



Original Contribution

Dietary coenzyme Q10 does not protect against cigarette smoke-augmented atherosclerosis in apoE-deficient mice

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ABSTRACT

Dietary coenzyme Q10 reduces spontaneous atherosclerosis in the apoE-deficient mouse model of experimental atherosclerosis. We have shown previously that exposure to sidestream cigarette smoke (SSCS) enhances atherosclerotic lesion formation in apoE-deficient mice. The aim of the present study was to determine if CoQ10 protected against SSCS-mediated atherosclerosis. Female apoE-deficient mice were fed a saturated fat-enriched diet (SFD) alone, or supplemented with 1% wt/wt coenzyme Q10 (SFD-Q10). Mice in each diet group were exposed to SSCS for 4 hrs/day, 5 days/week in a whole-body exposure chamber maintained at 35 ± 4 mg smoke particulates/m³. Mice kept in filtered ambient air served as controls. Mice were euthanized after either 6 or 15 weeks of SSCS exposure and following measurements were performed: i) lung 7-ethoxyresorufin-O-deethylase (EROD) activity; ii) plasma cholesterol and CoQ10 concentrations; iii) aortic intimal area covered by atherosclerotic lesions; and, iv) pathological characterization of lesions. Lung EROD activity increased in SSCS mice of both diet groups, confirming SSCS exposure. Plasma concentrations of CoQ10 in SFD-Q10-fed mice were increased markedly in comparison to SFD-fed mice. Plasma cholesterol concentrations and distributions of cholesterol in lipoprotein fractions were unaffected by SSCS exposure. Dietary supplementation with CoQ10 significantly reduced atherosclerotic lesions in control mice. As reported previously, exposure to SSCS increased the size of lesions in apoE^{-/-} mice at both time points. However, dietary supplementation with CoQ10 had no effect on atherosclerotic lesions augmented by SSCS exposure. The results suggest a role of oxidative processes in smoke-augmented atherosclerosis that are different than those mitigated by CoQ10.

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Introduction

Increased oxidative stress has been implicated in the development of vascular disease and its functional importance in the evolution of atherosclerosis has been long recognized [1–4]. Exposure to cigarette smoke is a major risk factor for atherosclerotic vascular diseases. While the mechanisms through which tobacco smoke contributes to development and progression of atherosclerotic disease are poorly understood, it is generally believed that a high oxidative stress associated with tobacco smoke exposure plays an important role in promoting disease [5–7].

Efforts to protect against oxidative stress and cardiovascular disease by antioxidant intervention, particularly alpha tocopherol, have yielded mixed results [4,8–10]. Various studies have indicated an important role of mitochondria in generating endogenous oxidative stress which in turn may influence development and progression of atherosclerosis [11–13]. Natural ubiquinone or Coenzyme Q10 (CoQ10) is a lipid-

soluble endogenous antioxidant in mitochondria that is composed of a quinoid moiety with isoprenoid side chain [14]. It is an essential component of the mitochondrial electron transport chain [15] and acts as a lipid antioxidant either directly in its reduced form, ubiquinol, or in recycling of radical forms of vitamin E [16]. Dietary CoQ10 is readily reduced to ubiquinol in the body which is the predominant form present in the plasma [17]. It possesses strong inhibitor activity against lipid peroxidation in tissues and membranes and is more efficient than vitamin E in protecting LDL oxidation [18]. Beneficial effects of dietary CoQ10 in cardiovascular disease have been reviewed [17,19].

In rodents, the main CoQ is CoQ9 which is a shorter chain homologue of CoQ10. Feeding CoQ10 to animals is known to increase the levels of both CoQ9 and CoQ10 in the plasma, tissues and mitochondria [20,21]. Studies have also shown that dietary supplementation with CoQ10 reduces the development of atherosclerotic lesion formation in atherosclerosis-prone apoE-deficient mice [22,23]. We have previously shown that exposure to sidestream cigarette smoke (SSCS) accelerates the development of atherosclerotic plaque formation in apoE deficient mice [24]. The present study was performed to determine if dietary CoQ10 supplementation will protect against SSCS-induced acceleration of atherosclerotic lesion formation in this model.

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Materials and methods

Animals

Female apoE-deficient mice (8–9 wks old) were purchased from the Jackson Laboratory (Bar Harbor, ME). Food and water were available to the mice *ad libitum*. Weekly body weights were maintained to assess any differences in growth rates. All procedures performed in this study had the prior approval of the University of Kentucky Institutional Animal Care and Use Committee.

Diets

Purified Teklad fat enriched diet (milk fat 21% wt/wt, cholesterol 0.15% wt/wt; TD 88137; SFD), and the same diet supplemented with 1% wt/wt coenzyme Q10 (CoQ10-SFD) were prepared by Harlan-Teklad, WI. Pure CoQ10 (natural ubiquinone, trans configuration) was supplied by Tishcon Corp (NY). Diets were obtained in batches prepared at 6 week intervals which were stored at 4 °C. Animals were divided into two diet groups of 30 each: one maintained on SFD and the other on CoQ10-SFD. Fresh diets were provided on alternate days.

Smoke exposures

Each diet group was divided into two equal subgroups: control and SSCS. SSCS groups were exposed for a total of 4 hrs/day, 5 days a week, for up to 15 weeks, as described previously [24,25]. Briefly, the inhalation exposures were carried out in a whole-body exposure chamber. Sidestream cigarette smoke was generated from the University of Kentucky 2R4F reference cigarettes. The total suspended particulate level in the chamber averaged 35 ± 4 mg/cubic meter. Sham control groups were exposed to filtered ambient air.

Exposure markers

Inhalation of SSCS by mice was monitored by urinary cotinine measurement by an ELISA and the measurement of 7-ethoxyresorufin-O-deethylase (EROD) activity in lung microsomes at the termination of the studies. Exposure to cigarette smoke induces this CYP1A1-linked enzyme which we have used to ascertain exposure of animals to cigarette smoke particulates [24].

Plasma measurement

Blood was collected from mice under light anesthesia and serum was obtained. Concentrations of total cholesterol by enzymatic assays were performed with commercially available kits (Wako Chemical Co). Lipoprotein cholesterol distributions among lipoproteins were determined by FPLC size-exclusion chromatography on 50 μ l of serum from individual mice as described previously [26]. Serum concentrations of total CoQ10 were quantified by HPLC [27].

Quantification of atherosclerosis

The entire aorta from arch to iliac bifurcation was carefully removed for plaque area measurement. Atherosclerosis was quantified by intimal area measurements of the atherosclerotic lesions by *en face* method, as described previously [28,29]. After removing the extraneous fat, atherosclerotic lesions on the intimal aortic surface of apoE^{-/-} mice were measured under a dissecting microscope, equipped with a Nikon digital camera and quantified with Image Pro (Media Cybernetics Inc.) software.

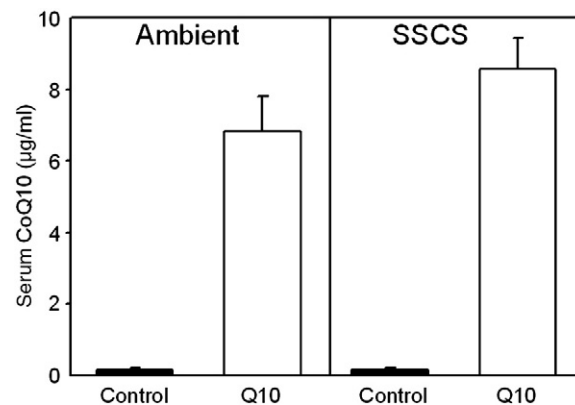


Fig. 1. Serum concentrations of CoQ10 in apoE^{-/-} mice exposed to ambient air and SSCS for 15 weeks. Mice were fed a saturated fat-enriched diet (closed bars) or one supplemented with CoQ10 (open bars). Histograms are means of $n = 10$ measurements and bars are SEMs.

Characterization of atherosclerotic lesions

Lesions were sectioned in the aortic sinus as described previously [28] and stained by histological and immunochemical techniques, as described previously [26,30].

Statistics

Mean and standard error of mean (SEM) were calculated for each parameter. Data was analyzed using Sigma Stat using parametric or nonparametric analysis as appropriate. $P < 0.05$ was considered statistically significant.

Results

Mice maintained on either SFD or CoQ10-SFD gained body weights at a similar rate between control and smoke-exposed animals in both diet groups (data not shown). Total serum CoQ10 concentrations were markedly increased by the dietary supplementation in groups exposed to both ambient air and SSCS (Fig. 1). These data demonstrate that there was efficient absorption of CoQ10 in mice and that SSCS exposure had no effect on the serum concentrations.

Dietary CoQ10 did not significantly influence the excretion of cotinine in urine in SSCS exposed mice (concentrations ranged from 1.6 to 3 μ g/mg creatinine). A ~5 fold increase in pulmonary EROD activity occurred in SSCS exposed mice on both diets, indicating effective inhalation of smoke particulates (Fig. 2).

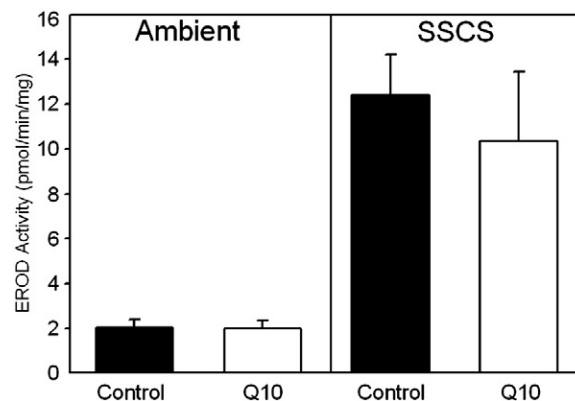


Fig. 2. Pulmonary EROD activity of apoE^{-/-} mice exposed to ambient air and SSCS for 15 weeks. Mice were fed a saturated fat enriched diet (closed bars) or one supplemented with CoQ10 (open bars). Histograms are means of $n = 10$ measurements and bars are SEMs.

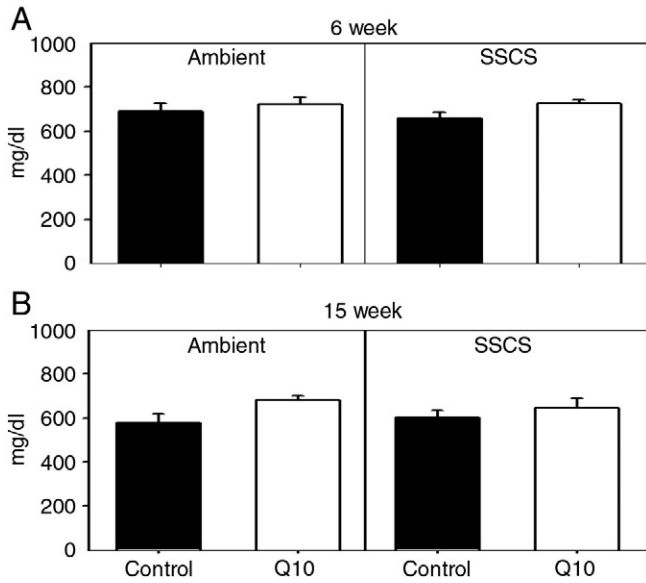


Fig. 3. Serum cholesterol concentrations. A. Serum cholesterol concentrations of mice exposed to either ambient air or SSCS and fed either a saturated fat enriched diet alone (closed bars) or one supplemented with CoQ10 (open bars) for 6 weeks. B. The same groups as represented in A, but exposed and fed for 15 weeks. Histograms represent means and bars are SEM.

As expected, serum cholesterol concentrations in all groups were greatly elevated through the study. Neither exposure to SSCS nor supplementation with CoQ10 had any significant effect on plasma cholesterol concentrations (Fig. 3). Also, the distribution of cholesterol among lipoproteins fractions was not influenced with either SSCS exposure or CoQ10 supplementation (data not shown).

After 6 weeks of feeding a saturated fat-enriched diet, the size of atherosclerotic lesions covering the aortic arch intima was not significantly influenced by supplementation with CoQ10. Exposure to SSCS significantly increased the size of atherosclerotic lesions at this interval, and this augmentation was not influenced by CoQ10 supplementation (Fig. 4A). Atherosclerotic lesion sizes increased in all groups by 15 weeks of feeding the modified diet. In the group exposed to ambient air, there was significant reduction in lesion size in mice fed a CoQ10 supplemented diet in comparison to non CoQ10 supplemented group. However, supplementation with CoQ10 had no significant effects on the SSCS augmented lesions after 15 weeks of exposure (Fig. 4B).

To determine whether supplementation of dietary CoQ10 and exposure to SSCS had overt effects on the characteristics of lesions, tissue sections from the aortic sinus were immunostained for macrophages and histologically stained using Gimori's trichrome. As shown in the examples in Fig. 5, the lesions that form under these conditions are composed predominantly of lipid-laden macrophages. Based on these stains, the exposure to SSCS or supplementation with CoQ10 had no overt effect on lesion characteristics.

Discussion

We have previously established a model of cigarette smoke-mediated atherosclerosis in apoE-deficient mice [24]. It should be noted that the concentrations of SSCS used for this study exceeded those encountered in the environmental tobacco smoke-contaminated environments such as bars and restaurants. However, no overt toxicity was observed and the increase in atherosclerosis was reproducible in different experiments. Hence, we have utilized this model to assess the effectiveness of dietary CoQ10 in protecting against increased atherosclerotic lesion formation by SSCS. The results showed that in spite of a substantial increase in total plasma CoQ10 concentrations, which is considered a useful measure of overall CoQ10 status [17], significant

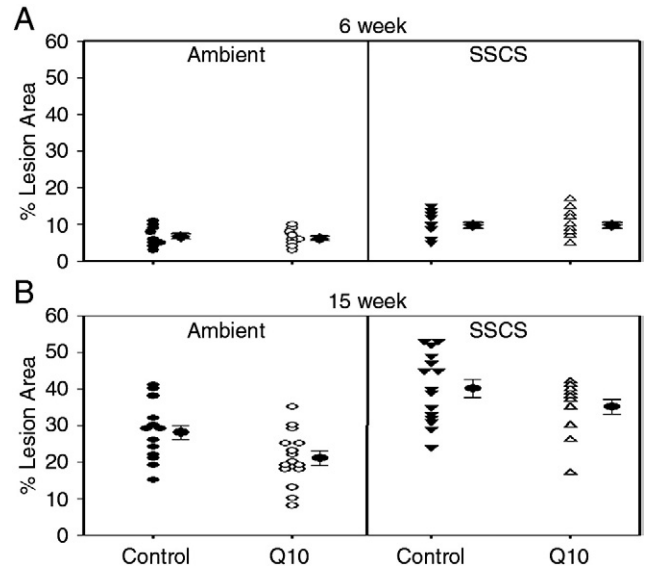


Fig. 4. Aortic intimal area covered by grossly discernable atherosclerotic lesions. A. Percent of the intimal surface covered by atherosclerotic lesions in mice exposed to either ambient air or SSCS and fed either a saturated fat enriched diet alone (closed bars) or one supplemented with CoQ10 (open bars) for 6 weeks. B. The same groups as represented in A, but exposed and fed for 15 weeks. The size of atherosclerotic lesions in individual mice is presented, in addition to means and SEMs.

protection was not obtained against smoke-mediated promotion of atherosclerosis. However, the lesion growth in unexposed animals was significantly reduced by dietary CoQ10.

The selection of CoQ10 for this study was based on its previously demonstrated ability to protect against atherosclerosis in apoE deficient mice exposed to ambient air [22] presumably by reducing the concentrations of lipid hydroperoxides in arteries [23]. Furthermore, recent findings demonstrated an involvement of mitochondrial DNA damage and protein nitration in smoke-mediated promotion of atherosclerosis in apoE^{-/-} mice [12,13]. We have also observed dysregulation of soluble epoxide hydrolase in aortic endothelial cells isolated from mice exposed to SSCS [31]. CoQ10 has been reported to protect against lipid and protein oxidation *in vivo* [17,18,21]. Since CoQ10 is an important membrane antioxidant in mitochondria and functions in maintaining mitochondrial bioenergetics by shuttling electrons from complex I or II to complex III [14,16,32], it was expected to protect against oxidative damage. Unavailability in tissues could not have been a factor for the negative finding because feeding animals CoQ10 in diet significantly increased its plasma concentrations several fold in the present study. Increased concentrations of CoQ10 in tissues and mitochondria of rodents following dietary supplementation has been documented [33]. Thus, it would be safe to assume that CoQ10 concentrations in mitochondria and tissues of our supplemented animals were also elevated which in turn would counteract endogenous and SSCS-induced oxidative stress.

An absence of an effect of CoQ10 supplemented diet on plasma cholesterol concentrations in control and SSCS exposed mice ruled out the possibility of hypercholesterolemia as a factor for the lack of protection against smoke-mediated atherosclerosis.

It has been reported that antioxidant supplementation is generally beneficial against cardiovascular diseases and antioxidant therapy protects against cigarette smoke-mediated cardiovascular dysfunction in humans [34,35]. It was, therefore, surprising to find a lack of protective effect of CoQ10 supplementation, particularly when, in agreement with an earlier report [22], supplementation of dietary CoQ10 reduced the extent of spontaneous atherosclerosis in apoE^{-/-} mice exposed to ambient air. These results raise the possibility of a divergence in the mechanisms that induce spontaneous atherogenesis and those responsible for SSCS augmented atherosclerosis.

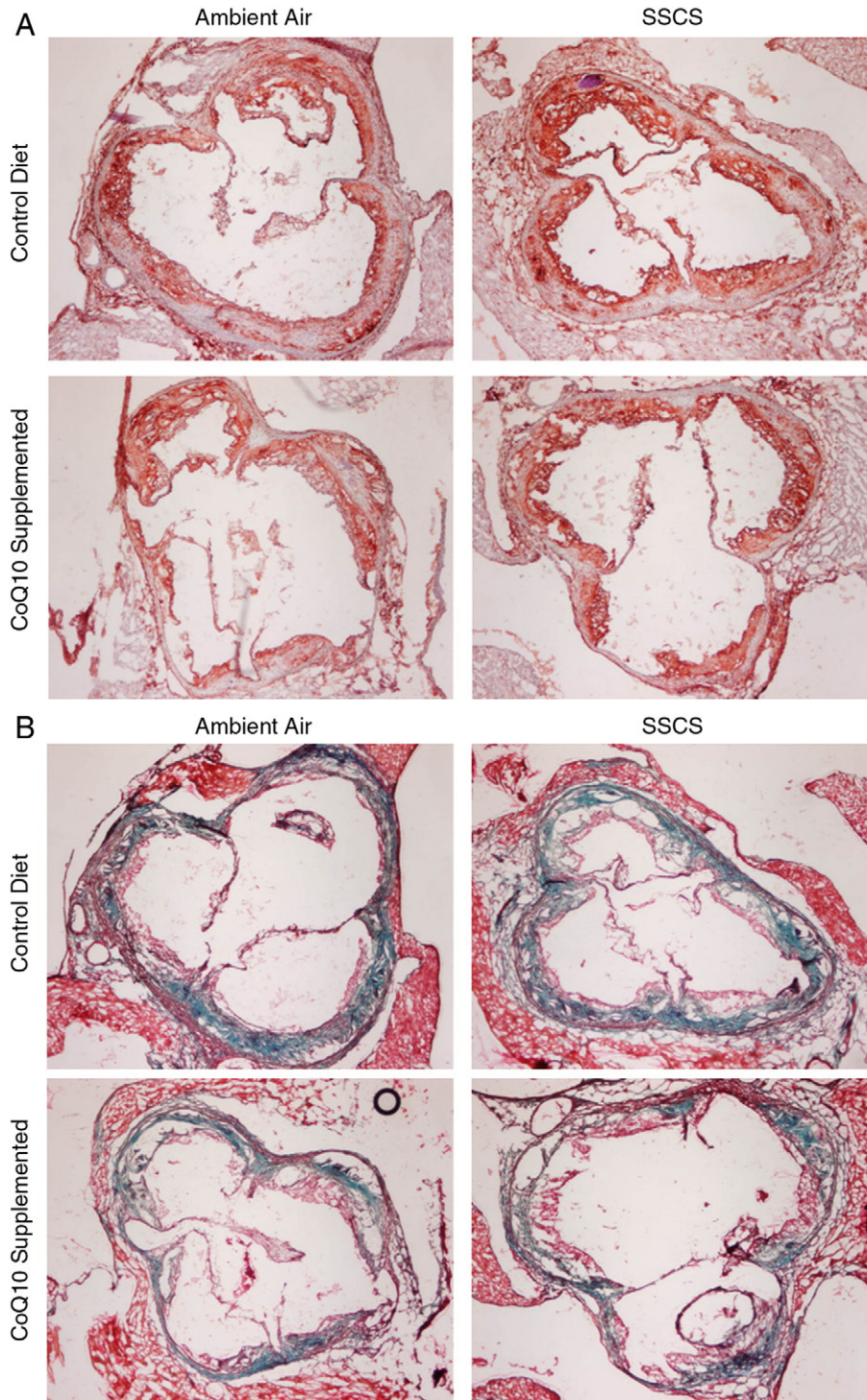


Fig. 5. Characterization of atherosclerotic lesions. A. Immunostaining for macrophages in the aortic roots for examples from each of the 4 groups. B. Histological staining using Gimori's trichrome.

It should be noted that the translational value of results obtained in animal models require special consideration. Although apoE-deficient mice have been extensively used as experimental model of human atherosclerosis, it is well established that animal models recapitulate only facets of human disease and fail to develop all components of mature human disease [36]. For example, atherosclerotic lesions generated in mice models are of limited complexity in comparison to human atherosclerotic plaques.

Cigarette smoke is a complex mixture containing over four thousand chemicals. In addition to its pro-oxidant activity, many of

its constituents are highly reactive and are capable of directly interacting with cellular macromolecules to cause damage. Thus it is possible that the acceleration of atherosclerosis by smoke exposure involves mechanisms other than and/or in addition to oxidative stress that were not mitigated by CoQ10 supplementation.

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