 3TC or TEN. ATP-linked respiration decreased in all three NRTI treatment groups (Fig. 5a–d), especially in the AZT-treated cells, which exhibited a dramatic decline. Meanwhile, TEN-treated cells also exhibited a decline in basal and maximal respiration. Therefore, we tested whether Q10 co-treatment improved the cellular respiration rate observed after NRTI treatment (Fig. 5c, d). Intriguingly, chronic co-treatment with Q10 not only reversed the AZT-induced decline in ATP-linked respiration but also increased maximal respiration (Fig. 5d).

Based on this result, we tested whether chronic NRTI treatment affects bioenergetic function in HAEC. In

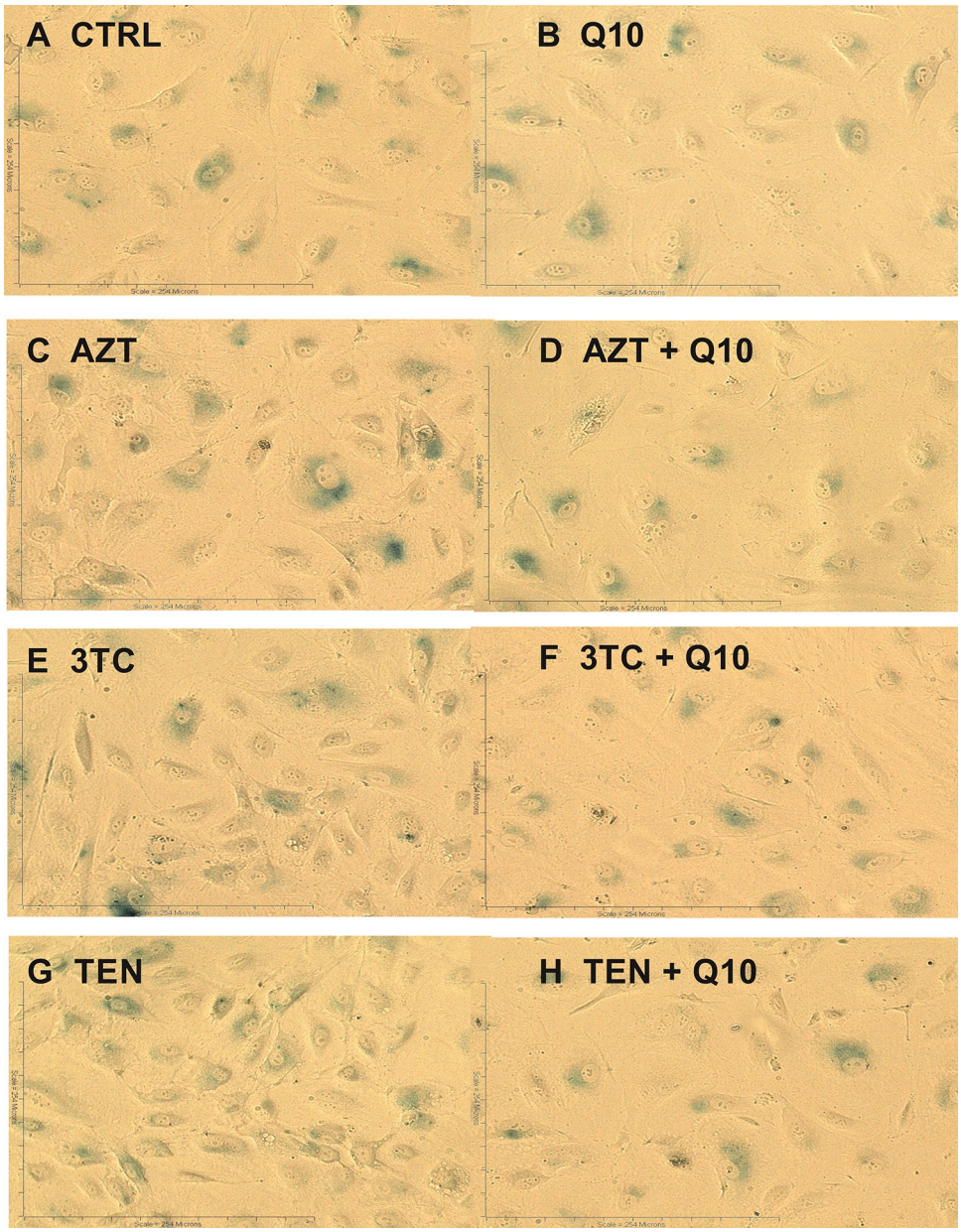


**Fig. 2** Senescence-associated β-galactosidase staining of endothelial cells. **a**–**h** HAEC were treated with NRTI with or w/o Q10 chronically, and the accumulated senescence-associated β-galactosidase (SABG) was detected by staining with X-gal. Senescent cells are depicted by a dark green–blue color. Images shown are from passage 8 and are representative of $n = 3$ experiments

endothelial cells, glycolytic flux is reportedly the predominant bioenergetic pathway contributing to ~ 85% of the total cellular ATP production [8]. To examine the metabolic change induced after chronic AZT ± Q10 treatment, we monitored ECAR, which is used as an indicator for the rate of glycolysis (Fig. 5e). After chronic treatment, the ECAR level was higher in Q10 and AZT + Q10 treated groups compared to AZT-treated HAEC. This result demonstrates that Q10 may prevent the decline in glycolytic flux caused by chronic AZT treatment.

## Discussion

Effective antiretroviral therapy has transformed HIV-1 infection from a fatal disease into a manageable but chronic condition demanding long-term administration of multiple drugs. However, the long-term administration of antiretroviral therapies has the potential to induce metabolic disruptions, such as hyperlipidemia, insulin resistance and abnormalities in glucose metabolism, and has

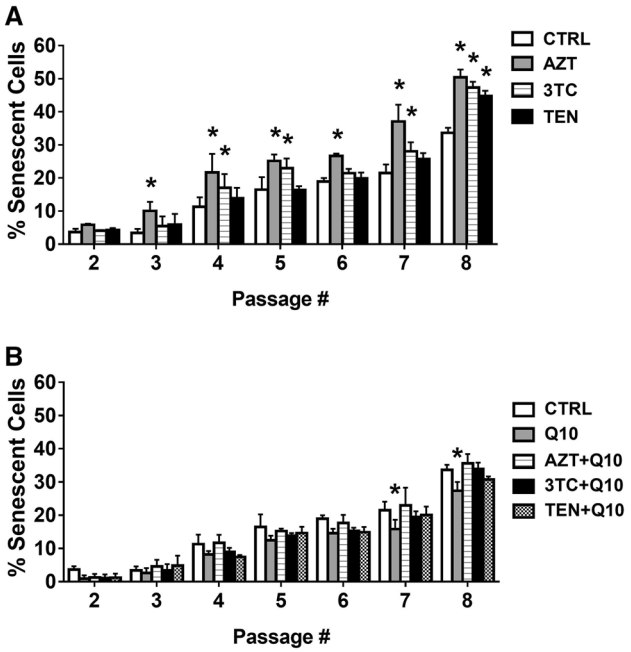


**Fig. 3** Q10 blocked the accumulation of senescent endothelial cells after chronic NRTI treatment. **a** HAEC were treated with 10 μM of NRTI or **b** 10 μM NRTI + 5 μM Q10 for 8 passages. The cells were stained for SABG accumulation, and the number of senescent cells were counted. $*p < 0.05$, compared to passage-equivalent control cells; repeated measures two-way ANOVA and Dunnett post hoc were used, $n = 3$. SABG = senescence-associated β-galactosidase. Images shown are representative of cells treated across 8 passages

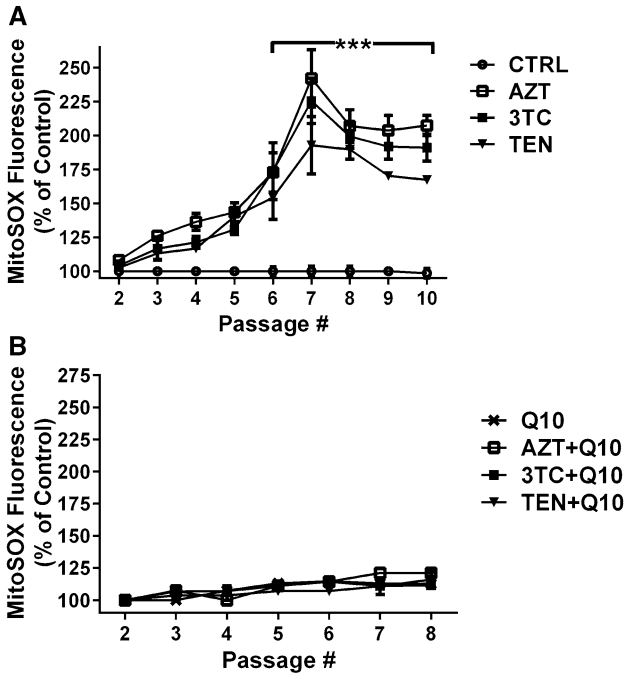


**Fig. 4** Q10 diminished NRTI-induced increase in mitochondrial ROS production after chronic treatment. **a** HAEC were treated with 10 μM NRTI for 10 passages. ROS production was determined using the MitoSOX fluorescent indicator, and the data were normalized to DNA content. Data are expressed as percent increase compared to controls. **b** HAEC were treated with 5 μM of Q10 ± 10 μM NRTI for 8 passages. ROS production was determined as in **a**. $*p < 0.05$, compared to equivalent passage control cells; repeated measures two-way ANOVA and Dunnett post hoc were used, $n = 3$–4

been shown to increase the risk for developing chronic cardiovascular diseases (CVD) among HIV patients [12, 17]. We and others have shown that NRTI treatment results in endothelial dysfunction both in vitro and in vivo [19, 22]. Oxidative stress, and even mitochondrial oxidative stress, are known contributors to endothelial dysfunction, and NRTI are recognized mitochondrial toxicants. Therefore, NRTI-induced mitochondrial oxidant injury may be a mechanism contributing to this drug-mediated endothelial dysfunction. However, the mechanism underlying NRTI-induced endothelial dysfunction and the chronic impact of NRTI treatment on mitochondria remains unclear. Thus, identifying a possible mechanism contributing to NRTI-induced endothelial dysfunction and potentially developing an adjunct therapy for reducing this dysfunction should be helpful in improving outcomes for antiretroviral therapy.

Functionally, mitochondrial respiration can be divided into electron transport and oxidative phosphorylation operating through the actions of complexes I–IV [16, 24]. We previously reported suppressed activities of complexes I–IV, decreased ATP production, and a stimulation of mitophagic activity 6–8 h after NRTI treatment, but these acute impairments recovered 24–48 h after dosing [38]. Our earlier findings thus suggested that the antioxidant system within mitochondria and repair via mitophagy may successfully compensate for the excess ROS produced after acute NRTI, but the impact of chronic NRTI administration was yet to be elucidated. It is well established that oxidative stress promotes ROS signaling in many pathologies, especially in aging-related diseases [10]. Previously, we found that NRTIs induce an increase in both cellular and mitochondrial ROS production, but this injury did promote apoptosis [18, 20]. Since oxidative stress is a known driving force for cellular senescence, we hypothesized that chronic NRTI treatment promotes premature endothelial senescence but that co-treatment with the mitochondrial antioxidant Q10 would block this progression.

In this study, HAEC chronically administered AZT, 3TC, or TEN exhibited a decreased PD and an increased senescent cell accumulation. At the same time, the production of mtROS increased across NRTI treatment groups. These findings suggested that chronic NRTI treatment induces excess mtROS production and promotes endothelial senescence. In support of our findings in endothelial cells, zidovudine (AZT) was reported to induce senescence in immortalized

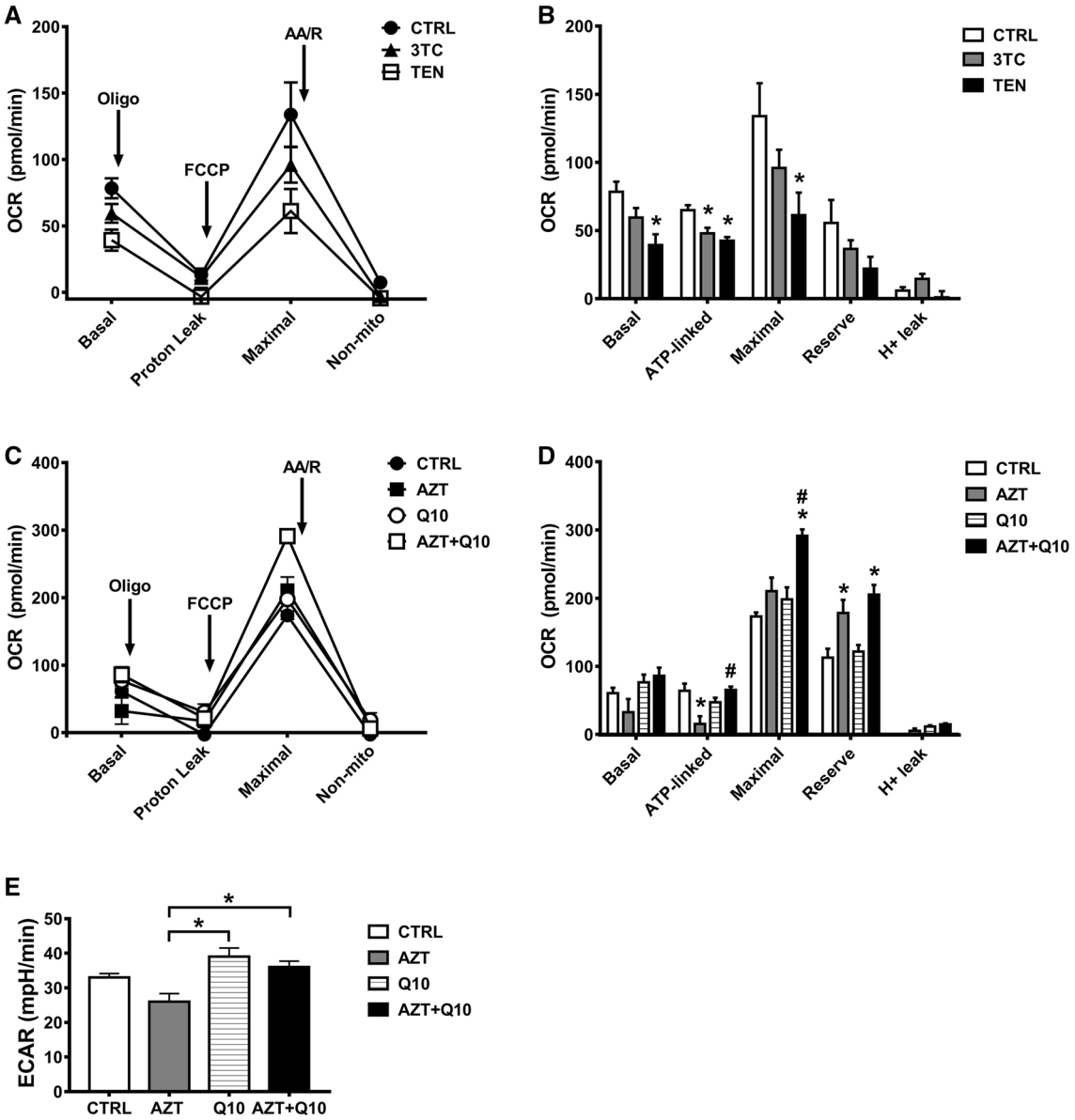


**Fig. 5** Q10 rescued NRTI-mediated suppression in ATP-linked respiration and the reduction in glycolytic flux after chronic treatment. **a**, **b** HAEC were treated with 10 μM NRTI as indicated for 10 passages and OCR was measured using an XF24 Extracellular Flux Analyzer. Oligomycin (Oligo), FCCP, and Antimycin A with Rotenone (AA/R) were added (as indicated by arrows) to assess rates of basal respiration, ATP-linked respiration, maximal respiration, reserve respiration, and proton leak. **c**–**e** HAEC were treated with 10 μM AZT, 5 μM Q10, or both for 10 passages and OCR and ECAR were measured. $^{*}p < 0.05$, compared to the control group; $^{\#}p < 0.05$, compared to AZT-treated cells; respiration rate was compared by using one-way ANOVA at each respiratory state with Dunnett post hoc, $n = 3$–5. *OCR* oxygen consumption rate, *ECAR* extracellular acidification rate

cancer cells, presumably by inhibiting telomerase activity [35]. In addition, stavudine and zidovudine, but not abacavir, didanosine, lamivudine nor tenofovir, induced mitochondrial dysfunction and senescence in human primary fibroblasts [2]. Tenofovir and emtricitabine promoted mitochondrial stress and premature senescence in human lung and cardiac fibroblasts in an mTORC1-dependent pathway [29]. Taken together, these reports suggest that NRTIs of varying types induce premature senescence in a number of cell types. Although specific drugs among the class of NRTI may induce senescence via differing mechanisms, a commonality among these reports is that mitochondrial dysfunction seems to prime cells for undergoing premature senescence. Thus, the premature induction of endothelial senescence may contribute to NRTI-induced vascular dysfunction.

An imbalance in mitochondrial function is a common predisposing factor for vascular diseases. For example, endothelial senescence is associated with an impairment of

mitochondrial biogenesis and an alteration in the expression of the mitochondrial components transcribed from mitochondrial DNA (mtDNA) [7]. Previously, we observed decreases in ETC complex I-IV activity after acute NRTI treatment [38]. In this study, all three treatment groups exhibited a suppression in ATP-linked respiration. ATP-linked respiration is mediated by oxidative phosphorylation. Its decline could be owing to low ATP demand, low substrate availability or ETC damage [15]. Although ATP demand in endothelial cells is accommodated mainly via glycolysis, in studies presented here, 25 mM glucose was supplied in the analysis medium to provide a carbon source for the cells. Therefore, an ETC inhibition seems the most likely cause for NRTI-induced reductions in ATP-linked respiration. In addition to these findings, TEN treatment resulted in a reduction in basal and maximal respiration that was not observed for 3TC or AZT treated cells. We do not have an explanation for these results, except to suggest that perhaps its mechanism of injury within mitochondria may be more complex than for the other two drugs. On the other hand, in AZT-treated cells, there was an increase in reserve respiration. However, the reserve respiration capacity (RRC) is the mathematical difference between maximal and basal respiration. Since AZT did not simultaneously increase the maximal respiration rate, the observed AZT-mediated increase in RRC could have simply resulted from a decreased level of basal respiration.

Because endothelial cells depend primarily on glycolysis for their ATP, the observed disturbance in mitochondrial respiration after NRTI treatment likely impacts endothelial function mainly through an alteration in redox signaling, rather than by reducing their ATP production. Mitochondria are the major intracellular source of ROS, even in endothelial cells, where they regulate signaling in numerous physiological and pathological processes, including inflammation [31]. Therefore, targeting mitochondrial redox signaling is an emerging therapeutic strategy for numerous pathologies [26].

Q10 is both a free radical scavenger and an important cofactor for electron transport [1, 11]. It may thus preserve mitochondrial respiration and function by removing excess mitochondrial ROS. Interestingly, Q10 co-treatment attenuated NRTI-mediated inhibition in population doubling and the accumulation of senescent cells and mtROS. The bioenergetic result here indicates that Q10 administered together with AZT not only rescued ATP-linked respiration, but also elevated the reserve and maximal respiration capacities. RRC fluctuates according to cellular ATP demands, helping the cells cope with acute insults or increased workload. Bioenergetic reserve respiration varies among cell types, but an increase in substrate availability, mitochondrial mass, and good ETC integrity typically expands its capacity. The mitochondrion is the only organelle equipped with its own genome, mtDNA. Thus, a decrease in mtDNA abundance or mtDNA mutations would critically impair mitochondrial ETC integrity. In support of this assertion, NRTI-induced mtDNA depletion was reported in adipocytes isolated from HIV patients, and this outcome associated with subcutaneous fat wasting [30]. Further study may thus be required for fully elucidating the impact of chronic NRTI treatment on the integrity of mtDNA.

It was peculiar that Q10 treatment together with AZT, but not Q10 treatment alone, increased RRC, perhaps suggesting the effect of co-treatment on HAEC may be more complicated than simply eradicating NRTI-induced ROS. This outcome led us to consider whether chronic use of Q10 with AZT induces a metabolic switch that increases RRC. Since endothelial cells are glycolytic, monitoring glycolytic flux, quantified by measuring glucose uptake and lactate excretion, represents a good estimation for their metabolic status. Using the Seahorse XF analyzer, we thus used an ECAR measurement to assess the excretion of lactic acid per unit time into cell culture media [37]. The observed increases in ECAR measured in the Q10 and Q10 + AZT treatment groups could suggest that Q10 elevates the metabolic status of endothelial cells. However, the production of carbon dioxide via respiration can also contribute to the acidification of the medium, thus complicating the interpretation of data [28]. Nevertheless, these findings may suggest a future direction of study, investigating a switch in the metabolic status of senescent endothelial cells and a thorough elucidation of the role of Q10 as an anti-aging supplement.

In summary, here we demonstrate that chronic NRTI treatment induces premature senescence and decreases mitochondrial bioenergetic function in endothelial cells. Taken together, these findings suggest a potential interaction between NRTI-induced mitochondrial damage and endothelial cell senescence, which may contribute to the mechanism of NRTI toxicity. A limitation of this study is that we did not examine the effect of chronic NRTI treatment on the mitochondrial genome, and damage to the mitochondrial genome is known to exacerbate cellular aging [32]. Evaluating NRTI induced effects on mtDNA copy number, mtDNA damage level, or mtDNA sequencing and the impact of Q10 co-treatment will be helpful in identifying possible mutation site(s) on mtDNA to derive a better understanding of NRTI pathogenesis. Importantly, our findings suggest Q10 as a possible candidate for adjunct therapy to alleviate the vascular side effects arising from chronic NRTI usage, but in vivo confirmation of these in vitro findings will be necessary.

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## Compliance with Ethical Standards

**Conflicts of interest** The authors have no conflicts of interest related to this study.

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