

Oxidative Damage, Skin Aging, Antioxidants and a Novel Antioxidant Rating System

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ABSTRACT

It is believed that oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to neutralize the reactive intermediates. Oxidative damage occurs because of both intrinsic and extrinsic mechanisms. Together, intrinsic and extrinsic damage are the primary causes of skin aging. The skin uses a series of intrinsic antioxidants to protect itself from free radical damage. Naturally occurring extrinsic antioxidants have also been widely shown to offset and alleviate these changes. Unlike sunscreens, which have an SPF rating system to guide consumers in their purchases, there is no widely accepted method to choose antioxidant anti-aging products. ORAC (Oxygen Radical Absorbance Capacity) and ABEL-RAC (Analysis By Emitted Light-Relative Antioxidant Capacity), are both accepted worldwide as a standard measure of the antioxidant capacity of foods, and are rating systems that could be applied to all antioxidant skincare products. The standardization of antioxidant creams could revolutionize the cosmeceutical market and give physicians and consumers the ability to compare and choose effectively.

INTRODUCTION

Oxidative changes are one of the primary causes of aging of the skin. Topical and oral antioxidants have been widely shown to improve and alleviate these changes.¹ The growing awareness of the importance of the use of topical antioxidants is creating the need for the evaluation and standardization of these products.

The utilization of Oxygen Radical Absorbance Capacity (ORAC) or Analysis By Emitted Light-Relative Antioxidant Capacity (ABEL-RAC), globally accepted standards of measurement of antioxidant capacity, in providing a foundation for the development of this type of numerical rating system, could revolutionize the cosmeceutical market and give both physicians and consumers the ability to compare and effectively choose between products.

The Science of Skin Aging

Oxidative damage occurs because of both intrinsic and extrinsic mechanisms. Intrinsic oxidation is due to the production and breakdown of adenosine triphosphate,¹ which is largely controlled by genetics. Extrinsic oxidative damage can be traced to environmental factors such as sunlight, smoking and other pollutants, an unbalanced diet, lack of exercise, stress and illness.¹

Oxygen is also a key player in free radical production, which leads to the signs of aging. Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily neutralize the reactive intermediates or easily repair the resulting damage. A free radical is defined as an atom or molecule with an unpaired electron.¹ These chemically reactive molecules are short lived and react at the place where they are created.¹ Free radicals are known to

promote oxidation of nucleic acids, proteins and lipids, and can damage intracellular structures, including DNA.²⁻⁴ Free radicals also interact with intracellular transcription factors, proteins that bind to specific sequences of DNA and thereby control the transcription of genetic information from DNA to RNA. They up-regulate transcription factors such as activator protein 1 (AP-1), and nuclear transcription factor-kappa B (NF-kB).^{2,5} AP-1 is responsible for the production of metalloproteinases that break down existing collagen, contributing to wrinkle formation.^{2,6} NF-kB up-regulates the transcription of pro-inflammatory mediators including interleukin-1 (IL-1), IL-6, and IL-8, and tumor necrosis factor alpha (TNF- α).^{2,7} These pro-inflammatory mediators serve to further activate the transcription factors AP-1 and NF-kB, resulting in additional damage.

It is the sum of these events that is responsible for skin aging. The free radicals that are the most biologically significant include superoxide anion, peroxy radical, peroxy nitrite radical and hydroxyl radical.¹ In addition, reactive oxygen molecules, which are not free radicals, are capable of initiating similar oxidative reactions that can generate free radicals. Together, the free radicals and the reactive oxygen molecules are called reactive oxygen species (ROS). Damage caused by ROS lead, in the skin, to melanocytic overproduction of melanosomes and to weakened elastin and collagen. These changes also lead to a slower turnover of new skin cells. The cumulative effect is skin wrinkling, laxness, fragility, dull appearance, mottled brown pigmentation and distinct dark spots. DNA mutations caused by oxidative changes may also produce abnormal keratinocytes leading in some cases to malignancy.

Reactive oxygen species, while leading to aging, also serve an important function of white blood cells by removing bacteria

and other foreign material. In this process, small amounts of intracellular and extracellular free radicals are formed. The human body has evolved the ability to produce enzymes and antioxidants which limit the damage that might be caused by these free radicals. During times of high oxidative stress, the body's natural antioxidants are not sufficient to control these free radicals, and excess damage can occur.

Oral and topical antioxidant supplementation serves to augment the body's natural supply of antioxidants, and is not meant to completely eradicate free radical production. Rather, it serves to prevent excess damage by augmenting the body's natural defenses.

Natural Antioxidants in the Body

The skin uses a series of intrinsic enzymatic and nonenzymatic antioxidants to protect itself from free radical damage.^{2,8} The mechanism by which an antioxidant protects and prevents changes in the vulnerable human tissue is by donating an electron to the unstable molecule.

Enzymatic antioxidants include catalase, glutathione, peroxidase, superoxide, dismutase, and glucose-6-phosphate dehydrogenase (G6PD). There are also non-enzymatic antioxidants, including vitamin C, vitamin E, co-enzyme Q10, glutathione and alpha lipoic acid.³ Both UV exposure and natural aging deplete these naturally occurring antioxidants from the skin. Without our natural antioxidant protection, free radicals are generated, which overwhelm the body's natural defense system resulting in signs of skin aging.

Topical Antioxidants

It has been demonstrated that topical antioxidant use can provide additional protection from oxidative damage, retard skin aging and improve skin appearance.^{2,9}

Retinoids

Retinoids are a derivative of vitamin A, are lipid soluble, and can easily penetrate the epidermis. Their biologic properties include antioxidant activity, increased fibroblast proliferation, increased production of collagen and hyaluronate, modulation of cellular differentiation and proliferation and decreased matrix metalloproteinase-mediated extracellular matrix degradation.¹⁰⁻¹²

Clinical effects of topical retinoids include fading of solar lentigines,^{10,13} improvement in facial wrinkling and decrease in roughness. Specific clinical recommendations for topical retinoid use include acne and the treatment and prevention of photoaging.

Vitamin C (L-Ascorbic Acid)

Almost all plants and animals synthesize vitamin C. Humans are an exception however, because of a lack of the enzyme, L-gulonolactone oxidase, that is necessary to produce it. Because of this, humans must obtain vitamin C through diet. It is found in citrus fruits and green leafy vegetables.¹⁰ Due to

human biologic control mechanisms, the amount of vitamin C that can be absorbed through diet is limited. Additional vitamin C sources could be provided through topical application. Vitamin C is necessary because it is one of the catalysts required for the formation of structural protein chains, such as collagen. It is also required to provide strength and stabilization for collagen. By functioning as a free radical scavenger, it may inhibit elastin biosynthesis therefore reducing elastotic material accumulation in photodamaged skin. Vitamin C has several other important functions including reduction in pigment synthesis¹⁴ and improvement in epidermal barrier function.^{1,15,16}

Vitamin E

Vitamin E is the body's major lipid phase antioxidant and exists in eight molecular forms.^{1,17,18} All of these isomers are available from dietary sources. However, human beings use predominantly alpha-tocopherol. The major function of vitamin E is to prevent lipid peroxidation, which is important for protecting the lipid structures and also for protecting proteins from oxidation.

Studies have shown that topically applied alpha-tocopherol protects skin against UV-induced erythema,^{1,19} UV-induced lipid peroxidation,^{1,20} UV-induced photo-aging changes,^{1,21,22} UV-induced immunosuppression,^{1,20,23,24} UV-induced photocarcinogenesis^{1,23,25} and inhibition of melanogenesis.^{1,26} Vitamin E is found primarily in the lower levels of the stratum corneum^{1,27,28} and evidence suggests that it is the predominant physiologic barrier antioxidant in the stratum corneum.^{10,29} Vitamin E in the stratum corneum is the body's first line of defense against the oxidative stress of sunlight and pollutants, which can easily deplete the vitamin E stores in the skin. This depletion necessitates the regeneration of vitamin E in order to maintain natural antioxidant protection in the skin.

It has been shown that topical application of vitamin E can be used to restore the antioxidant defense barrier of the skin.^{10,30,31} Evidence supports a co-dependent relationship between the antioxidant actions of vitamins C and E.^{1,32-34} Vitamin C has been shown to protect vitamin E from oxidation^{1,35,36} and is capable of regenerating, or recycling, the activity of vitamin E.^{1,37} The non-enzymatic antioxidant glutathione functions to maintain the exogenous antioxidants, such as vitamins C and E, in their reduced or active forms.

Pycnogenol

Pycnogenol is a standardized French maritime pine bark extract consisting of oligomeric proanthocyanidins (OPCs) known to have potent anti-inflammatory, photoprotective, antioxidant and anticarcinogenic activity.³⁸ Topical application has also been shown to decrease pigmentation caused by UV exposure,³⁸ accelerate wound healing and reduce scar formation.^{10,39} It is thought to have the capacity to stabilize collagen and elastin, improving the elasticity, flexibility and appearance of the skin.⁴⁰ It has been used for at least 500 years as a medicinal extract. As noted, Pycnogenol is rich in phenolic and polyphenolic

flavonoids primarily in the form of monomeric and oligomeric procyanidins, OPCs.^{30,41}

OPCs are found in French maritime pine bark extract, grape seed extract, grape skin, bilberry, cranberry, black currant, green tea, black tea, blueberry, blackberry, strawberry, black cherry, red wine, red cabbage and red apple skins.³⁸ Pycnogenol is significantly more potent than vitamin C and vitamin E. It can recycle vitamin C, regenerate vitamin E and can also enhance the activity of endogenous antioxidant enzymes.³⁸

Tea Polyphenols

The tea plant, *Camellia sinensis*, is a potent source of polyphenols. Green tea contains primarily monomeric catechins (flavonols), including epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). Of these catechins, EGCG is the most abundant and the most biologically active.^{1,42,43} Black tea contains predominantly polymeric polyphenols (flavanols).^{1,42}

Tea has been extensively studied and has been shown to have antioxidant, anti-inflammatory, and anticarcinogenic properties, and has been shown to be effective with both topical and oral administration.^{43,44} In addition, tea has been found to have the ability to suppress UV-induced carcinogenesis.^{43,45} Tea also provides protection against other UV-mediated changes such as sunburn, photoaging, and immunosuppression.^{40,43,46} Tea polyphenols are also strong antioxidants, more powerful than vitamin C and vitamin E.^{1,47}

Coffea Arabica Extract

Coffee fruit extract is a product derived from the unripened fruit of the coffee plant, *Coffea Arabica*. The harvest time is extremely important to obtain the maximum antioxidant activity by avoiding mycotoxin contamination, which often occurs later in the ripening process.⁴⁸ The extract contains several known active components including condensed proanthocyanidins, polyphenols, chlorogenic acid, caffeic acid, ferulic acid, quinic acid and trigonelline.^{40,43,48-50} Several preliminary studies have shown that this extract has potential in improving dyspigmentation, erythema and photoaging.⁵¹ Testing of antioxidant strength by ORAC has shown that coffeeberry outperforms common antioxidants such as green tea extract, vitamin C, pomegranate and vitamin E.⁴³

Sunscreens: Are They Enough?

After sun avoidance behavior and sun-protective clothing, sunscreens are considered to be the gold standard for protecting the skin from photodamage.^{1,52} Recently it has been discovered that sunscreens provide much less protection than expected, due to variation in application thickness. Sunscreens are tested at 2 mg/cm², however controlled studies of actual sunscreen use revealed that they were applied to skin at only 0.5 mg/cm² or less.^{1,53,54} Sunscreen ingredients may become free radicals

themselves when activated by UV radiation.^{1,55} In addition, it has been found that sunscreen chemicals may be absorbed into the skin,⁵⁶ potentially causing harm.¹ DNA damage, due to UV exposure, occurs at suberythemal levels of irradiation. Clearly, sunscreen use without antioxidant supplementation provides users with a false sense of security. Although sunblock products have an accepted standard rating system, which is intended to enable users to make an informed choice as to the level of protection they desire, it is clear that the addition of a topical antioxidant offers an additional level of protection. Topical antioxidants, however, do not currently have an accepted standardized rating system in place.

ORAC and ABEL-RAC: Measuring Antioxidative Activity

The ORAC assay was originally created at the National Institute on Aging by Richard Cutler, PhD. Its use was later moved to the U.S. Department of Agriculture (USDA) where Ronald Prior, PhD worked with Brunswick Laboratories to continue its development. Brunswick Laboratories has recently patented the ORAC assay, which is accepted by the USDA for comparing the activity of natural substances against reactive oxygen species.^{48,57,58} The ORAC assay, ORAC-sc (skin care) and ORAC-fn (food and nutrition), measures how well water-soluble and lipid-soluble components of a natural substance protect a standardized target from oxidation by peroxy nitrite, hydroxyl radicals, superoxide anion and singlet oxygen, and generates a score based on comparison with an antioxidant control.⁴⁸ Specifically, the ORAC assay, measures hydrogen-atom transfer reactions and simulates in-vivo antioxidant action. It is therefore considered to be a more biologically relevant assay than other methods of measuring antioxidant potency.⁵⁹ It is currently being used on biological samples, hydrophilic and lipophilic emulsions and commercial skincare products.⁶⁰

Another form of antioxidant potency testing called ABEL-RAC is performed by Knight Scientific, Ltd, based in Plymouth, England, where it was founded in 1990 by Jan and Robert Knight. The ABEL-RAC testing method uses Pholasin, a light-emitting protein produced by marine rock-boring mollusk *Pholas Dactylus*, which emits light under the stimulus of free radicals, ROS, and peroxidase enzymes. The antioxidant capacity of substances is measured per milligram or per microliter per challenge for finished products. Their research dates back to 1979, and provides another standard by which antioxidant potency can be measured.

Universal adoption of ORAC or ABEL-RAC testing could help educate the public on the importance of antioxidant capacity in skincare products. Future expansion of the acceptance and use of antioxidant testing for skincare products should include a numerical rating system keyed to antioxidant potency to enable physicians and consumers to refine their judgments about the relative benefits of available antioxidant products.

CONCLUSION

Oxidative changes caused by intrinsic and extrinsic mechanisms are the primary causes of aging of the skin. Along with the body's natural antioxidants that protect from free radical damage, there are a number of available topical antioxidant ingredients. Recognition of the importance of an antioxidant regimen in skin care is growing among dermatologists and other medical professionals. As the abundance of available antioxidant products continues to expand, it is becoming more challenging for both physicians and consumers to judge their effectiveness and choose the best product. A standardized numerical system representing antioxidant potency must be developed in order to provide a basis for product comparison. The ABEL-RAC or ORAC testing may provide the foundation for the development of a validated antioxidant rating system in which both physicians and consumers can judge available antioxidant skincare products.

DISCLOSURES

Dr. Palmer is an educator for Allergan and Intendis, but has no financial interests in the companies.

Dr. Kitchin has no conflicts of interest.

The authors have no affiliation with Brunswick Laboratories or with Knight Scientific, Ltd.

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