Oxidative Stress, the Damage-Accumulation Theory of Skin Aging, and the Role of Antioxidants in the Future of Topical Skin Protection

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The major cause of skin aging is a lifetime of damage accumulation from oxidative stress. This scientific review looks at the effects of both internal and external sources of oxidative stress: intrinsic oxidative stress from toxic free radicals produced as a result of cellular metabolic energy production, and extrinsic oxidative stress as a result of continuous exposure to a toxic environment and poor lifestyle choices. In this article, the most toxic free radicals are reviewed, and understanding of the role of molecular oxygen as a toxic, pollutant, mutagenic, biradical, dangerous, poisonous gas is discussed. The importance of antioxidants, their capacity to inhibit skin aging, and an in-depth look at various quantitative methods (eg, oxygen radical absorbance capacity, environmental protection factor, Trolox) for determining topical antioxidant skin protection capacity are reviewed.

Oxidative stress can be defined as the stress and resulting damage caused to living cells as a result of exposure to reactive oxygen species (ROS),[1] which are reactive oxygen-based molecules that may be in the form of free radicals, such as superoxide or hydroxyl radical, or they may be non–free-radical molecules such as hydrogen peroxide or ozone.[2] The one common denominator to all of these molecules is oxygen. Many of us are accustomed to the notion that oxygen is that one element essential for life and simply take for granted its goodness and necessity for most living organisms. Very few of us realize that oxygen is actually a toxic, pollutant, mutagenic, biradical, dangerous, poisonous

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gas.[2] In essence, oxygen is actually a double-edged sword, both the boom and bane of life on earth. If you are currently challenging this theory as you read this article and are questioning the toxicity of oxygen, one only has to think of everyday examples of oxygen's assault on nearly all materials. For example, an unpainted iron porch railing will rust, a sliced apple will brown rapidly, and a forest on fire will experience free-radical–mediated oxidative combustion. Oxygen can corrode the toughest of metals, so its effect on living cells is significant.

HISTORY OF OXYGEN

Oxygen has not always been the problem it is today. The earth is approximately 4.55 billion years old. For the first 2 billion years, oxygen was bound up in iron compounds, and there was none of it to breathe. Instead there was methane, so oxygen was of little consequence to the earliest developing life forms. Initially, the first forms of life on earth were known as anaerobes, living organisms that evolved in an essentially oxygen-limited environment. These early life forms utilized hydrogen from the methane to run all metabolic activity. For the methane-breathing anaerobes, oxygen was a poisonous gas. Approximately 2.5 billion years ago, these initial anaerobes (ie, blue-green algae [cyanobacteria]), developing in the ancient "organic soup" when our planet was covered with water, processed water to get the hydrogen they needed for metabolism.[2] Take hydrogen away from water and the resulting by-product is oxygen. When these ancient anaerobes began photosynthesis, they, in the process of removing hydrogen from water, actually produced dioxygen, which began to slowly pollute the earth's atmosphere. As the earth's atmosphere began to increase in oxygen content, these anaerobes had 3 choices: retreat to an oxygen-free environment, evolve to live in the presence of this toxic substance, or, as irony would have it, meet their own demise. What then happened is probably the single most important evolutionary event in the history of life on earth, the evolution of anaerobes into aerobic organisms, organisms that actually utilize toxic oxygen for metabolic energy production. Aerobes evolved to harness the energy of oxygen and actually use it to their benefit, a critical step in the pathway of life on earth as oxygen became more abundant in the earth's atmosphere (up to 21% where it is today) and the most prevalent element in the earth's crust (more than 50%).[2] Anaerobes are still important life forms, although most life on earth today is aerobic. Anaerobes are difficult to grow in a laboratory, and we are just beginning to learn more about them through their DNA, although *Propionibacterium acnes,* for example, is significant in the skin.

FREE RADICALS: AGENTS OF AGING

Molecular oxygen is also a double free radical, which explains the reactive nature of this molecule.[2] Atomic and molecular orbitals of negatively charged electrons circling a positively charged nucleus are filling the orbitals (or shells) according to a law known as Hund's rule (each orbital is filled with 1 electron before it receives 2) and a principle known as the Pauli exclusion principle (no 2 electrons can have the same 4 quantum numbers).[3] The fourth quantum number relates to the electron "spin" number; as electrons are filling molecular orbitals, they are trying to aggregate in pairs with opposite spins to achieve stability. Free radicals are atoms or molecules with 1 or more unpaired electrons that create instability in their structure.[2] The element hydrogen, with 1 proton and 1 electron, is the simplest free radical. Free radicals are always trying to either give away or gain an electron to achieve molecular stability. Although there can be hydrogen-, carbon-, sulfur-, nitrogen-, and oxygen-centered free radicals, oxygen, because of its unique molecular structure, is most often the center of free radicals that are most important in medicine and biology. Reactive nitrogen species (RNS) is a collective term used to designate reactive radicals and nonradicals that contain the element nitrogen. In most cases, RNS are also ROS, since nonoxygen RNS occur significantly less in medicine and biology. In Table 1, the most common oxygen-based free radicals in the human body are shown.[2] Some free radicals produced in cells are so reactive (eg, the hydroxyl radical) that they do not have an easily measurable lifespan. In fact, hydroxyl radicals are so reactive that they will react with nearly every other biological molecule in the cell instantaneously.[2] However, not all ROS are free radicals. For example, singlet oxygen, hydrogen peroxide, and ozone are all considered ROS but are technically not free radicals,

TABLE 1

Reactive Oxygen Species

Free Radicals	Nonradicals
Hydroxyl (OH)	Hydrogen peroxide (H_2O_2)
Alkoxyl (RO)	Ozone (O_3)
Superoxide (O_2^-)	Singlet oxygen ($^1\Delta gO_2$)
Peroxyl (RO_2)	Peroxynitrite[a] ($ONOO^-$)
Hydroperoxyl (HO_2)	
Oxygen (O_2)	
Nitric Oxide[a] (NO)	

[a]Could equally be called a reactive nitrogen species.

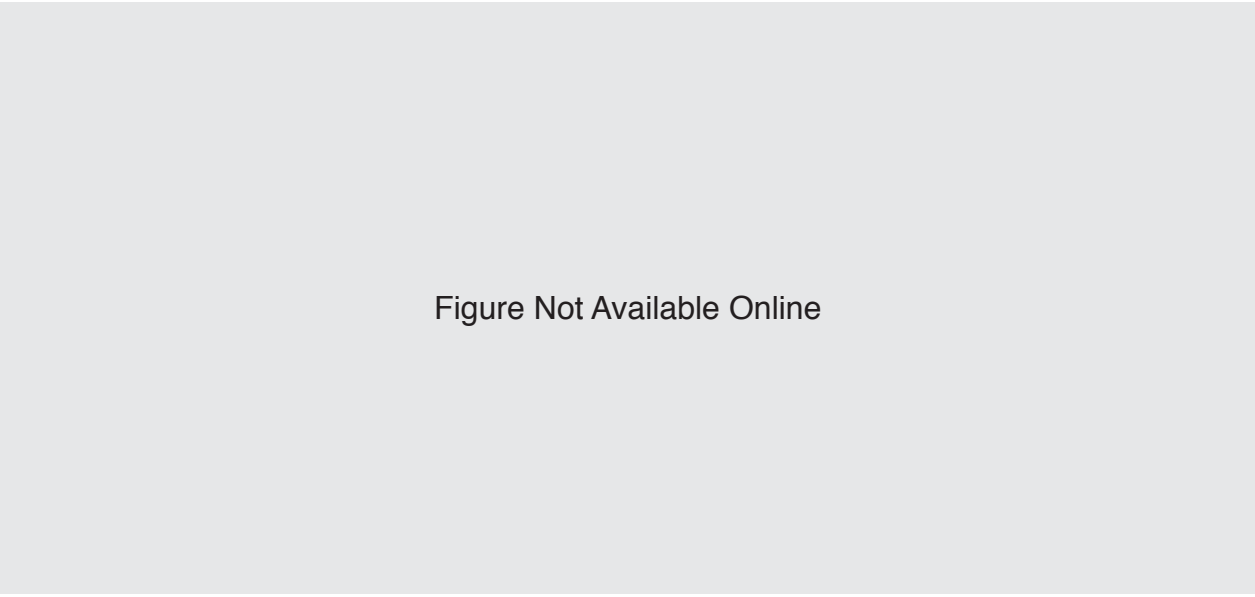

Figure 1. Oxidative stress, the major cause of skin aging. Illustration courtesy of ScienceMedia.

as they do not possess a molecular structure with unpaired electrons. These oxygen-based molecules are reactive because of other molecular structural instabilities.

Dangerous to living cells, ROS react with other cellular molecules, organelles, and structures, such as DNA, lipids, proteins, and sugars, to cause damage. Cells have many built-in mechanisms to alleviate oxidative stress or to eliminate or repair the damage caused by ROS. Antioxidant defenses include: (1) agents that catalytically remove ROS, such as enzymes like superoxide dismutase, catalase, and peroxidase; (2) proteins that minimize the availability of pro-oxidants such as iron and copper ions; (3) proteins that protect biomolecules from damage (eg, heat-shock proteins); and (4) low–molecular-weight ROS scavenging agents such as vitamin C, vitamin E, naturally occurring ubiquinone or ubiquinol or synthetic idebenone, and polyphenols.[2] Other defenses include specifically targeted cellular repair enzymes that repair cellular oxidative damage postoccurrence. The problem is that these built-in defenses are not 100% efficient and diminish in their capacity as a function of aging itself (ie, creating a self-destructing, self-perpetuating cycle).[4] It is the accumulation of this damage over an organism's lifespan that is a fundamental cause of aging.

OXIDATIVE STRESS: EXTERNAL AND INTERNAL SOURCES

Oxidative stress comes from 2 main sources: externally, as a result of exposure to our toxic environment (extrinsic), and internally, as a result of metabolic energy production (intrinsic) (Figure 1).[5] Externally, oxidative stress in the skin is produced primarily by UV radiation promoting the generation of free radicals on a large-scale basis. Other external sources of oxidative stress include air pollution, ozone, cigarette smoke, and even oxygen itself. One can only imagine that the skin bears the brunt of external oxidative stress since it is the organ designated to protect the human body from the environment. Internally, cells must create energy to run all cellular metabolism, and this is done by a process known as aerobic respiration. In this process, foodstuff is oxidized (electrons removed) and these electrons are transported along the Krebs cycle and the electron-transport chain in the cellular mitochondria. This flow of electrons produces the energy adenosine 5'-triphosphate necessary to run all cellular metabolism.[6] But where do the electrons ultimately go? They are transferred to molecular oxygen, which is supplied to the cells by the hemoglobin in blood (transferred from the lungs). In the process, oxygen, already a free radical, becomes an even more toxic free radical after it picks up 1 additional electron, the superoxide radical. Superoxide is toxic (superoxide theory of oxygen toxicity), therefore cells have built-in processes to enzymatically add electrons to dioxygen to reduce superoxide to hydrogen peroxide and subsequently to harmless water.[2] The problem is that these processes are not 100% efficient, and some stray superoxide radicals react with DNA, lipids, and proteins to cause damage. This damage accumulates and ultimately expresses itself as premature aging. Evidence of this damage includes inflammation, damage to telomere structure, mitochondria, cell function, and cancer. Therefore, aging can be directly related to metabolic energy production. This is the reason that a hummingbird has a lifespan of only a few years and a tortoise may live more

than 100 years; the rate-of-living hypothesis states that the metabolic rate of a species ultimately determines its life expectancy.[1] For the hummingbird, energy demand is enormous to meet its lifestyle. Therefore, superoxide generation is high, elimination efficiency is not perfect, and damage accumulation is rapid, resulting in a short lifespan. For the tortoise, just the opposite applies. Lifespan extension is possible via caloric restriction. Another method to extend lifespan is using certain antioxidants to modulate the gene-expression levels of telomerase or telomere length-maintenance genes in oxidatively stressed cells (D.H. McDaniel, MD, oral communication, June 2009).

As previously emphasized, oxidative stress is both internal and external. The skin, the largest organ in the human body and the primary barrier to the environment, must also produce energy to function. This is why it is one of the first organs of the human body to show signs of aging, in essence a double jeopardy because it is under oxidative stress assault both externally and internally. The same does not apply to internal organs such as the heart and liver, as they are not exposed to the toxic oxygen-laden environment; conversely, only small concentrations (nothing close to atmospheric concentration) of oxygen are delivered to the internal organs' cells for metabolic energy production, and thus these organs are not under exogenous threats from the environment.[2] Because the skin is visible every day, it is the organ we are most concerned with for its appearance and for the effects of aging. As oxidative stress damage accumulates on a cellular level, it begins to express itself clinically in the change in appearance of the skin, including fine lines, wrinkles, uneven skin tone, and loss of skin elasticity (Figure 2).

ANTIOXIDANTS

Antioxidants (AOs) are molecules that prevent damage to cells from oxidative stress and are thus vital defenses in the continuous fight against aging.[7] In addition, AOs function by scavenging or neutralizing toxic free radicals before they cause harm to cell structures. The details of the pathways of such radical scavenging capacity are beyond the scope of this article, but AOs essentially fit into several functional classes: enzymes, sacrificial low–molecular-weight AOs, and metal chelators. Antioxidant compounds can be further classified into families based on type, such as vitamins, enzymes, respiratory chain, chelating agents, hormones, botanicals, and plasma proteins.

PROTECTIVE CAPACITY OF ANTIOXIDANTS

The ability of AOs to protect cells against oxidative stress has been widely researched. AOs have become so popular

Figure 2. As oxidative stress damage accumulates, it begins to express itself clinically in the change of the skin, such as fine lines, wrinkles, pigmentation, and loss of skin elasticity (A), as opposed to smooth, youthful, radiant skin (B). Illustration courtesy of ScienceMedia.

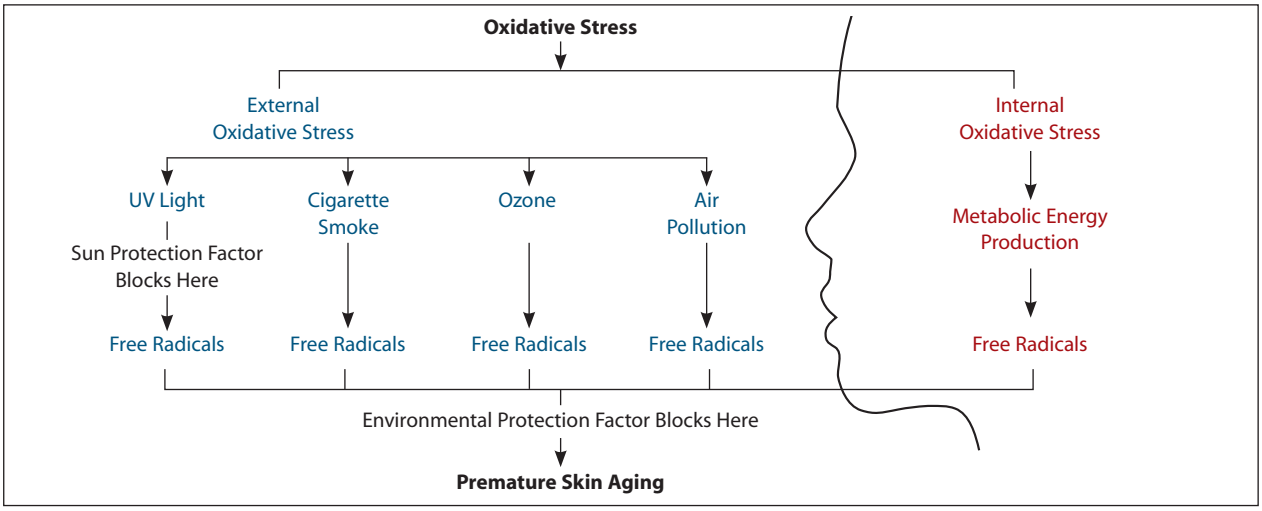

Figure 3. The causes of premature skin aging. Environmental protection factor versus sun protection factor and the differences in mechanisms of protection.

in modern skin care products because of the lack of efficient topical skin-protection products in the marketplace. Total topical skin protection is lacking a vital component in the widely accepted sun protection factor (SPF) skin-protection system used throughout the world.[8] The reason that SPF is not completely efficient is due to the fact that sunscreens do not work by a mechanism of scavenging free radicals. To the contrary, sunscreens function to prevent the formation of free radicals by either absorbing UV light and converting it to heat energy (organic sunscreens such as octylmethoxy cinnamate and oxybenzone) or scattering, reflecting, and blocking UV light (inorganic or physical blocking agents such as titanium dioxide or zinc oxide). In both cases, this is accomplished by first intercepting the UV light before it causes toxic free radicals in vivo.[9,10] The problem with SPF is that no sunscreen is 100% effective at blocking UV light (not even high SPF), and therefore some UV rays do penetrate the epidermis and dermis to cause free radicals and damage. Once free radicals are formed, SPF sunscreens are useless in preventing further damage. In addition, no sunscreen affords protection against other environmental sources of free radicals such as air pollution, ozone, cigarette smoke, and even oxygen itself. Therefore, total topical skin protection is lacking a key element: a protective capacity against free radicals (Figure 3).

McDaniel et al[8] proposed such a topical protective capacity term in 2005: environmental protection factor (EPF), which is the measure of an AO's ability to protect human skin from oxidative stress (ie, a ROS scavenging capacity measurement for AOs). Antioxidant protection is becoming a fundamental part of topical skin care protection because the manufacturers of sunscreens and skin care products are starting to realize that AOs play an important role in skin protection that is quite different from SPF. An SPF provides a primary shield against the barrage of UV radiation that is very damaging if not intercepted, but another shield is necessary to protect against free radicals, a secondary shield from UV rays that penetrate the UV shield and a primary shield from other sources of free radicals previously mentioned (Figure 4). The fact that rates of every type of skin cancer have risen in the last 30 years when, at the same time, sunscreen use has become more compliant than ever illustrates the need for additional protection.[11]

The EPF concept as a method for measuring AO

Figure Not Available Online

Figure 4. Environmental protection factor combined with sun protection factor equals the total topical skin protection. Illustration courtesy of ScienceMedia.

TABLE 2

Environmental Protection Factor: Idebenone Versus Common Antioxidants

Oxidative Stress Test	Idebenone	Vitamin E	Kinetin	Ubiquinone	Vitamin C	Lipoic Acid
Sunburn cell assay	20	16	11	6	0	5
Photochem radical scavenging capacity	20	20	10	15	20	5
Low-density lipoprotein oxidation	16	10	20	5	3	4
Cell membrane oxidation	19	17	10	12	12	20
UV-induced DNA cross-linking	20	17	17	17	17	7
Total score: Environmental protection factor	95	80	68	55	52	41

capacity was based on a series of 5 different cell biological in vitro and clinical in vivo methods. At the time, the AOs vitamin C, vitamin E, α-lipoic acid, coenzyme Q10, kinetin, and idebenone were researched. The scoring system was based on a maximum score of 100, and the results were presented at the American Academy of Dermatology meeting[12] and in the *Journal of Cosmetic Dermatology* in 2005.[8] Idebenone, with an EPF of 95, was found to be the most efficient AO tested at the time (Table 2). Since 2005, others have started to apply the oxygen radical absorbance capacity (ORAC), originally developed for food use, to topical cosmetic products in order to rate oxidative stress protection capacity.[13] Figure 5 provides ORAC values for some common fruits. It is interesting to note that a new, natural antioxidant extract derived from the whole coffee fruit has a reported ORAC value of 102,300 μmol Trolox equivalent 100/g.[14]

Both systems have their pros and cons. For example, EPF is cost prohibitive for most companies under its current testing protocol, but is more predictable of clinical outcome because it uses living human skin and cultured keratinocytes in determining AO capacity. On the other hand, ORAC is very cost efficient with high throughput assays, but real clinical outcome in living skin is less predictable because all the testing is done ex vivo with analytical analysis.[15] For example, the molecule idebenone does not perform well in ORAC testing because it is formulated in products in its oxidized state and requires an active respiratory chain to be cycled into its active state. In vivo, its performance was unsurpassed compared to most common AOs.[16]

There is no doubt that the future of total topical skin

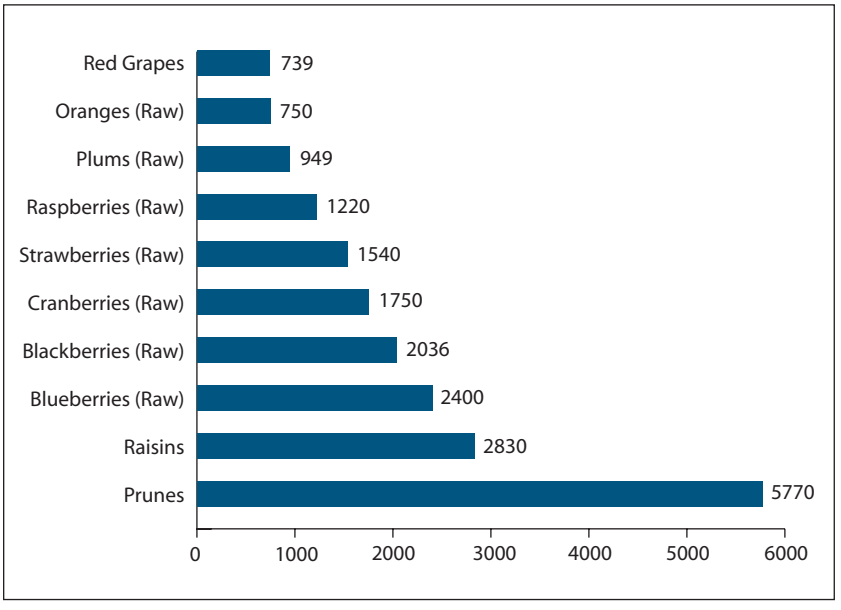

Figure 5. Oxygen radical absorbance capacity per 100 g in various fruits.

protection will follow a course that incorporates the vital role that AOs play in the fight against aging. This may result in a modification of EPF or ORAC or some other AO protection capacity standard, but no scientist or physician can deny the need for such a protection capacity to measure cosmetic or over-the-counter SPF drug products containing AOs in the future.

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