

Comparative Topical Absorption and Antioxidant Effectiveness of Two Forms of Coenzyme Q₁₀ after a Single Dose and after Long-Term Supplementation in the Skin of Young and Middle-Aged Subjects

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Abstract

Coenzyme Q₁₀ is an endogenous antioxidant found in the skin along with vitamins A, C and E. Coenzyme Q₁₀ is used increasingly in cosmetic products and is advertised as a skin energizer, protector against skin aging, skin repairer, and an anti-wrinkling agent. As the outermost layer of skin, the stratum corneum is the interface between the body and the environment and requires antioxidants to protect it and the epidermis and dermis below it. Nutrient levels in the stratum corneum correlate with those in the skin. Two forms of coenzyme Q₁₀ (pure coenzyme Q₁₀ and a yeast-based coenzyme Q₁₀) were investigated in a commercial vehicle. Two groups of subjects were tested aged 21-29 and 51-70, respectively. Coenzyme Q₁₀ absorption in the stratum corneum was determined after a 1-hour application and ethanol extraction. Significantly more yeast coenzyme Q₁₀ was absorbed than the pure coenzyme Q₁₀ and the middle-aged subjects absorbed about twice as much coenzyme Q₁₀ as did the younger subjects. Skin antioxidants were significantly increased by yeast coenzyme Q₁₀ but not by pure coenzyme Q₁₀. Peroxides declined in the stratum corneum after twice daily application of coenzyme Q₁₀ with both forms but the decrease was greater with the yeast form. The older subjects had significantly higher baseline levels of lipid peroxides than did the younger group, indicating an increase in skin oxidative damage with age. The yeast coenzyme Q₁₀ is the superior form of coenzyme Q₁₀ for human skin application.

Keywords: Coenzyme Q₁₀, human skin, lipid peroxides, antioxidants, stratum corneum

INTRODUCTION

The ubiquinones or coenzyme Q are a family of lipid-soluble benzoquinones that are widely distributed in living organisms and present in the hydrophobic interior of the phospholipid bilayer of virtually all cellular membranes. The quinone head can alternately assume three different redox states: ubiquinone (Q), the fully oxidized form; ubisemiquinone (•QH), the partially reduced form which is also a free radical; and ubiquinol (QH₂), the fully reduced form. The isopropenoid side chain has various lengths (30-50 carbon atoms). The different forms of coenzyme Q are designated by the number of isopropenoid units in the side chain (2-10), with there being 9 or 10 in mammals and 2 in bacteria.

Coenzyme Q₁₀ is the human form of coenzyme Q, and it is ubiquitous in human tissue. The level is highest in organs with high rates of metabolism such as the heart and liver [1].

Coenzyme Q can play multifunctional roles in cells, three of which have been well documented. First, the most studied function is that of coenzyme Q in its quinone form. It transfers electrons in the mitochondrial electron transport chain from complexes I and II to complex III, during which protons are passed to the outer mitochondrial compartment thus generating a transmembrane electrochemical gradient [2]. Secondly, it is now well established that the quinol form of coenzyme Q acts as a potent antioxidant in the inner mitochondrial membrane. It inhibits lipid

peroxidation either by scavenging free radicals directly or by reducing the α -tocopheroxyl radical to α -tocopherol [3]. Thirdly, it acts as a pro-oxidant in that autoxidation of the semiquinone form is the major intracellular source of superoxide and hydrogen peroxide generation [4]. Thus coenzyme Q can be both an antioxidant and a pro-oxidant.

The skin is the body's first line of defense against the radicals formed by UVA light from the sun. These radicals cause oxidative damage to lipids, proteins and DNA. The first barrier in the skin is the stratum corneum, which is composed of surface lipids, corneocytes and intercellular lipids. In the skin, the reduced form of coenzyme Q, ubiquinol-10, acts as an antioxidant, with 10-fold higher levels in the

epidermis than in the dermis [5]. After UV exposure, mouse skin ubiquinol-9 was depleted more rapidly than α -tocopherol, suggesting that coenzyme Q₉ is the first line of defense [6].

Oxidative stress is thought to play a role in the aging process, which is a combination of chronological aging and photoaging [7]. Using human fibroblasts it could be shown that prolonged cell culture mimics chronological aging and that coenzyme Q₁₀ significantly increased cell proliferation [1]. Coenzyme Q₁₀ was also found to increase the life span of the worm *C. elegans* by reducing oxidative stress, namely by decreasing superoxide [8]. Photoaging is caused by both UVA and UVB. Photoaged skin is characterized by wrinkles and a lack of tensile strength, which is normally provided by the dermis. Hyaluronic acid is lowered during photoaging, which also causes disorganization of the dermal matrix due to the degradation of collagen fibers. Coenzyme Q₁₀ reduced the detrimental effects of UVA on dermal fibroblasts, which maintain the dermal matrix [1]. The level of coenzyme Q₁₀ declines in heart and brain with age [9, 10]. It increases in the stratum corneum from childhood to maturity and then decreases with age [11]. Coenzyme Q₁₀ also decreases in the epidermis in a linear fashion with age [1] and thus the skin is a tissue that would benefit from supplementation.

In order to act as an antioxidant, coenzyme Q₁₀ needs to penetrate into the skin. When a solution of 1% coenzyme Q₁₀ in olive oil was topically applied to rats, coenzyme Q₁₀ was found to reach levels in living rat skin of 8 μ g/g after 2 hours and 15 μ g/g after 4 hours. There also was a dose-response relationship between the amount of coenzyme Q₁₀ applied and the coenzyme Q₁₀ skin concentration [12]. In porcine skin, coenzyme Q₁₀ administered in ethanol vehicle penetrated into the stratum corneum, with 20% penetrating further into the viable layers of the epidermis and 2% into the dermis [1].

In this study we investigate two forms of coenzyme Q₁₀ to determine which form has the greater absorption and antioxidant efficacy after topical application to young and older humans.

EXPERIMENTAL

Subjects

Eight subjects (4 males and 4 females) aged 21-29 years (average 24 ± 3) and 8 subjects (3 males and 5 females) aged 51-70 years (average 56 ± 7) participated with informed consent. None had any current history of dermatological problems, and they were told to avoid excessive sun exposure during the study. No subjects were taking cholesterol-lowering drugs as determined by a medical history questionnaire.

Skin treatment absorption study

Five young subjects and 4 middle-aged subjects participated in this study. A 75 mg portion of ointment containing either the yeast or the pure form of coenzyme Q₁₀ (ubiquinone) was smeared on a 25-mm diameter circular area of the inner wrist (randomly chosen) and allowed to absorb for one hour. The stratum corneum coenzyme Q₁₀ of the volunteers was extracted from skin by applying a 25-mm diameter glass cylinder to skin and then rinsing that area three times with 1 mL of anhydrous, non-denatured ethanol [13]. The 3 mL ethanol extract was concentrated 10 times by evaporation and reconstitution with 300 μ L of ethanol. This procedure oxidizes ubiquinol to ubiquinone. Extracts were stored at -20°C until assayed.

Skin treatment long-term study

The subjects were given (blinded) two kinds of lotion (coenzyme Q₁₀ and yeast coenzyme Q₁₀), which they applied to the same arm (ventral forearm) in a dose of approximately 75 mg twice every day (morning and evening) for one month using a 1 mL syringe and dispensing 0.1 mL. This applies 75 μ g of coenzyme Q₁₀. Extraction was done as described for the absorption study in the morning after the last evening application. No lotion was then applied for 30 days and the extraction was repeated. There was no washout period.

Coenzyme Q₁₀ (oxidized form, ubiquinone) and all reagents were obtained from Sigma Chemical Co., St. Louis, MO, USA. Yeast coenzyme Q₁₀ was 8% coenzyme Q₁₀ by weight in a glycoprotein matrix

and was obtained from Phamachem Labs, Kearny, NJ, USA. Jergens Ultra Healing Lotion (Andrew Jergens Co., Cincinnati, OH, USA) was used as the vehicle. This lotion does not contain any added vitamin antioxidants. The ointments with USP coenzyme Q₁₀ and yeast coenzyme Q₁₀ were prepared by weighing. For example 10 mg of coenzyme Q₁₀ was mixed with 10 grams of the lotion or 125 mg of 8% yeast coenzyme Q₁₀ was mixed with 10 grams of the lotion to make a 1 mg coenzyme Q₁₀/g lotion. The lotion was melted in a furnace at 95°C for 2 minutes, uniformly mixed with coenzyme Q₁₀ and sealed during storage.

Assays

Coenzyme Q₁₀ Assay. Ubiquinone was used as the standard. A Hewlett Packard Series 1050 High Performance Liquid Chromatograph was equipped with a Perkin Elmer cartridge C18 column (4.6 mm x 3.5 cm). The mobile phase solvent was 25% isopropanol/75% ethanol and the detector wavelength was 275 nm. The flow rate was 2.0 mL/min.

Antioxidant (AOX) Assay [14]

The AOX reagent working solutions were 300 mM acetate buffer (pH 3.6), 10 mM 2,3,6-tripyridyl-s-triazine (TPTZ) in 400 mM HCl, and 20 mM FeCl₃. AOX reagent was prepared fresh daily by combining 20 mL of acetate buffer, 0.3 mL of FeCl₃ and 0.3 mL of TPTZ. A standard solution of 1 mM α -tocopherol in methanol was prepared and standard dilutions from 50 μ M to 500 μ M were made for the standard curve. Methanol was utilized as the blank. The automated reaction was performed in a temperature-controlled spectrophotometer (Spectronic Genesys 5, Milton Roy, Rochester, NY). A disposable cuvette containing 2.0 mL of the AOX working reagent was placed in the spectrophotometer at a wavelength of 593 nm and constant temperature of 37°C for 6.0 minutes and the absorbance was recorded. 50 μ L of blank, standard or sample were added to separate cuvettes that were covered tightly with parafilm and inverted 5 times before they were replaced in the spectrophotometer for 6.0 minutes and the absorbance was recorded again. Each sample was measured in duplicate.

Lipid hydroperoxides and hydrogen peroxide assay [15]

Reagent (1) was 25 mM ammonium iron (II) sulfate in 110 mM perchloric acid. Reagent (2) was 100 mM sorbitol and 125 μ M xylenol orange in 110 mM perchloric acid. The working reagent was prepared by mixing 1 volume of Reagent (1) with 100 volumes of Reagent (2). Fresh standards were prepared each day for use from 0.025 M stock solution of H_2O_2 to yield 1-100 μ M standards. Nanopure water was used as the blank. One mL of working reagent was pipetted into each cuvette and 10 μ L of blank, standards and samples were added to the appropriate cuvettes. The solutions in the cuvettes were mixed thoroughly and incubated for 30 minutes at room temperature. Absorbance was read at 560 nm.

Statistics

Results are given as mean values and standard errors of the mean. Statistical differences between treatments were evaluated by a paired Student's t test and between groups by a Student's t test using Sigma Stat 3.01 for Windows (Systat, Richmond, CA, USA). A $p < 0.05$ was considered significant.

RESULTS

The skin absorption study data is shown in the bar graph of **Figure 1**. The yeast form had 34% more absorption than pure coenzyme Q_{10} in the young subjects, and 23% more in the middle-aged subjects. The older subjects absorbed significantly more of the yeast form than pure coenzyme Q_{10} , as did all subjects taken together ($p < 0.05$). The older subjects absorbed over twice as much coenzyme Q_{10} as the young subjects, independent of the form, with $p < 0.001$ in the Student's t test.

The antioxidants assayed according to the ferric reducing ability of plasma (FRAP) procedure used in this study include small molecules such as vitamin C, tocopherol, uric acid and larger molecules such as albumin and bilirubin. The reduced form of coenzyme Q_{10} , ubiquinol, would also be analyzed by this antioxidant assay. The amounts of antioxidants measured in the

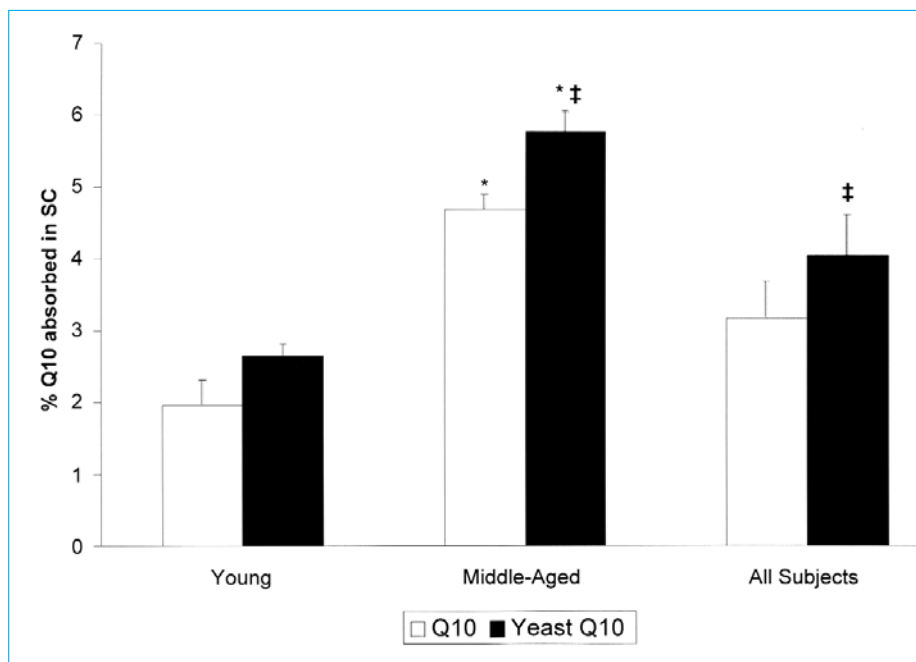


Figure 1: Percent coenzyme Q_{10} (Q_{10}) absorption in human stratum corneum (SC) after 1 hour of topical application of two different forms of coenzyme Q_{10} to young and middle-aged subjects. Results are reported as the mean \pm SEM, * $p < 0.001$ vs. younger group, † $p < 0.05$ vs. Q_{10} .

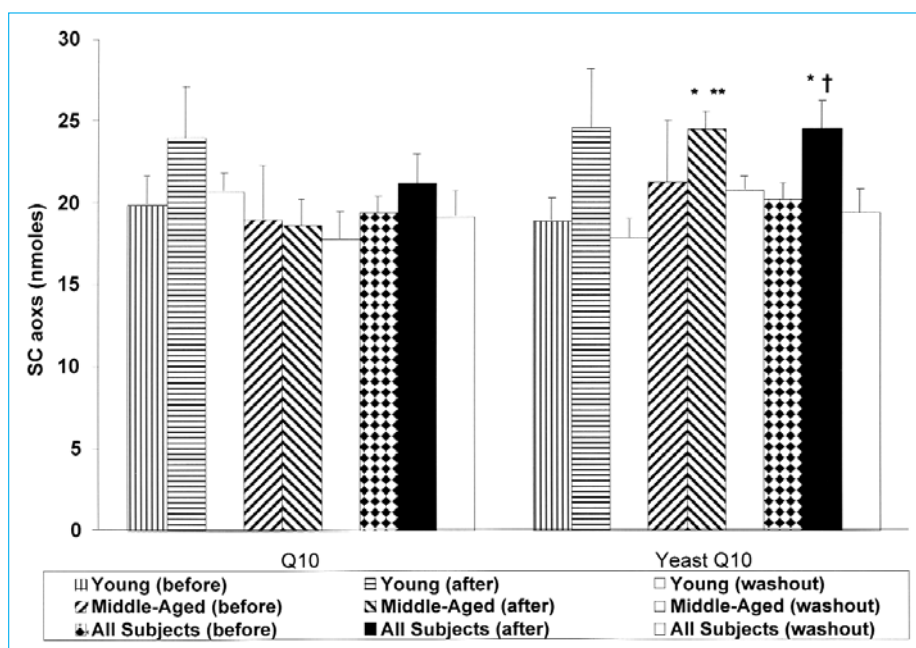


Figure 2: Human stratum corneum (SC) antioxidants (nmole units) before and after 1 month of topical application of two forms of coenzyme Q_{10} (Q_{10}) to young and middle-aged subjects. A washout period of 1 month with no treatment followed the coenzyme Q_{10} application. Results are reported as the mean \pm SEM. * $p < 0.05$ vs. washout, † $p < 0.01$ vs. after coenzyme Q_{10} in the middle-aged group, ‡ $p < 0.05$ vs. before and after washout.

stratum corneum are found in **Figure 2**. The inter-subject variation was large and not all subjects showed an increase after

topical application of the antioxidants or a decrease after the washout period. However, six of the eight young subjects

had an increase in antioxidants following yeast application and seven of the eight young subjects experienced a decrease in antioxidants after the washout period following yeast application. The young subjects experienced a greater change in stratum corneum antioxidants following application and washout than did the middle-aged subjects, irrespective of the form of coenzyme Q₁₀ applied. In fact, the middle-aged subjects had a small, non-significant 2% decrease in skin antioxidants after application of the pure coenzyme Q₁₀ but a 13.3% increase after the yeast form. In middle-aged skin the levels of antioxidants after application of the yeast form were significantly higher than after pure coenzyme Q₁₀ ($p < 0.01$). Moreover, in the middle-aged group the skin antioxidants were significantly increased after application of the yeast form compared with after the washout period ($p < 0.05$). Considering all the subjects together, the yeast form caused a significant increase in antioxidants compared with baseline and after washout ($p < 0.05$). Washout returned the stratum corneum antioxidants to the baseline levels.

The results of the lipid hydroperoxides and hydrogen peroxide assay are depicted in **Figure 3**. The inter-subject variations are

very large with this assay. The yeast coenzyme Q₁₀ caused the largest changes in peroxides; for young subjects the level decreased by 61% ($p < 0.01$), and washout increased the value by 80%. In comparison, pure coenzyme Q₁₀ produced a 33% decrease in peroxides ($p > 0.05$) and washout increased the value by 37%. There was a significant 53% decrease after topical application of pure coenzyme Q₁₀ in middle-aged subjects ($p < 0.01$). For middle-aged subjects the yeast form produced a 62% decline in peroxides ($p < 0.01$), and washout increased peroxides by 44%. With all subjects combined, the yeast form produced significant differences after application ($p < 0.01$) and again after washout ($p < 0.05$). The pure coenzyme Q₁₀ combined groups also experienced a significant reduction (45%) in peroxides ($p < 0.01$). Washout did not completely return the levels to those of the baseline; the peroxide levels were always lower than baseline but not significantly different.

DISCUSSION

Although many scientific articles have been written on the medical benefits of oral coenzyme Q₁₀ [16, 17], little has been

published on the effects of topical application. However, there are numerous cosmetic formulations containing coenzyme Q₁₀ on the Web, with claims of skin repair and regeneration as well as anti-wrinkling, anti-aging and antioxidant effects. Although a number of articles comparing the human plasma bioavailability of various forms of coenzyme Q₁₀ (powder, gel, emulsified, hydrosoluble) have been published, there have been no published reports comparing the topical effects of different coenzyme Q₁₀ forms. We have shown that significantly more of the yeast form of coenzyme Q₁₀ is absorbed than of the pure coenzyme Q₁₀ after one hour of application in the middle-aged group and in both groups combined. A larger population of younger subjects might have revealed a significant difference between the forms. Twelve hours after the last application (overnight) in the 30-day study no coenzyme Q₁₀ was found in the stratum corneum. This agrees with the results of an earlier study of 60 days' duration with a 0.05% coenzyme Q₁₀ lotion [18]. Overnight the coenzyme Q₁₀ had moved from the stratum corneum to deeper epidermis layers and to the dermis. Middle-aged subjects absorb more of the lipophilic coenzyme Q₁₀ probably due to differences in hydration of the stratum corneum, which is less for the older subjects [19], and the corneocyte size, which is smaller for older subjects. More benzoic acid, a more hydrophilic molecule than coenzyme Q₁₀, was also absorbed in subjects aged 45-55 years than in those aged 20-30 years [20]. Antioxidants in the skin were measured by the antioxidant (AOX) assay and include ethanol-soluble antioxidant vitamins. Thus the antioxidant assay measured the total low molecular weight antioxidant capacity/defense in the skin. This assay has previously been used for food and beverage antioxidant analysis [21], and for plasma [14]. To our knowledge this is the simplest and cheapest measure of stratum corneum antioxidant capacity. Our results indicate that chronic application of coenzyme Q₁₀ in the yeast form provides significantly more antioxidants to the stratum corneum and surface lipids in both young and middle-aged subjects. Young subjects experienced a greater increase in stratum corneum antioxidants than did the older subjects. In fact the middle-aged subjects had no increase at all in

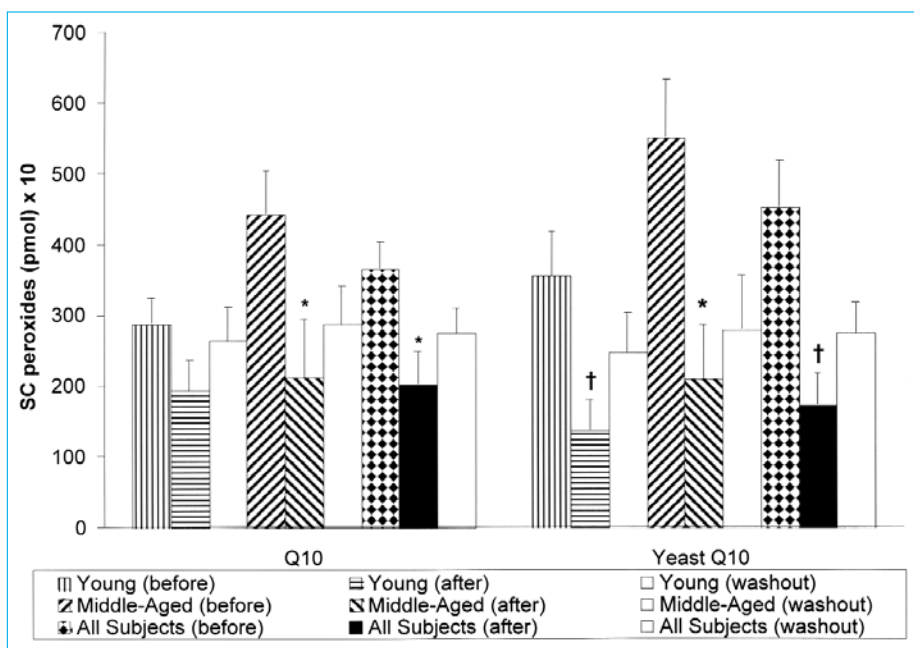


Figure 3: Human stratum corneum (SC) peroxides (pmole x 10 units) before and after 1 month of topical application of two forms of coenzyme Q₁₀ (Q10) to young and middle-aged subjects. A washout period of 1 month with no treatment followed the coenzyme Q₁₀ application. Results are reported as the mean \pm SEM, * $p < 0.01$ vs. before, † $p < 0.05$ vs. before and washout.

skin antioxidants with pure coenzyme Q₁₀. There was no difference in baseline stratum corneum antioxidants between the young and older subjects in the present study. This agreed with the finding of other workers that there is no clear correlation of age with changes in antioxidant concentrations [22].

Peroxides in the stratum corneum provide a measure of oxidative damage and stress and consist of lipid hydroperoxides and hydrogen peroxide. Baseline peroxides were significantly higher in the elderly subjects than in the younger subjects with 4,980 and 3,230 pmoles, respectively (p<0.05). This clearly indicates more oxidative damage to the stratum corneum of elderly subjects. Lipid peroxidation was found to increase with age in human skin [23]. In addition, the same group showed that superoxide dismutase, catalase, and glutathione peroxidase (antioxidant enzymes) decrease with age in skin fibroblasts. Also hydrogen peroxide and DNA damage increase with age in fibroblasts [24]. Our results confirm this finding, as lipid peroxides and the hydrogen peroxide resulting from decreased catalase activity were elevated with age in stratum corneum. Coenzyme Q₁₀ significantly lowered peroxides and the yeast form was more efficacious. Washout did not result in an elevation of stratum corneum peroxides back to baseline levels, indicating a lingering antioxidant effect of topical coenzyme Q₁₀. Hoppe et al. found that topical coenzyme Q₁₀ (0.3%) decreased the level of oxidation in the skin as measured by weak photon emission [1]. Coenzyme Q₁₀ also decreased UVA-induced increases in weak photon emission of skin [25]. Although there are numerous reports in the literature on topical antioxidants and skin protection from UV-induced oxidative stress, this is the first report indicating a decrease in oxidative damage in non-stressed skin as a result of coenzyme Q₁₀ antioxidant application.

CONCLUSION

Antioxidants (especially vitamin E) are routinely added to cosmetic formulations and advertisements tout the benefits. Certainly, human studies have convincingly demonstrated large photo-protective effects of both natural and synthetic anti-

oxidants when topically applied before UV exposure. Oxidative stress has also been linked to skin aging in numerous published studies. We have shown that coenzyme Q₁₀, most effectively in the yeast form, is capable of improving the antioxidant capacity of stratum corneum and decreasing the amount of oxidative damage in non-stressed skin after topical application. We hypothesize that the yeast matrix increases coenzyme Q₁₀ absorption and stabilizes coenzyme Q₁₀. An *in vitro* stability study on reduced coenzyme Q₁₀ performed in our laboratory showed that the yeast matrix provided increased stability. Since stratum corneum levels correlate strongly with percutaneous absorption [26], our findings indicate that human skin should be correspondingly affected. We also conclude that older subjects have substantially more oxidative damage to skin than younger subjects. This demonstrates the need for topical application of antioxidants throughout the human lifetime.

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