Concentration and distribution of ubiquinone (coenzyme Q), the endogenous lipid antioxidant, in the rat lens: effect of treatment with simvastatin

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Purpose: Ubiquinone (Ub) is the only known endogenously synthesized lipid soluble antioxidant. It is synthesized from intermediates in the cholesterol metabolic pathway. Our goal was to identify the Ubs and determine the concentration and distribution of Ubs in the rat lens and the effect of treatment with simvastatin, a cholesterol synthesis inhibitor, on lens levels.

Methods: Intact lenses and separated lens fractions from young rats were homogenized in organic solvents, the Ubs recovered, and identified by HPLC analysis. Rats were fed Ub-10 to determine effects of supplementation on tissue levels. Sprague-Dawley (SD) and Chbb:Thom (CT) rats were treated with simvastatin, an inducer of cataracts in CT rats, to determine its effects on lens Ubs.

Results: Ubiquinone-9 (9 isoprenes in its hydrocarbon tail) was the main Ub in the rat lens. The intact lens contained about 3.0 µg Ub/g lens wet weight of which 80-90% was Ub-9 and the remainder Ub-10. No reduced Ubs were detected. Although the epithelial fraction contained the highest Ub concentration (about 8 µg/g), the cortex and nucleus combined accounted for about 90% of the lens' total content. Dietary supplementation with Ub-10 markedly increased the Ub-10 concentration in liver but not lens. Treatment with simvastatin decreased lens Ubs of both SD and CT rats by about 20%.

Conclusions: The abundance of mitochondria in lens epithelium likely accounted for its high level of Ubs; but, finding most of the lens' total Ub in the cortex plus nucleus also suggests roles in maintaining the fiber cell membrane. The decrease in lens Ubs caused by simvastatin is interpreted to reflect a response to drug induced cellular stress rather than to inhibition of the cholesterol synthesis pathway.

Because oxidation of lenticular membrane and cytosolic components is believed to underlie cataractogenesis in humans [1] there has been great interest in the lens' natural antioxidants [2-7] and the possibility of delaying cataract development by dietary supplementation with antioxidants [6,8-10]. Glutathione is the principal natural water soluble antioxidant in the lens, present at millimolar concentrations [3,5,6]. Although glutathione has received much attention, there is no available information on ubiquinone (coenzyme Q), the only known endogenous lipid antioxidant in the lens.

Ubiquinone (Ub) is a lipid consisting of a polyisoprene hydrocarbon chain attached to parabenzoquinone containing methoxy groups on carbons 2 and 3 and a methyl group on carbon 5. The polyisoprene chain contains 9 monounsaturated isoprenes (each 5 carbons) in rats and 10 isoprenes in humans [11]. Although concentrated in the inner mitochondrial membrane, Ub is widely distributed in cellular membranes [12] and has at least several functions in the cell [13,14]. Ubiquinone shuttles electrons between components of the electron transport chain (from Complex I and II to Complex III/bc1) and is an antioxidant with apparently multiple functions. It scavenges lipid peroxyl radicals [15], protects polyunsaturated fatty ac-

ids from oxidation [16], scavenges ascorbate free radicals [17], and regenerates reduced α-tocopherol [18]. In addition, Ub is an obligatory cofactor for mitochondrial uncoupling proteins [19] which enhance state-4 respiration [20]. Its capacity to stimulate state-4 respiration, and thereby decrease generation of reactive oxygen species, is believed to antagonize the oxidative stress and loss of dopamine producing brain cells associated with Parkinson's disease [20]. Treatment with ubiquinone-10 prevented dopamine cell loss in a primate model of Parkinson's disease [20], and delayed progression of this disease in human patients [21].

Our interest in Ub originated from the possibility that there could be a connection between formation of Ub and induction of cataracts in rats treated with simvastatin (Zocor), a cholesterol lowering drug which inhibits the rate controlling enzyme, HMG CoA reductase, in cholesterol biosynthesis [22,23]. Farnesyl pyrophosphate, an intermediate in the cholesterol synthesis pathway [23], along with p-hydroxybutyric acid are substrates for the synthesis of Ub [14]. Treatment with simvastatin-induced cataracts strain specifically in rats [22]. It induced cataracts in Chbb:Thom (CT) but not Sprague Dawley (SD) rats [22]. Compared with untreated control rats, rates of cholesterol synthesis in lens markedly increased in treated SD, but not CT, rats. We suggested that something formed in increased amounts due to upregulation of the sterol synthesis pathway might have protected the SD rat lenses from

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simvastatin-induced stress. We speculated that the something was ubiquinone. Therefore, the main goals of this study were to describe, for the first time, the identity, concentration, and distribution of Ub in the lens, to determine if lens concentrations of Ub changed in SD and CT simvastatin treated rats and to test whether dietary supplementation with Ub can influence lens levels of this antioxidant.

METHODS

Reagents, animals, and lenses: Standards of ubiquinone-6, ubiquinone-9, and ubiquinone-10 for HPLC analyses were from Sigma (St. Louis, MO). Most routine chemicals were also from Sigma. Ubiquinone 10 for dietary supplementation studies was from the Vitaline Corp. (Wilsonville, OR). Cost prohibited supplementation with pure Ub-9 or Ub-10. SD rats were purchased from Hilltop Lab Animals (Scottdale, PA) and CT rats were from our own breeding colony. CT rats were initially obtained from Boehringer-Ingelheim (Biberach an der Riss, Germany). Swiss H1A mice were from Hilltop Lab Animals. Human lenses were obtained from the National Disease Research Interchange (Philadelphia, PA). Bovine lenses were from cattle slaughtered for human food by local abattoirs.

Treatment of rats: Beginning at 20 days of age, rats received 200 mg of simvastatin/day by gavage for 16 days as done before [22] or were untreated (controls). Rats were sacrificed by carbon dioxide inhalation 22 h after the final dose of simvastatin. Mice were also sacrificed by carbon dioxide inhalation. Pulverized Vitaline tablets contained 0.114 g of ubiquinone-10 and 0.114 g of dl-vitamin E per g total. This is the same product shown to delay progression of Parkinson's disease in human patients [21]. Ground rat chow was supplemented with 17.5 g of pulverized tablets per kg to give approximately 2 g of Ub-10 and 2 g of vitamin E. Control chow was supplemented with 2 g/kg of dl-vitamin E (Sigma). Rats were treated for 17 days beginning at 20 days of age. Estimating that the rats eat about 10% of their body weight per day in chow, treated rats would have received about 200 mg/kg body weight of Ub-10 per day. All animals were treated in full compliance with the Association for Research in Vision and Ophthalmology resolution on usage and treatment of animals in research.

Extraction of ubiquinone from tissues: The method of Aberg et al. [11] was used to recover Ub from tissues. The principal ubiquinone in rat tissues is Ub-9, accounting for 80-90% of the total in most tissues [11]. Intact young rat lenses (in groups of 4) and rat lenses manually divided into separate pools of capsule (epithelium), cortical, and nuclear fractions (each pool from 8-10 lenses) were weighed and then homogenized in 1 ml of 0.25 M sucrose to which 10 μg or 20 μg of Ub-6 was added as internal standard. Eighteen ml of methanol was added and the Ub immediately extracted with 12 ml of petroleum ether (boiling point 35-60 °C). According to Aberg et al. [11], the rapid extraction into petroleum ether prevents oxidation of reduced ubiquinones and removes the need to add a protecting agent, such as butylhydroxytoluene, to the extraction system. This claim was evaluated (see Results). The petroleum ether was evaporated under nitrogen and

the residue usually dissolved in 110 μl of ethanol. Bovine lens and a pool of 10 intact mouse lenses were similarly extracted. Individual human lenses were divided into capsule and lens body, the fractions weighed, homogenized and extracted as described above. Aliquots of rat liver and heart (0.35-0.54 g) were similarly extracted. The residues from the liver and heart extracts were dissolved in 1.0 ml of ethanol. Rat heart was examined to confirm the HPLC elution time of oxidized Ub-9, since the oxidized form of Ub-9 accounts for most of the total ubiquinone in this tissue [11]. Aliquots of reference Ub-9 and Ub-10 were also reduced with sodium borohydride as described by Tang et al. [24] to confirm the elution time of the reduced forms of the coenzymes and to assess recovery of reduced Ub-9 and Ub-10 carried through the homogenization and extraction procedure.

HPLC quantitation of ubiquinone: HPLC was performed on a Beckman System Gold with a 128 solvent monitor and a 166 detector (Beckman Coulter, Fullerton, CA). The HPLC system was adapted from Rousseau and Varin [25]. Aliquots (100 μl) of the Ub extracts representing 10% or 91% of the total sample were injected onto a Waters, Spherisorb ODS 2-5 m, 150x3.2 mm column (Supelco, Bellfonte, PA), eluted at 1 ml per min with 70:30 methanol:ethanol and monitored at 275 nm. Elution times for Ub-6, Ub-9 reduced, Ub-10 reduced, Ub-9, and Ub-10 were approximately 1.5, 3.9, 4.8, 6.0, and 8.7 min, respectively. Areas under the peaks corresponding to elution times of oxidized and reduced Ubs were estimated by triangulation and masses of the unknowns estimated by comparison to the area of known masses of the added internal standard (Ub-6). Corrections were made for the presence of trace amounts of a contaminant in the internal standard with an elution time similar to Ub-10. This contaminant was equivalent to 0.024 μg of Ub-10 per 10 μg Ub-6 in one lot of Ub-6 and 0.077 μg in another. Changes in instrument sensitivity made during the separations (i.e., changes in attenuation) were accounted for in the calculations. Since the correction applied to the measured mass of Ub-10 due to the presence of the contaminant is assumed to be constant from sample to sample, there could be considerable error in estimating the concentration of Ub-10 in rat lenses. However, the significance of the error to estimation of lens total ubiquinone should be minor since Ub-10 accounts for only about 15-20% of the total in rat lens and other rat tissues. Time from tissue homogenization until injection onto the HPLC column for all samples was generally less than 2 h. Procedures were performed in the absence of direct lighting or sunlight because of potential photodegradation of ubiquinones.

Ubiquinones separated by HPLC can be quantitated from both ultraviolet [11,25,26] and coulometric (electrochemical) detection [24,27]. The advantage of coulometric detection is its much greater sensitivity in recognizing the reduced forms of Ubs. However, UV detection is more universally applicable and still permits recognition of the reduced Ubs. We selected UV detection at 275 nm [24,26], rather than 210 nm [11] because extracted cholesterol does not absorb at 275 nm. The identity of reduced Ubs was confirmed by repeating the HPLC separations with detection at 291 nm [26,28]. The absorbance

of reduced Ubs approximately doubles at 291 nm relative to 275 nm, while the absorbance of oxidized Ubs decreases at 291 relative to 275 nm.

RESULTS

Lens oxidized and reduced ubiquinones compared: Standard Ub-6, Ub-9, and Ub-10 eluted at about 1.6, 5.8, and 8.8 min, respectively (Figure 1A). When the eluents were monitored at 291 nm, absorbance by identical masses of these oxidized ubiquinone standards decreased by about 30% relative to that at 275 nm (Figure 1A). The principal ubiquinone of rat liver is reduced Ub-9 [11], eluting at about 3.9 min (Figure 1B). As expected, absorbance of this peak more than doubled at 291 nm compared with that at 275 nm, confirming its identity as reduced Ub-9. The principal ubiquinone of rat heart is oxidized Ub-9 [11] eluting at about 6.0 min (Figure 1C). Its absorbance decreased as expected at 291 nm relative to than at 275 nm. Aliquots of ubiquinone extracted from whole bovine and rat lenses were separated by HPLC in duplicate, with the eluents first monitored at 275 nm and then repeated at 291 nm. The absorbance of the principal ubiquinone of bovine lens (eluting at about 8.7 min) and the principal ubiquinone of rat lens (eluting at about 5.7 min) decreased at 291 nm relative to that at 275 nm, confirming their identities as oxidized ubiquinone 10 and 9, respectively (Figure 2A,B). There were no obvious increases in the absorbance at 291 nm, relative to 275 nm, by any of the separated substances (Figure 2), indicating the absence of detectable reduced ubiquinones in lens tissue. When mixtures of reduced and oxidized Ub-9 and Ub-10 were carried through the homogenization and extraction procedures, the ratio of reduced Ub-9 to oxidized Ub-9 were essentially the same before and after extraction, while the ratio of reduced to oxidized Ub-10 slightly decreased (Figure 3). The ratios of reduced to oxidized Ub-9 before and after extraction were 0.91 and 1.17, respectively (Figure 3A), while the ratios of reduced to oxidized Ub-10 before and after extraction were 0.41 and 0.34, respectively (Figure 3B). Thus, although some of the reduced ubiquinones may be selectively lost during processing of the lens samples, most should have been recovered. We observed that reduced Ub-9 is the major Ub in rat liver (Figure 2B), as reported by others [11].

We found rat lenses to typically contain 80-85% oxidized Ub-9 and 15-20% oxidized Ub-10. The apparently greater than 15-20% contribution of Ub-10 to the total in rat lens indicated by the Ub-10 peak size in Figure 2B is due to the presence of an impurity in the Ub-6 internal standard with an elution time like that of Ub-10. The calculated concentration of lens Ub-10 was corrected for the presence of the impurity (0.035 μg in this assay). Lens substances which eluted before Ub-9 are unidentified.

Concentration and distribution of ubiquinones in lenses: Mouse and rat lenses contain about 3 μg of ubiquinone (mainly Ub-9)/g wet lens (Table 1). Reduced ubiquinone 9 and 10 (ubiquinols) were not detected. Prior to opening of the eyes, rat lenses (11 days of age) contained 2.26 μg of Ub/g lens, 92% was oxidized Ub-9, and 8% oxidized Ub-10. No reduced
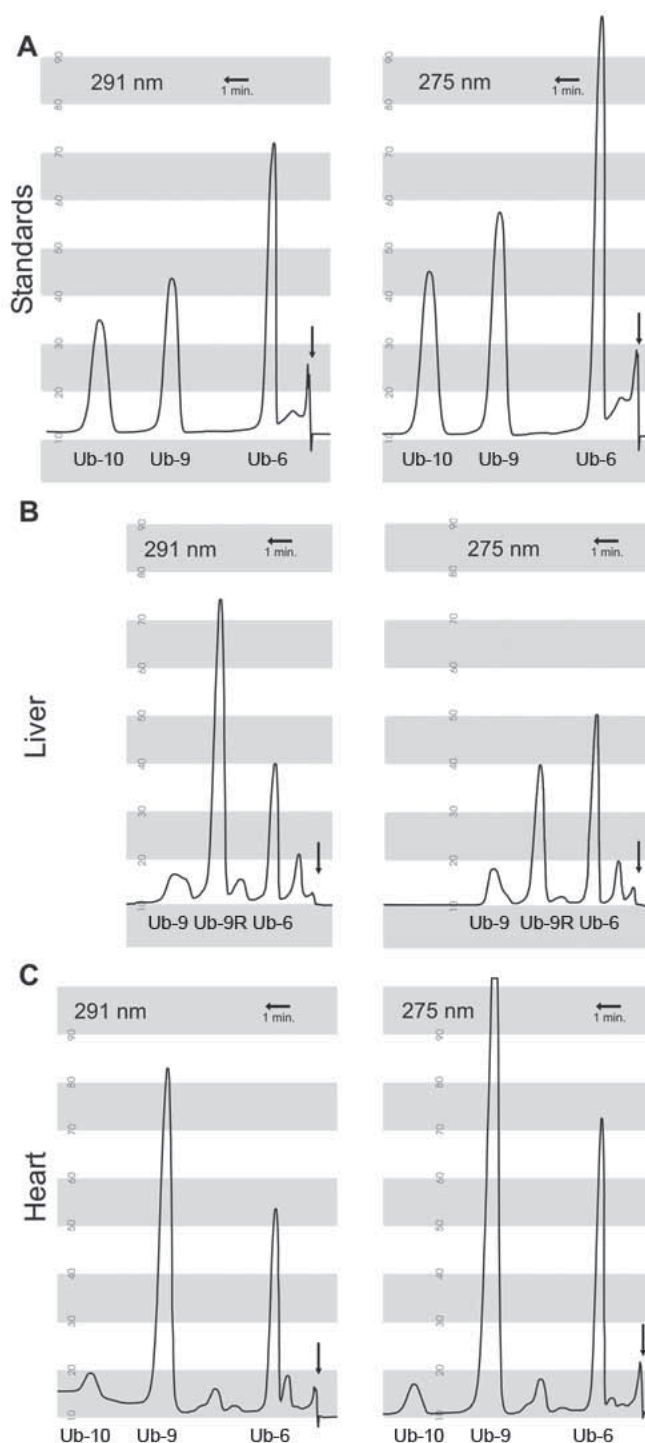

Figure 1. HPLC profile of ubiquinones examined at 275 nm and 291 nm. UV absorbance of oxidized ubiquinones are less at 291 nm compared with 275 nm. Reduced ubiquinones absorb more at 291 nm than 275 nm [26]. Peaks for ubiquinone-6 (Ub-6), ubiquinone-9 (Ub-9), reduced ubiquinone-9 (Ub-9R; ubiquinol-9), and ubiquinone-10 (Ub-10) are labeled. **A**: Absorbance of standards at 275 and 291 nm. **B**: Absorbance of rat liver ubiquinones at 275 and 291 nm. **C**: Absorbance of rat heart ubiquinones at 275 and 291 nm. Vertical arrows mark the solvent front. Horizontal arrows mark one minute. Tracings are from right to left.

ubiquinone was detected. Human and bovine lenses both contained 0.3 to 0.4 µg (solely Ub-10)/g lens. Although the epithelium of rat lenses contained a 3-4 times higher concentration of total ubiquinone than the cortex and nucleus (Figure 4A), it accounted for only about 10% of the total ubiquinone in the whole lens (Figure 4B). The nuclear region contained about 60% of the total.

The ubiquinone content of the human lens fiber cell mass appeared to increase with age (Table 2). The contribution of the epithelial fraction to the lens total decreased with age, from about 45% of the total Ub in the 16 year old lens to about 15% in the 70 year old lens.

Feeding young Sprague-Dawley rats chow containing 2% Ub-10 (about 200 mg/kg/day) for 17 days produced a 25 fold increase in the Ub-10 concentration in liver (from about 2.5 µg/g to 62 µg/g; Figure 5A,B). Supplementation did not change the concentration of the liver's main Ub, reduced Ub-9 (ubiquinol-9). It remained at about 180 µg/g liver. The increase in liver total Ub, from 224 µg/g to 285 µg/g, was not quite statistically significant. The concentration and composition of Ubs in whole lens was unaffected by supplementation (Figure 5C,D). Oral treatment with 200 mg of simvastatin/kg/day for 16 days led to about a 20% decrease in the concentration of total ubiquinone in the lenses of both SD and CT rats (Figure
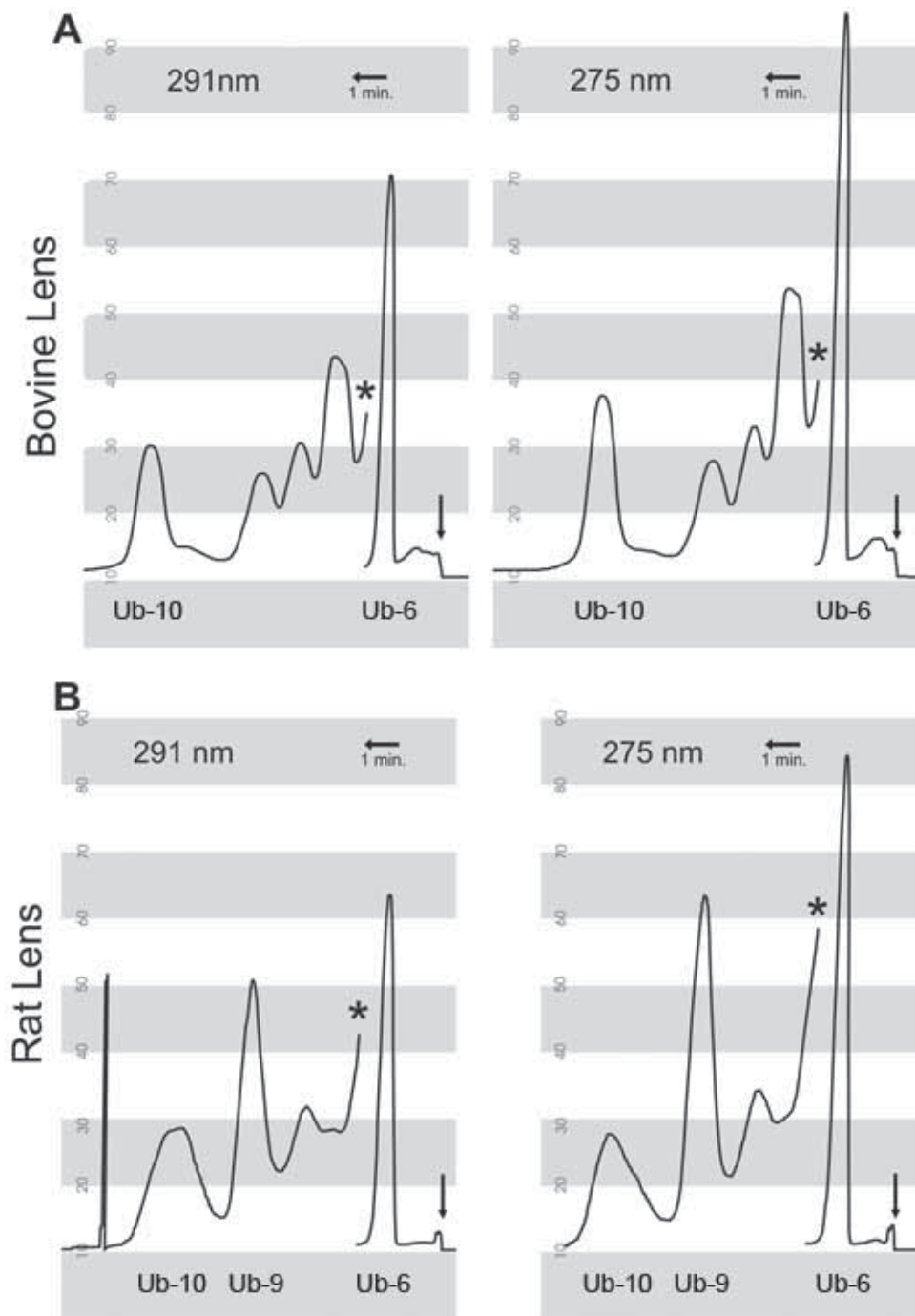

Figure 2. HPLC profile of lens ubiquinones examined at 275 nm and 291 nm. **A**: The separations at 275 and 291 nm each show bovine lens ubiquinones recovered from the equivalent of 1.2 g of a whole lens homogenate. Detector sensitivity was increased by twenty fold (asterisk) following elution of the internal standard (6.06 µg of Ub-6). **B**: The separations at 275 and 291 nm each show rat lens ubiquinones recovered from 48 mg of a whole lens homogenate. Detector sensitivity was increased by 62.5 fold (asterisk) following elution of the internal standard (4.545 µg of Ub-6). The concentration of Ub-10, estimated from the area under the curve, was corrected for the presence of a Ub-10-like impurity in the internal standard. Peaks eluting prior to Ub-9 (5.7 min) are unidentified. Vertical arrows mark the solvent front. Horizontal arrows mark one minute. Tracings are from right to left.

6). The decrease became statistically significant when the pooled control SD plus CT lenses was compared to the pooled treated SD plus CT lenses. The percent contribution of Ub-9 and Ub-10 to the total was not significantly different between control and simvastatin treated rats.

DISCUSSION

The identity and concentration of ubiquinones measured in rat lenses should accurately describe the status in the lens, since ubiquinone compositions and levels measured in other rat tissues were similar to those reported by Aberg et al. [11]. For example, we found rat liver contained about 220 μg of Ub-9/g which was about 80% reduced, while Aberg et al. [11]

reported 130 μg of Ub-9/g liver which was about 85% reduced. Measurement of ubiquinones in the lens required higher analytical sensitivity than for liver. Typically 70-100 mg of lens tissue (a pool of 4 or more young rat lenses) was extracted and 91% (0.1/0.11 ml) was injected onto the HPLC column. Instrument sensitivity after elution of the internal standard (usually 9.1 μg of Ub-6) was increased by 25 to 100 fold in order to recognize the lens' endogenous ubiquinone.

Intact rat lenses contained between 2.5 to 3.0 μg/g wet lens of total ubiquinone of which 80-90% was Ub-9 and the remainder Ub-10. Since a similar level was found in lenses of rats prior to opening of the eyes (11 day of age), light per se does not appear to degrade lens Ub. No reduced ubiquinones
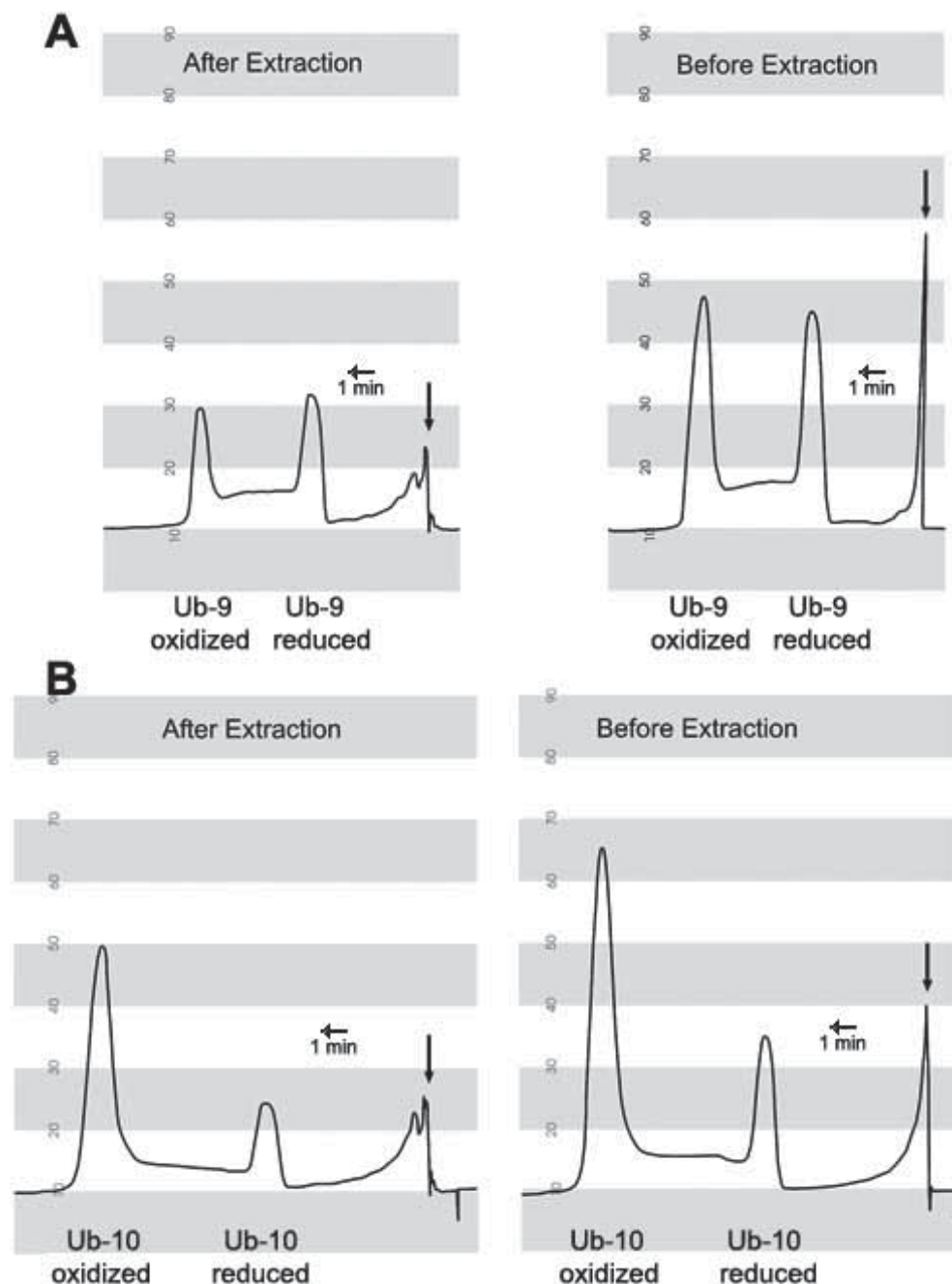

Figure 3. Recovery of reduced ubiquinone. Mixtures (10 μg total) of reduced and oxidized Ub-9 (**A**) and reduced and oxidized Ub-10 (**B**) were subjected to direct HPLC analysis (Before Extraction) and HPLC analysis after being carried though the homogenization and extraction procedure (After Extraction) used to recover lens ubiquinones. The ratio of reduced to oxidized was compared before and after extraction. Vertical arrows mark the solvent front. Horizontal arrows mark one minute. Tracings are from right to left.

were detected in lens at any rat age. Since ubiquinones are membrane components [13] and lens membranes account for perhaps 4% of lens volume [29,30], the concentration in membrane might approach 100 μg/g of membrane. Even higher concentrations of ubiquinones could be found in membranes of the epithelium. In spite of the high concentration of ubiquinones in the epithelium, the cortex and nucleus accounted for about 90% of the total content in the rat lens and about 80% in the older human lens. We suggest that the ubiquinones of the cortex and nucleus originated from both salvage of ubiquinones from mitochondria that were imported into the lens mass with differentiated epithelial cells and from de novo synthesis. Essentially all of the cholesterol synthesis in the lens occurs in elongating cortical fiber cells [31] and the isoprene tail of ubiquinones is derived from intermediates in the cholesterol synthesis pathway [14].

Ubiquinone may function in the lens as an antioxidant and/or modulator of membrane order as it does in other tissues [32]. The levels measured in lens were 5 to 10 times greater than the next most abundant lipid antioxidant, α-tocopherol. α-Tocopherol was shown to be widely distributed in the young rat lens at concentrations ranging from 0.2 to 0.5 μg/g lens [33]. In spite of the relatively high concentration of ubiquinone, the absence of detectable reduced ubiquinone in the lens confounds assigning it an antioxidant function since the reduced form is required to support this role [11,13,24]. Perhaps the presence of low concentrations of reduced ubiquinone that escaped detection in a small fraction of the whole lens might have been adequate to support a role as an antioxidant. In heart, only 10-20% of the ubiquinone is reduced (Figure 1C and [11]). About 90% of the lens' ubiquinone was present in the cortex plus nucleus that lack the mitochondria needed to regenerate the reduced forms. Therefore, the ubiquinone present in these lens regions might represent nonregenerable, spent antioxidant which has other functions, such as modulating fluidity of cortical and nuclear membrane.

Because there has been marked interest in the possibility of improving the lens' defense against oxidation by dietary supplementation with antioxidants, we tested whether supple-

TABLE 1. CONCENTRATION OF UBIQUINONE IN LENSES OF VARIOUS SPECIES

Species	Age	Pools	Lenses/pool	Wet lens (μg/g)
Mouse	adult	1	10	3.32
Rat	50 days	6	4	2.91 ± 0.19
Rat	11 days	1	13	2.26
Bovine	adult	1	2	0.286
Human	16-75 year	3	1	0.426 ± 0.067

There was marked variability in the concentration of total ubiquinone in lens between the different species. In the wet lens column, the values are means±the standard errors of the mean. In the Bovine row, the cortex contained 0.269 μg/g and the nucleus contained 0.304 μg/g.

TABLE 2. DISTRIBUTION OF UBIQUINONE-10 IN HUMAN LENSES

Age	Ub-10 (ng)/ epithelium	Ub-10 (ng)/ lens body
16	28.3	34.6
45	16.1	49.7
70	14.3	82.8

The epithelium capsule accounted for an average of 8% of the lens' total weight. Only Ub-10 was present in these lenses.
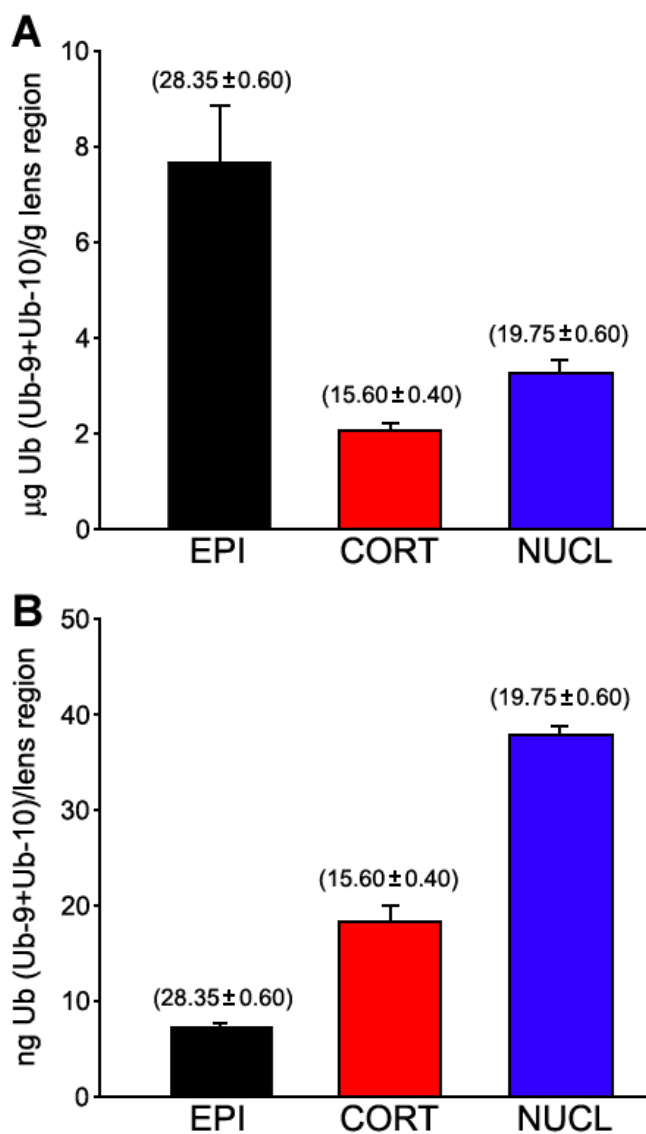

Figure 4. Concentration and content of ubiquinones in rat lens regions. Four separate pools of capsules (epithelium), cortex, and nucleus, each from 8-10 lenses of 50 day old Sprague-Dawley rats, were weighed and homogenized. The ubiquinones were then extracted and quantitated by HPLC. **A**: Concentration (μg/g lens region) of total ubiquinones (Ub-9 plus Ub-10). **B**: Content (ng/lens) of total ubiquinones (Ub-9 plus Ub-10). Numbers in parentheses are the percentage of Ub-10 of the total ubiquinone; the remainder was Ub-9. Error bars represent the standard error of the mean.

menting the rat's diet with a high level of Ub-10 could alter the ubiquinone content of liver and lens. We found that supplementation greatly increased the Ub-10 concentration in liver without changing lens levels. These findings are consistent with those of others showing that dietary supplementation with Ub-10 significantly increases blood and liver concentrations, but not that of heart, kidney, or brain [27]. Crane, in his review of coenzyme Q10 (Ub-10), describes that membrane levels of ubiquinones may be normally saturated and therefore resistant to further increase by supplementation [13].

Our initial interest in ubiquinones and the lens came from the suggestion that upregulation of ubiquinone synthesis and levels in the lens might be a factor in protecting Sprague-Dawley (SD) rats from the simvastatin induced cataract. Treatment of young Chbb:Thom (CT), but not SD, rats with simvastatin for two weeks produced irreversible damage to the lens which resulted in lens opacification within one to three additional weeks [22]. Sprague-Dawley, but not CT, rats re-
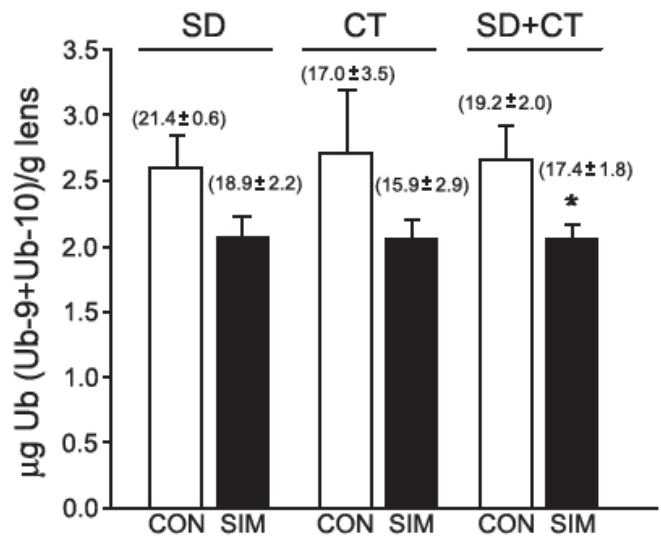

Figure 6. Effect of treatment with simvastatin on lens concentrations of ubiquinones. Twenty day old Sprague-Dawley (SD) and Chbb:Thom (CT) rats were given 200 mg of simvastatin/kg body weight per day for 16 days by gavage or were untreated. CON represents the untreated controls and SIM represents simvastatin treated. SD control and treated groups each consisted of 5 pools of whole lenses, 4 lenses/pool. The CT control group consisted of 5 pools of 4 lenses/pool. The CT treated group contained 4 pools of 4 lenses/pool. Values in parentheses are mean percent Ub-10±SEM; the remainder was Ub-9. The asterisk indicates that the difference (decrease) in the total concentration of ubiquinones in the lens of control and treated rats (Sprague Dawley and Chbb:Thom data pooled) was statistically significant to the P(t) level of 0.039 as measured by the Students t test (two tailed).
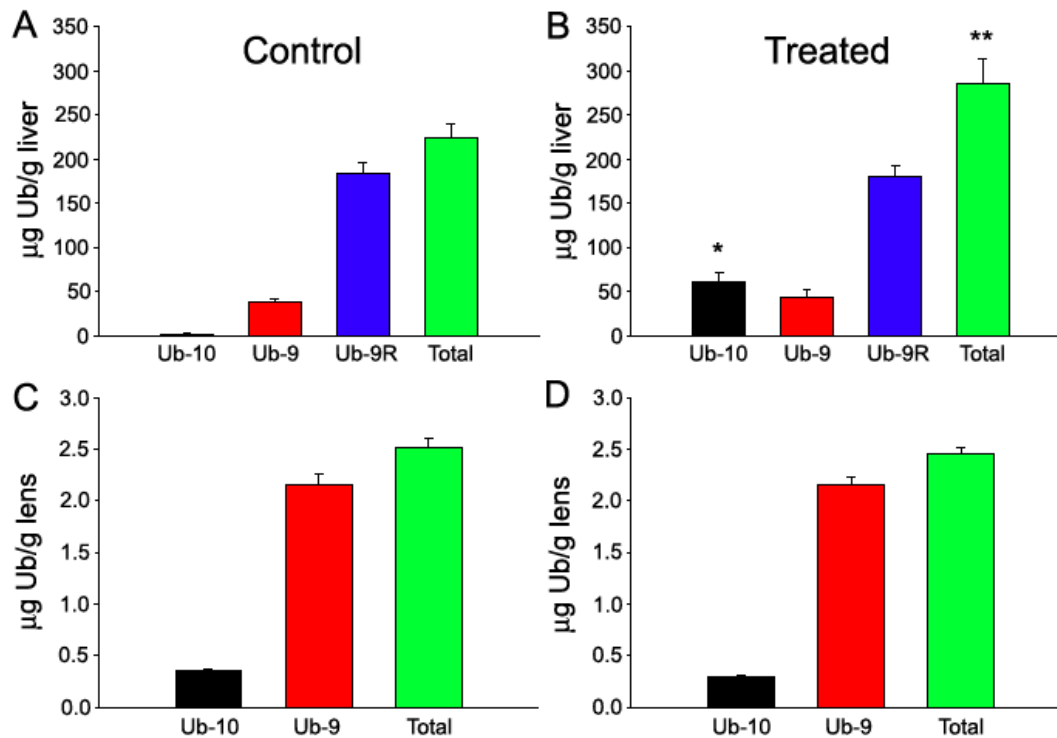

Figure 5. Effect of dietary supplementation with ubiquinone-10 on the ubiquinone levels of rat liver and lens. Starting at 20 days of age, Sprague-Dawley rats were fed chow containing Ub-10 for 17 days. The dose was approximately 200 mg Ub-10/kg body weight per day. Samples of liver from individual rats (0.35-0.54 g) and pools of whole lenses (4-6 lenses) were homogenized and the ubiquinones extracted. The ubiquinones were fractionated as ubiquinone-10 (Ub-10), ubiquinone-9 (Ub-9), and ubiquinone-9 reduced (Ub-9R; ubiquinol-9). **A**: Liver from control rats (n=6). **B**: Livers from treated rats (n=6). The single asterisk indicates that the concentration of Ub-10 in the liver of treated rats is statistically different from the concentration in the liver of untreated-control rats to a P(t) value of less than 0.0001 measured by the Students t test. The double asterisk indicates that the difference in total Ub concentration in the liver of treated compared to control was note quite statistically significant (to only a P(t) of 0.09). This is because the level of reduced Ub-9, which accounts for the major Ub in rat liver, was not increased. **C**: Lens from control rats (3 pools of 4-6 lenses). **D**: Lens from treated rats (4 pools of 4 lenses). Error bars represent the standard error of the mean.

sponded to simvastatin treatment by a marked (4-5 fold) increase in the rate of lens cholesterol synthesis following one and two weeks of treatment. Cholesterol synthesis by CT rat lenses remained at basal levels with treatment. Failure of CT rat lenses to upregulate HMG CoA synthase, the enzyme which supplies the substrate for the rate controlling enzyme (HMG CoA reductase), is believed to explain the inability of these lenses to upregulate cholesterol synthesis. We speculated that something generated from the cholesterol synthesis pathway in increased amounts protected the SD lens from damage [22]. That substance might have been ubiquinone.

The synthesis of ubiquinones begins with the attachment of isoprene diphosphate to p-hydroxybenzoic acid [14]. Since the isoprenes arise from intermediates in the cholesterol synthesis pathway, upregulation of the pathway could lead to higher concentrations of isoprenes and perhaps increased ubiquinone synthesis. This of course assumes that the concentration of isoprenes is rate limiting in this synthesis. Our findings did not support this assumption. Levels of ubiquinones actually decreased by about 20% in lenses of both SD and CT rats treated with simvastatin for 16 days (Figure 6). Others have shown that treatment of both humans and rats with statins produces similar decreases in ubiquinone levels in other tissues (e.g., blood, liver, and heart) [13,34-36]. These investigators assumed that the decreases in tissue ubiquinone levels were due to statin inhibition of cholesterol synthesis. Unlike us, they did not simultaneously measure statin effects on rates of cellular cholesterol synthesis. We suggest that the decrease in ubiquinone levels in lenses of both the SD and CT rats reflects a response to stress induced by simvastatin. Oxidative stress in rats can cause a decrease in tissue levels of Ub-9 and Ub-10 [25]. Statins can have pronounced effects on cells independent of inhibiting cholesterol biosynthesis [37,38], and, in fact, proteomic studies show that treatment of rats with high dose simvastatin induced changes in many sets of cellular stress proteins [39].

ACKNOWLEDGEMENTS

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