Acta Dermatovenerol APA

Acta Dermatovenerologica
Alpina, Pannonica et Adriatica

2012;21:33-36
doi: 10.2478/v10162-012-0009-0

Intrinsic skin aging: The role of oxidative stress

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Abstract

Skin aging appears to be the result of two overlapping processes, intrinsic and extrinsic. It is well accepted that oxidative stress contributes significantly to extrinsic skin aging, although findings point towards reactive oxygen species (ROS) as one of the major causes of and single most important contributor; not only does ROS production increase with age, but human skin cells' ability to repair DNA damage steadily decreases over the years. We extrapolated mechanisms of intrinsic oxidative stress in tissues other than skin to the skin cells in order to provide effective anti-aging strategies and reviewed the literature on intrinsic skin aging and the role of oxidative stress.

Received: 21 February 2012 | **Returned for modification:** 27 February 2012 | **Accepted:** 25 May 2012

Introduction

Human skin, like all other organs, undergoes changes due to aging. Skin aging appears to be the result of two types of aging, "intrinsic" and "extrinsic". "Intrinsic" structural changes occur as a consequence of physiological aging and are genetically determined. However, it is very difficult, if not impossible, to separate "intrinsic" aging from a variety of other factors clearly contributing to aging, such as smoking, sun exposure, alcohol consumption, dietary habits, and other environmental and lifestyle factors (1, 2). Hereditary genetic influences are actually believed to contribute no more than 3% to aging, making epigenetic and post-translational mechanisms the most important pathways of aging (3, 4). Consequently, the rate of aging is significantly different among different populations and even among different anatomical sites in a single individual.

Many theories have tried to explain the aging process, but the most plausible of these concentrate on DNA damage and the concomitant repair process, which induce genome-wide epigenetic changes leading to cell senescence, loss of proper cell function, and genomic aberrations (5). Whether many post-translational mechanisms of skin aging are independent pathways or a consequence of DNA damage and the resulting epigenetic changes remains to be established.

"Intrinsic" (genetically determined) and "extrinsic" (UV- and toxic exposure–mediated) skin aging processes overlap and are strongly related to increased generation of free radicals in the skin. The underlying mechanism of both processes is increased oxidative stress, which is probably the single most harmful contributor to skin aging, leading to loss of cells and the extracellular matrix as the most prominent features of chronologically aged skin (6). Oxidative stress is the imbalance between ROS production and antioxidative defense (Table 1).

The clinical manifestations of intrinsic aging are fine wrinkles, thin and transparent skin, loss of underlying fat leading to hollowed cheeks and eye sockets, dry and itchy skin, inability to perspire sufficiently, hair graying, hair loss or hirsutism, and thinning of nail plates (7).

Most studies of intrinsic skin aging are derived from observations of tissues other than skin. The mechanisms of intrinsic aging apply more or less to all proliferating and terminally differentiated cells (8). It is widely accepted that intrinsic aging is primarily caused by accumulated damage due to free radical reactions and by reactive oxygen species (ROS)–induced damage to critical cellular macromolecules (6).

Generation of reactive oxygen species and oxidative stress

Free radicals are a vital part of the metabolism and are essential for life, playing roles such as killing microbes in macrophages. Intrinsic aging depends on the homeostasis between free radical production and the effectiveness of defense and repair systems. In order to understand the basic principles of intrinsic skin aging, the biochemistry of free radical formation is briefly presented. There is no doubt that oxygen (O_2) is essential for life (9). Humans and other aerobes need O_2 because they have evolved electron transport chains (ETC) and other enzyme systems utilizing O_2 and can tolerate its toxic byproducts through antioxidant defense. The predecessors of the anaerobic bacteria that exist today followed a "blind" evolutionary path of restricting themselves to environments devoid of O_2. It could be argued that the evolution of multi-cellular aerobes and antioxidant defense mechanisms are intimately related (10). Even present-day aerobes suffer oxidative damage. Free radicals important for living organisms include hydroxyl ($OH^{\bullet}$), superoxide (O_2^{-}), nitric oxide ($NO^{\bullet}$), thyl ($RS^{\bullet}$), and peroxyl ($RO_2^{\bullet}$).[14] Peroxynitrite ($ONOO^{-}$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and ozone (O_3) are not free radicals but can easily lead to free radical reactions.

The term reactive oxygen species (ROS) is often used to include not only free radicals but also the non-radicals ($^1O_2$, $ONOO^{-}$, H_2O_2, O_3). Reactive oxygen species are reactive molecules that contain an oxygen atom (11). The essence of metabolic energy production is in food oxidation: electrons in the respiratory chain are accepted by electron carriers, such as nicotinamide dinucleotide (NAD+) and flavins (flavin mononucleotide/FMN and flavin adenine dinucleotide/FAD). The resulting reduced nicotinamide adenine dinucleotide (NADH) and reduced flavins ($FMNH_2$ and $FADH_2$) can be re-oxidized in mitochondria, producing large amounts of ATP

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(12). Generation of ROS in mitochondria is therefore a byproduct of cell respiration, due to electron leakage in the electron transport chain (ETC) during oxidative phosphorylation (13) (Fig. 1).

$O_2 + e^- \dashrightarrow O_2^{\bullet -}$	superoxide anion	*(1)*
$O_2^{\bullet -} + e^- + 2H^+ \dashrightarrow H_2O_2$	hydrogen peroxide	*(2)*
$H_2O_2 + e^- + H^+ \dashrightarrow H_2O + OH^{\bullet}$	hydroxyl radical	*(3)*
$OH^{\bullet} + e^- + H^+ \dashrightarrow H_2O$	water	*(4)*
Sum:		
$O_2 + 4e^- + 4H^+ \dashrightarrow 2H_2O$		*(5)*

Figure 1 | Endogenous sources of ROS production: stepwise reduction of molecular oxygen via one-electron transfer in mitochondria.

There are two main sources of ROS: a mitochondrial source (which plays the principal role in aging) and a non-mitochondrial source (which has a different, sometimes specific role, especially in the pathogenesis of age-related diseases). Most studies suggest that the majority of intracellular ROS production is derived from mitochondria (14). However, some authors are skeptical due to the lack of firm experimental evidence (15). At least in the liver, peroxisomes and the endoplasmic reticulum have a greater capacity to produce ROS (15).

The mitochondrial source of ROS is represented by the electron transport chain and the nitric oxide synthase reaction (16). The rate of mitochondrial respiration is responsible for the rate of generation of ROS: the higher the metabolic rate, the shorter the maximum lifespan of cells, with some exceptions to this rule (17). A Fenton reaction is an example of the non-mitochondrial source of ROS and involves H_2O_2 degradation, which is catalyzed by free bivalent iron ions and leads to the generation of $OH^{\bullet}$.

$$Metal^{(n)} + H_2O_2 \rightarrow Metal^{(n+1)} + HO^{\bullet} + HO^-$$

(Fenton reaction) *(Reaction 6)*

$$H_2O_2 + {}^{\bullet}O_2^- \xrightarrow{Metal^{(n+1)}} O_2 + HO^{\bullet} + HO^-$$

(Haber–Weiss reaction) *(Reaction 7)*

Superoxide, ascorbic acid, and α-tocopherol also play important roles as reducing agents in Reaction 6, in which the metal ions are recycled. Reaction 7 is the Haber–Weiss reaction, which supports the notion that transition metals play an important role in the formation of hydroxyl radicals. It should be borne in mind that the body's iron content increases with age (18, 19). Sources of H_2O_2 are mitochondria (superoxide dismutase reaction), peroxisomes (acyl-CoA oxidase reaction), and amyloid β of senile plaques (superoxide dismutase-like reactions) (16, 20). Sources of superoxide ($O_2^{\bullet -}$) are mitochondria, microsomes that contain cytochrome P450 enzymes, a respiratory burst of phagocytic cells, and others.

The production of mitochondrial superoxide radicals occurs primarily at two enzymatic complexes in the electron transport chain: complex I (NADH dehydrogenase) and complex III (ubiquinone–cytochrome c reductase) (16). Under normal metabolic conditions, complex III is the main site of ROS production (21). With respect to human aging, the weak point of this otherwise elegant system lies in the formation of free radical semiquinone anion species ($^{\bullet}Q^-$), which occurs as an intermediate in the regeneration of coenzyme Q (16). Once formed, $^{\bullet}Q^-$ can readily and non-enzymatically transfer electrons to molecular oxygen with the subsequent generation of a superoxide radical. The generation of ROS therefore becomes predominantly a function of metabolic rate and, as such, this rate can be indirectly correlated with the corresponding rate of oxidative stress (22). Analyses of the control of oxidative phosphorylation-electron transport chain activity suggest that the system appears to be primarily pull-regulated rather than push-regulated: putting in more NADH at the beginning of the respiratory chain does not drive up respiration, but shuts it down by restricting the availability of ADP (23). When there is an abundant, non-limiting amount of ADP available, mitochondria are said to be operating in state 3 respiration. When ADP is absent, there is no ATP production and the proton transduction mechanism becomes backed up, which is called state 4 respiration. Because the proton-motive force declines in state 3 compared to state 4, free-radical production would be expected to be considerably elevated in state 4 compared to state 3. This effect is interesting because it is actually the exact opposite of the postulated link between energy metabolism and free-radical production (aging) (23). The flux through the electron transport chain is relevant to the aging process because it is related to the rate of production of ROS. Small reductions in metabolic flux through the electron transport chain occur at the cost of increased upstream substrate levels (24). This increased concentration of reduced up-

Table 1 | The (im)balance between ROS production and antioxidative defense.

Increased free-radical production		Decreased antioxidative defense
Endogenous	Exogenous	
• increased mitochondrial leakage	• environment (UVR exposure, pollution, pesticides, radiation, etc.) • increased $^3O_2$ concentration	• mutation or reduced activity of enzymes (catalase, SOD, glutathione peroxidase) • reduced biokinetics of antioxidant metabolism • reduced intake of antioxidants from food • reduced bioabsorbtion of antioxidants from food • others
• increased respiration	• lifestyle	
• elevation in O_2 concentration	• strenuous exercise: increased physical activity of an untrained individual	
• inflammation	• smoking	
• others	• increased intake of dietary compounds prone to increase initiation rates: - increased metal ion intake (e.g., Fe, Cu, Cr) - polyunsaturated lipids - easily peroxidized amino acids (e.g., lysine)	
	• diseases and chronic illnesses	
	• chronic inflammation	
	• psychological and emotional stress	
	• others	

stream substrates allows a larger generation of ROS (16). Aerobic metabolism requires the constant removal of excess electrons through the reduction of oxygen (25). The need for oxygen as an electron acceptor is the sole purpose of breathing. Inevitable byproducts of this process are $O_2^{-\bullet}$, H_2O_2, and $HO^\bullet$. This happens mainly by complexes I and III (23, 24) of the electron transport chain, the most important sources of endogenous free radicals. About 10 oxygen molecules are processed by each human cell daily and the leakage of partially reduced oxygen molecules is about 1 to 5%, yielding about 2×10 superoxide and hydrogen peroxide molecules per cell per day (10, 12, 26). Based on the amount of oxygen damaged and altered nucleotides detected in human urine, it has been estimated that approximately 2×104 oxidative DNA lesions occur per human genome every day (27). Assuming that the repair of each excised adduct involves replacing one to five nucleotides, then oxygen-induced damage to DNA results in the replacement of 2×105 nucleotides per human cell per day (28). Each human cell receives 10,000 ROS hits per day, which equals 7 trillion insults per second per person.

Estimates of oxygen consumption in direct reactions to generate free radicals vary (23). However, typically cited values are around 1.5 to 5% of the total consumed oxygen (29, 30). These estimates have been questioned by Hansford et al. (30) and Staniek and Nohl (31, 32), who suggested that H_2O_2 production rates are less than 1% of consumed O_2. However, even if we accept a conservative value of 0.15%, this still represents a substantial amount of free radicals (23). As mentioned above, the rate of generation of H_2O_2 depends on the state of the mitochondria as determined by the concentration of ADP, substrates, and oxygen (33, 34).

A steep increase in electron-transfer chain activity produces a linear increase in ATP production but an exponential increase in ROS formation. Cells can produce the same amount of ATP for less ROS by having a larger number of mitochondria running at a lower rate of electron-transfer chain activity. Heart cells, for example, have thousands of mitochondria, whereas skin cells have fewer mitochondria per cell. Whether skin cells suffer more ROS-induced damage is to the best of our knowledge not yet established.

Oxidative damage and intrinsic skin aging

Skin cells are constantly exposed to ROS and oxidative stress from exogenous and endogenous sources. The difference between the two skin types is decay in the capacity of lipid membrane turnover of chronologically older skin (35, 36). Not only does ROS production increase with age but human skin cells' ability to repair DNA damage steadily decreases over the years (37). Reducing free radical production in the first place is far more efficient than trying to neutralize free radicals after they have been produced (Fig. 2).

The energy required by skin cells comes from three sources: mitochondrial oxidative phosphorylation, glycolysis, and the creatine/phosphocreatine system. All three major energy sources are affected by intrinsic and extrinsic skin aging and offer potential entry points for intervention strategies to decelerate the skin aging process (8). Due to impaired mitochondria with age, less energy is produced by mitochondrial oxidative phosphorylation, although the number of mitochondria does not change with age. Higher energy demand needs higher energy production via non-mitochondrial pathways, such as glycolysis. With advancing age, energy production is mostly anaerobic. Primary keratinocytes derived from elderly donors show higher glucose uptake and increased lactate production, which indicates suboptimal utilization of glucose and a shift in metabolism towards increased glycolysis (8).

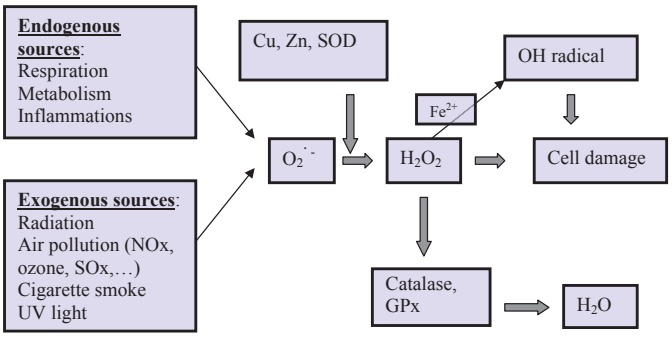

Figure 2 | Cellular generation of reactive oxygen species and antioxidant defense system.

Normal human dermal fibroblasts have a limited lifespan in vitro and cease proliferating after a fixed number of cell divisions. This process by which cells stop proliferation is called cellular senescence (38). Senescence is also characterized by a decrease in total cell numbers. It is not yet clear whether aging causes mitochondrial damage or vice versa. A loss of mitochondrial functions can cause premature senescence of the skin cells. This has been demonstrated in a reduction in the level of oxidative phosphorylation in human fibroblasts, which caused a reduction in cell proliferation and premature senescence (39). In addition to the well-established influence of ROS on proliferation and senescence, a reduction in the level of oxidative phosphorylation is causally related to reduced cell proliferation and the induction of premature senescence. Changes that occur with senescence can effect mitochondrial respiration. Using a human fibroblast model of in vitro senescence, Zwerschke et al. analyzed age-dependent changes in the cellular carbohydrate metabolism (40). The authors showed that senescent fibroblasts enter into metabolic imbalance associated with a strong reduction in the levels of ribonucleotide triphosphates, including ATP, which are required for nucleotide biosynthesis and hence proliferation. ATP depletion in senescent fibroblasts is due to dysregulation of glycolytic enzymes and ultimately leads to a drastic increase in cellular AMP, which is shown to induce premature senescence (40). With increasing passage number, senescent fibroblasts show a loss of membrane potential and a decline in ATP production (40, 41). A significant decrease in mitochondrial membrane potential in ex vivo samples of human dermal fibroblasts from elderly donors was recently found, accompanied by a significant increase in ROS levels. Respiratory activity was not significantly altered with donor age, probably reflecting genetic variation (42). It seems that long-term exposure of cells to ROS initiates a vicious cycle resulting in a reduced capacity of stress response, a reduction in ATP synthesis, and a further increase of ROS production in the affected cells (43).

Skin tissues engage in and derive energy mostly using aerobic glycolysis. Despite the presence of oxygen, there is preferential conversion of glucose to lactate (44). This results in the production of substantial amounts of lactate, which is carried to the liver by the bloodstream and converted back to glucose (the Cory cycle). Skin has a strong preference for the metabolism of glucose rather than fatty acids or ketone bodies, although alternative citric acid cycle intermediates such as glutamine are also actively utilized (45). Interestingly, of the relatively small amount of oxygen that is metabolized by the skin, the majority is supplied to the epidermis and upper dermis by diffusion from the atmosphere.

Because the majority of ATP in the skin is generated by glycolysis, mitochondria may be less important for ATP generation, although they may still have a pivotal role in aging (46, 47).

In conclusion, excess production of ROS and reduced antioxidant activity with advanced age significantly contribute to chronological aging. Oxidative damage is the major cause and single most important contributor to skin aging.

Acknowledgment

Parts of this manuscript were published in the paper Dahmane R, Poljšak B. Free radicals and intrinsic skin aging: basic principles. Health Med. 2011;5:1647-54.

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