

# In vivo supplementation with coenzyme Q<sub>10</sub> enhances the recovery of human lymphocytes from oxidative DNA damage<sup>1</sup>

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## SPECIFIC AIMS

This study was designed to investigate whether supplementation with CoQ<sub>10</sub> protects DNA against oxidative damage in lymphocytes oxidized in vitro with compounds inducing different oxidizing profiles; DNA recovery from oxidative damage was also studied. The hypothesis that CoQ<sub>10</sub> supplementation might modulate the repair enzyme activity will be tested on whole cell extract lymphocytes.

## PRINCIPAL FINDINGS

### 1. Coenzyme Q<sub>10</sub> in vitro enrichment inhibits the formation of DNA strand breaks induced by atmospheric oxygen

The incubation of lymphocytes under atmospheric oxygen leads to a formation of DNA strand breaks (SBs) detectable within the first hour of exposure. Both ubiquinol-10 and ubiquinone-10 are able to prevent DNA SB formation, the enriched lymphocytes resulting in less damage and exhibiting a faster DNA repair rate than that of nonenriched control lymphocytes (Fig. 1a). Conversely, the ubiquinol-10 or ubiquinone-10 neither prevents the endogenous formation of oxidized purine and pyrimidine bases nor affects their repair (Fig. 1b, c). When cells are treated with Ro19-8022, oxidized purine bases are specifically formed, even though a small amount of DNA SBs and modified pyrimidine bases is also detectable. Enrichment of lymphocytes with ubiquinol-10 or ubiquinone-10 did not prevent formation of oxidized purine bases, the kinetic of DNA repair being similar in control and ubiquinol-10- or ubiquinone-10-enriched cells.

### 2. Coenzyme Q<sub>10</sub> in vivo supplementation increases ubiquinol-10 endogenous levels of lymphocytes, inhibits DNA SB formation, and enhances DNA SB recovery from oxidative damage induced by atmospheric oxygen

The orally intake of 100 or 300 mg/day of coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) for two consecutive weeks increases the endogenous CoQ<sub>10</sub> cellular content by 45% and 144%, respectively. Reminiscent of the situation occurring in

vitro, DNA of enriched lymphocytes is less damaged by the exposure to oxygen, as indicated by the lower amount of DNA SBs formed in CoQ<sub>10</sub>-enriched cells compared with native lymphocytes and the ones isolated from washout plasma. The extent of DNA SB formation inversely relates to the concentration of CoQ<sub>10</sub> in plasma and cells (Fig. 2a, b).

DNA SBs accumulated in native and washout lymphocytes were repaired at the similar rate of endogenous DNA SBs in CoQ<sub>10</sub>-enriched lymphocytes. Conversely, in vivo supplementation of lymphocytes with CoQ<sub>10</sub> neither prevents the formation of endogenous oxidized purine and pyrimidine bases nor affects their repair.

### 3. CoQ<sub>10</sub> supplementation enhances DNA repair enzyme activity

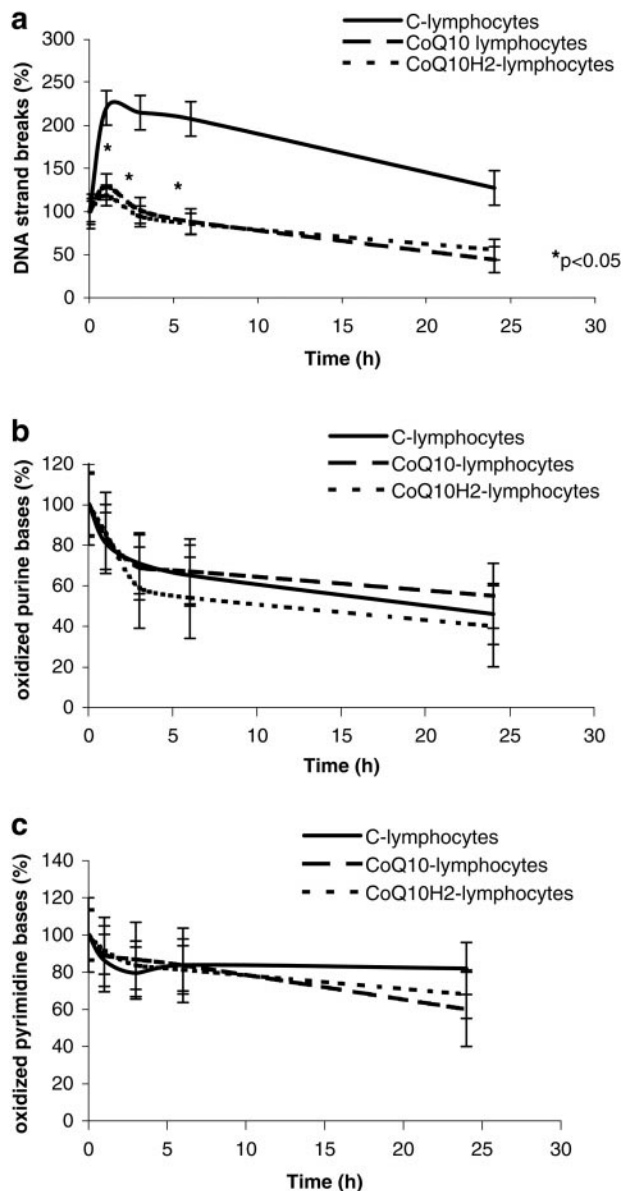
The activity of DNA repair enzymes has been assessed by incubating lymphocyte extracts containing DNA enzyme repair with a substrate consisting of oxidized purine bases derived from lymphocytes previously exposed to Ro 19-8022. DNA repair enzymes introduce breaks at sites of oxidized purines and enzyme activity is reflected by the number of DNA breaks. CoQ<sub>10</sub> supplementation enhances DNA repair activity, which is markedly higher in cellular extracts from CoQ<sub>10</sub>-enriched lymphocytes than in that of native and washout lymphocytes,  $148 \pm 25$  au. vs.  $55 \pm 15$  au. and  $94 \pm 21$  au.,  $P < 0.05$ , respectively.

## CONCLUSIONS

There are at least two ways to explain the effect of antioxidants on the recovery from oxidative DNA damage. Thus, the ability of an antioxidant to enhance the recovery may be due to a stimulation of the activity of repair enzymes or a protection against oxidation. Our results show that both ubiquinol-10 and ubiquinone-10 are capable of reacting with oxygen, as indicated by no additional formation of DNA strand breaks in enriched lymphocytes when exposed to atmosphere. The ability

<sup>1</sup> To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.00-0694fje>; to cite this article, use *FASEB J.* (April 6, 2001) 10.1096/fj.00-0694fje

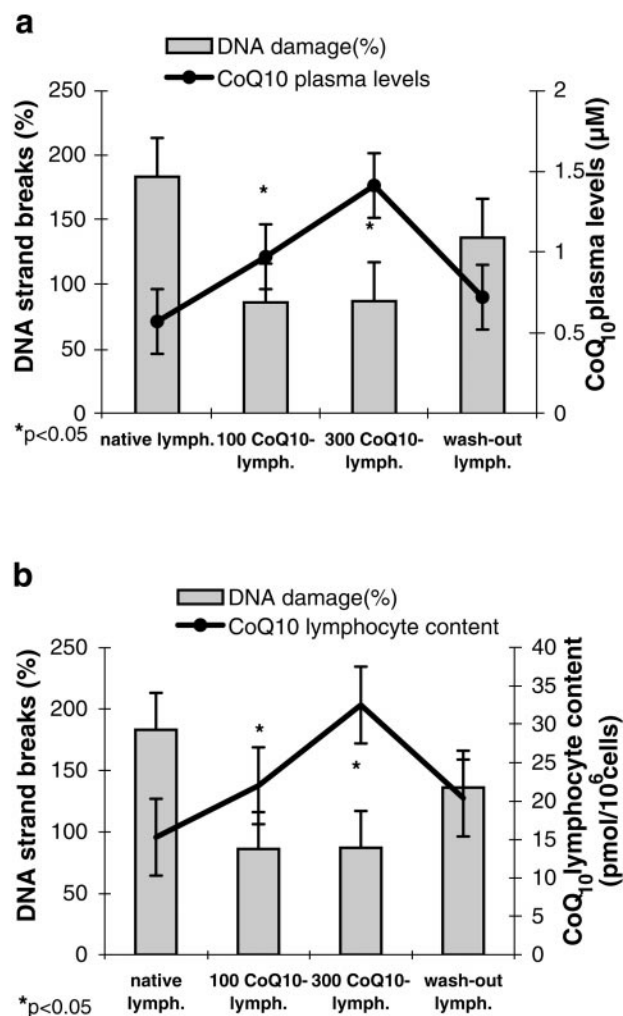
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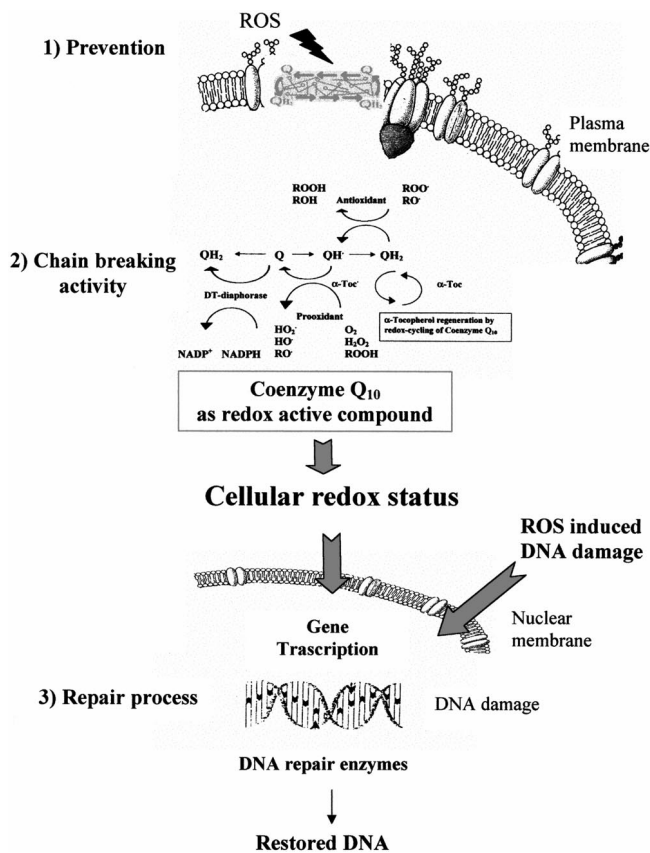
**Figure 1.** Kinetics of repair of DNA strand breaks (a), oxidized purine bases (b), and oxidized pyrimidine bases (c) in ubiquinone-10 or ubiquinol-10 in vitro enriched and control lymphocytes exposed to oxygen atmospheric. Lymphocytes suspended in Petri dishes with RPMI 1640 containing 10% FCS ( $1.0 \times 10^6$  cells/ml) were incubated at 37°C under normal atmosphere (5% of  $\text{CO}_2$ ) for 24 h. At regular interval times (0, 1, 3, 6, 24 h), aliquots of the samples were collected for the Comet assay. The kinetics of DNA repair were calculated as a percentage of the basal value. Results are expressed as mean  $\pm$  sd of values obtained from four separate experiments. Control lymphocytes vs.  $\text{CoQ}_{10}$  and  $\text{CoQ}_{10}\text{H}_2$ -enriched lymphocytes,  $P < 0.05$ .

of  $\text{CoQ}_{10}$  to increase the DNA repair rate is probably due to an inhibition of additional damage by protecting the cells against further oxidation. Such an effect is likely ascribed to the known antioxidant activity of ubiquinol-10. On the contrary, the capability of ubiquinone-10 to protect DNA from oxidative damage remains unclear. We hypothesize that the enrichment of cells with ubiquinone-10 yielded an ordering and condensing effect on cell membranes, and thus may have

restricted the number of radicals capable of reaching the cells' DNA. The assessment of repair capacity in a sample extract indicates that lymphocytes in vivo enriched with  $\text{CoQ}_{10}$  are endowed with a high capacity of DNA repair compared to native cells. Changes in the redox state of transcriptional factors have been proposed as a mechanism regulating the extent of DNA binding activity, which in turn modulates various events occurring in cells, including proliferation and apoptosis. The redox mechanism implicated in enzyme *trans*-activation could explain the property of ubiquinol-10 in enhancing the DNA repair enzyme activity and protecting DNA from oxidative damage. After  $\text{CoQ}_{10}$  supplementation, most of the antioxidant is present in its reduced form in plasma and lymphocytes (85–98% and



**Figure 2.** DNA strand break formation after oxygen exposure in relation to  $\text{CoQ}_{10}$  content in plasma (a) and lymphocytes (b). The antioxidant concentrations and DNA strand break formation were assayed for each subject before starting the supplementation (native lymphocytes), after 1 wk of supplementation with 100 mg/day of  $\text{CoQ}_{10}$  (100  $\text{CoQ}_{10}$  lymphocytes), after 1 wk of supplementation with 300 mg/day (300  $\text{CoQ}_{10}$  lymphocytes), and at 1 wk after the last supplementation (washout lymphocytes), respectively. Data were obtained by analyzing plasma or lymphocytes isolated from six donors and results are expressed as mean  $\pm$  sd. Native lymphocytes vs. 100 and 300  $\text{CoQ}_{10}$ -enriched lymphocytes,  $P < 0.05$



**Figure 3.** Hypothetical scheme illustrating the role of CoQ<sub>10</sub> in the prevention of DNA oxidative damage. 1) CoQ<sub>10</sub> may prevent DNA damage by either directly scavenging ROS or restricting the number of ROS able to reach DNA due to its ordering and condensing effect within the lipid bilayer of cell membrane; 2) CoQ<sub>10</sub> inhibits lipid peroxidation by a chain-breaking mechanism, preventing the propagation of peroxidation processes that can generate harmful radicals able to reach DNA, and by recycling α-tocopherol; 3) CoQ<sub>10</sub> may play a role in the *trans*-activation of DNA repair enzymes by affecting the cellular redox status involved in the regulation of transcriptional factors, such as NF-κB, and of regulatory proteins governing the transcriptional regulation of formamidopyrimidine (Fpg) DNA glycosylase, a base excision repair protein. ROS, reactive oxygen species; Q, ubiquinone-10; QH<sub>2</sub>, ubiquinol-10; QH·, ubisemiquinone-10

65–70%, respectively), and can be involved into redox reactions. In conclusion, we demonstrated the ability of *in vitro* and *in vivo* CoQ<sub>10</sub> supplementation in inhibiting oxidative DNA damage and enhancing DNA repair enzyme activity in cultured lymphocyte. On the basis of our results and consistent with data previously shown by others, we propose a hypothetical scheme illustrating the preventive role of CoQ<sub>10</sub> against DNA damage (Fig. 3). However, further investigations will be required to clarify the precise mechanism(s) by which CoQ<sub>10</sub> may modulate gene expression of DNA repair enzymes. [F]