Cellular Resilience: Antioxidant Approaches to Combat Skin Stress

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Thesis mentor: Associate Professor Lara Batičić, PhD

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1. Professor Robert Domitrović, PhD (Committee Head)
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3. Professor Vlatka Sotošek, MD, PhD

The graduation thesis contains 31 pages, 8 figures, 75 references.

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List of abbreviations and acronyms

ATP	Adenosine triphosphate
CAT	Catalase
CYP450	Cytochrome P450
7-DHC	7-dehydrocholesterol
DNA	Deoxyribonucleic acid
ER	Endoplasmic reticulum
GPx	Glutathione peroxidase
HNO_3	Peroxynitrous acid
HO_2•	Hydroperoxyl radical
H_2O_2	Hydrogen peroxide
MFRTA	Mitochondrial free radical theory of aging
mtDNA	Mitochondrial DNA
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NO•	Nitric oxide radical
NOS	Nitric oxide synthase
O_2	Singlet oxygen
O_2•	Superoxide anion radical
•OH	Hydroxyl radical
$25(OH)D_3$	25-hydroxyvitamin D3
$1,25(OH)_2D_3$	1,25-dihydroxyvitamin D3
OXPHOS	Oxidative phosphorylation
p53	Tumor suppressor protein
R•	Alkyl radical
RC	Respiratory chain
RH	Unsaturated fatty acid
RNS	Reactive nitrogen species
ROH	Hydroxy fatty acid
ROO•	Peroxide radical
ROOH	Hydroperoxide
ROS	Reactive oxygen species
SOD	Superoxide dismutase

SOD1	Superoxide dismutase 1
SOD2 or MnSOD	Manganese superoxide dismutase
SOD3	Superoxide dismutase 3
UVA	Ultraviolet A
UVB	Ultraviolet B
UVC	Ultraviolet C
UVR	Ultraviolet radiation
vitamin D_3	Cholecalciferol
vit C•	Ascorbyl radical
vit E•	Tocopheryl radical
WHO	World Health Organization

1 Introduction

In the dynamic landscape of modern living, the human skin constantly copes with an array of environmental stressors that can erode its structural integrity and functional vitality. From UV radiation and pollution to lifestyle factors, the skin is exposed to numerous challenges that can accelerate aging processes and contribute to the onset of various dermatological conditions (1,2). Recognizing the role of cellular resilience in maintaining skin health, this thesis explores the complex interplay between skin stress and the potent protective mechanisms afforded by antioxidant approaches. Cellular resilience, the ability of skin cells to withstand and recover from stress-induced damage, emerges as a fundamental aspect of skin health. As oxidative stress becomes an ever-present concern, given its role in skin aging, inflammation, and the development of disorders, a deeper understanding of the cellular machinery involved in maintaining resilience becomes imperative. Antioxidants, with their ability to neutralize reactive oxygen species and reduce oxidative damage, stand out as promising candidates for improving cellular defenses against external aggressors (3). This thesis explores the multifaceted dimensions of cellular resilience within the context of skin stress. It investigates the complex cellular and molecular processes that control the skin's response to stress, emphasizing the role played by antioxidants in modulating these responses. By examining the latest advancements in antioxidant research and their application to skincare, this work aims to contribute to the growing knowledge surrounding strategies to improve skin resilience and reduce the impact of stressors on skin health. Through a comprehensive review of relevant literature and empirical studies, this thesis seeks to provide a perspective on the integration of antioxidant approaches in skincare products to promote lasting cellular resilience for healthier skin.

2 Aims and Objectives

This review paper aims to get an overview of the mechanisms of cellular resilience, investigating the complex interplay between oxidative stress and the skin aging process. Specific objectives include clarifying the role of endogenous and exogenous factors in generating reactive oxygen species (ROS), exploring the dynamics of oxidative stress in skin aging, and assessing the efficacy of enzymatic and non-enzymatic antioxidants in attenuating skin stress.

3 Literature Review

3.1 Oxidative stress

Oxidative stress is a widely acknowledged principle within the field of medicine. Given the simultaneous occurrence of various antioxidative and oxidative processes in both physiological and pathological conditions across diverse organ systems and cells, many attempts have been made to apply this concept translationally in a broad spectrum of human diseases and physiological processes. The disruption of the redox homeostasis (balance between free radicals and antioxidants) can cause premature aging and the onset of diseases (4).

The general definition of oxidative stress involves a disproportion of oxidants and antioxidants due to excessive production of free radicals caused by both endogenous and exogenous factors, resulting in an interference with redox signaling and regulation and/or potential harm at the molecular level due to the complex interplay of these processes. The basic concept in the context of a metabolic system is that a baseline redox tone is established by maintaining a consistent redox balance at a given set point. Any deviation from this stable redox equilibrium triggers a stress response. This implies that shifting to the opposite end of the balance means reductive stress. Distinctions are made between deviations beyond normal physiological levels, termed oxidative distress, and physiological variations, referred to as oxidative eustress. Redox control and physiological redox signaling are mainly regulated by oxidative eustress. The redox equilibrium is sustained at a selected setpoint, establishing a fundamental redox tone (5,6).

3.1.1 Free radicals

Free radicals are categorized into two types: reactive oxygen species (ROS) and reactive nitrogen species (RNS). These radicals possess an unpaired electron within an atomic orbit, making them highly unstable and reactive. By trying to eliminate the unpaired electron, they function as either oxidants or reductants, actively accepting or donating electrons from other molecules, respectively (7). In the process of becoming stable the free radicals are causing intracellular chaos in the molecules getting attacked. DNA, proteins, carbohydrates, and lipids, which are biologically relevant molecules are getting damaged (7,8).

3.1.1.1 Historical overview

First evidence of the existence of free radicals was reported in the 20th century with the first experiment of a free radical reaction conducted in 1894. In the 1950s, oxygen toxicity, X-irradiation, and premature aging was first hypothesized to involve a free radical reaction (9,10).

In 1895, the German physicist Röntgen made a groundbreaking discovery by identifying a new form of rays, which he named X-rays (9,11). Initially, the potential risks associated with radiation and radioactive materials were not fully understood, despite early signs of biological harm. Most of what we know about impacts of radiation has been acquired since the second World War. Recent studies have affirmed that even minimal exposure to radiation can lead to cell changes, contributing to the development of cancer. Progress in the early 20th century involved understanding how ionizing radiation affects individual elements within biological systems and the underlying mechanisms. Ionizing radiation directly affects crucial biological components such as DNA, proteins and lipids, causing the creation of radical cations, radical anions, and excitations. Moreover, ionizing radiation can indirectly impact biological molecules by generating free radicals like •OH (hydroxyl radical), resulting in the formation of H_2O_2 (hydrogen peroxide), atomic oxygen, and HO_2• (hydroperoxyl radical). These reactive substances then interact with biological molecules. The substantial damage that radiation can cause on DNA shows that DNA plays a key role in the cellular effects of radiation (9,12).

For most people, oxygen is an inconspicuous component of the air they breathe. However, oxygen has not always been a component of the air as we know it today. Oxygen started to accumulate in the atmosphere in small quantities around 50 million years ago, predating the great oxygenation event. This increase in oxygen offered new opportunities for life (13). Indeed, oxygen is indispensable for aerobic organisms, as they generate energy through oxidation reactions to maintain vital functions in their bodies. However, oxygen also possesses toxic properties, making it a double-edged sword. All living things developed a complex defense system against the Earth's oxygen-rich atmosphere to be able to survive nevertheless. However, this defense system is effective only within the normal atmospheric oxygen pressure. Exposure to higher oxygen pressures can lead to significant damage (9). In 1954, Gerschman et al. put forward a theory suggesting that oxygen poisoning and radiation injury might share a common basis of action. They proposed that there could be a similarity in how both radiation and high pressure oxygen exposure reduced the survival of mice, possibly by creating harmful oxidizing free radicals (9,14).

Two years later, Harman suggested that free radicals are generated during aerobic respiration, contributing to the degenerative processes associated with biological aging (9,15). While there have been numerous theories to explain the aging phenomenon (9,16), none has gained universal acceptance. Harman's theory, however, has gained growing recognition as a potential explanation for the free radical reactions underlying aging (9,17). Recognizing free radical reactions as contributors to aging suggests that trials designed to restrict or inhibit them could potentially lower the rate of age-related oxidative damage, resulting in a reduced aging rate (9,18).

3.1.1.2 Reactive oxygen species

ROS are molecules with the ability to exist independently, characterized by one oxygen atom and one or multiple unpaired electrons. This category includes the superoxide anion radical (O_2•), hydroxyl radical (•OH), hydroperoxyl radical (HO_2•), and singlet oxygen (O_2). Small amounts of ROS are generated during physiological cellular processes such as aerobic respiration or inflammatory reactions. ROS primarily have a signaling function and serve in cell differentiation and apoptosis, influencing the natural aging process. Elevated production of free radicals occurs due to prolonged exposure to ultraviolet radiation (UVR), chronic stress and inappropriate dietary habits. Under normal physiological circumstances, there is a so-called redox homeostasis, which means that free radicals are created and eliminated at equal rates. Excessive free radical formation leads to oxidative stress, causing molecular and cellular damage. In vitro, ROS induce chemical modifications and harmful effects on DNA, proteins, carbohydrates and lipids. These modifications influence the development of various diseases associated with free radical-mediated processes (19).

Proteins can undergo oxidative modifications in three main ways: specific changes to certain amino acids, peptide cleavage through free radical actions, and the creation of links between proteins due to reactions with products of lipid peroxidation (20). When proteins are damaged in this manner, they can contain highly reactive groups that may harm cell membranes and various cellular functions. The peroxyl radical is commonly seen as the free radical responsible for oxidizing proteins. This oxidative damage to proteins can impact signal transmission, enzyme activity, heat resistance, and susceptibility to breakdown, ultimately contributing to the aging process.

Numerous experiments strongly indicate that DNA and RNA can be harmed by oxidative damage. There is substantial evidence suggesting that, particularly in cases of aging

and cancer, DNA is a significant target for such damage (21). Studies have shown that mtDNA, in particular, is more prone to oxidative damage, and this vulnerability is linked to various diseases, including cancer (7).

3.1.1.3 Reactive nitrogen species

RNS encompass the free radical nitric oxide (NO•) and peroxynitrous acid (HNO_3). In physiological concentrations, NO• serves cell signaling. However, when there's an excessive amount of NO•, it causes oxidative stress (22). This happens when NO• reacts with O_2•, forming HNO_3. HNO_3 is involved in a process called nitration, where tyrosine residues in proteins are altered, affecting the function of the proteins (known as nitrative stress) (23). In situations of oxidative stress, where there's too much NO• being produced, nitrative stress plays a role in the development of various diseases (23,24).

3.1.1.4 Role of mitochondria in cellular resilience

Mitochondria play a crucial role as a source of ROS in most human cells. The ROS produced by damage to the mitochondria in various health conditions also has significance in transmitting redox signals from the mitochondria to the rest of the cell (25,26). Therefore, understanding how mitochondria generate ROS is essential for comprehending several critical biomedical aspects (Figure 1). The first indication of ROS production by the respiratory chain (RC) was reported in 1966, and subsequent groundbreaking research by Chance et al. demonstrated that hydrogen peroxide (H_2O_2) is generated by mitochondria (27,28). It was later confirmed that this H_2O_2 is a breakdown product of O_2• produced in mitochondria. Simultaneously the presence of mitochondrial specific superoxide dismutase (SOD2 or MnSOD) was discovered, which underlines the significance of mitochondrial O_2• production (29,30).

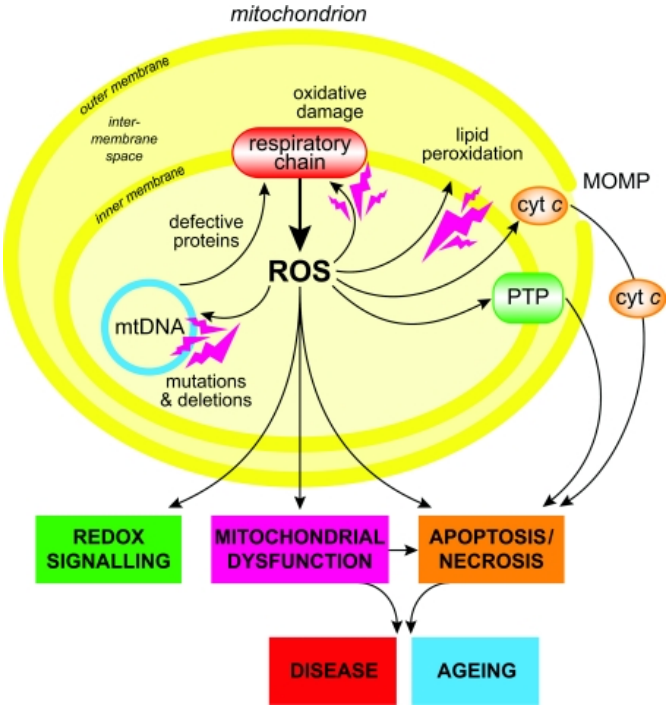

Figure 1. Overview of mitochondrial ROS production (30)

The mitochondrial RC plays a significant role in generating ROS within cells. The discovery of this central role of mitochondria supported Harman's free radical theory (15,31), which was expanded by this discovery to the mitochondrial free radical theory of aging (MFRTA) and proposes that mitochondrial DNA (mtDNA) mutations are the initial and primary events in the aging process. According to the MFRTA (Figure 2), aging is attributed to the harmful effects of ROS. This theory proposes a cycle where ROS toxicity initiates a sequence of events. The mtDNA damage leads to malfunction in the RC, which, in turn, increases the production of ROS. This elevated ROS production further contributes to damage to mtDNA, creating a self-amplifying cycle of deterioration.

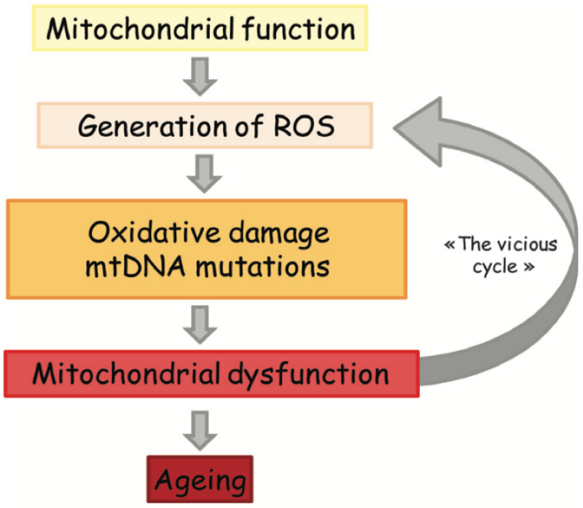

Figure 2. Schematic representation of the MFRTA (32)

Researchers have noticed that mutated mtDNA molecules tend to accumulate with age. An alternative proposal suggests that the accumulated damage, possibly induced by ROS, may surpass the repair machinery, leading to a build-up of mutated mtDNA. In line with the MFRTA (Figure 2), the buildup of damage sets off a vicious cycle, causing a rapid increase in mutated mtDNA over time.

Even though ROS are produced in various parts of cells, 90% of cellular ROS originates from mitochondria (25). Oxidative phosphorylation (OXPHOS) is a crucial process in the mitochondrial network that links the oxidation of nicotinamide adenine dinucleotide (NADH) or to synthesize ATP (adenosine triphosphate) (Figure 3). During this process, NADH provides electrons to the RC, consisting of four complexes. Electrons are transferred by these complexes step by step to ultimately reduce O_2 and form water (Figure 3). However free radicals can also form at certain points of the RC, when electrons escape from NADH and reduce O_2, creating free radicals. It's predicted that the first and third complex are the main sites for ROS production (33). Subsequently O_2• is formed, which can further transform into •OH and H_2O_2 (30).

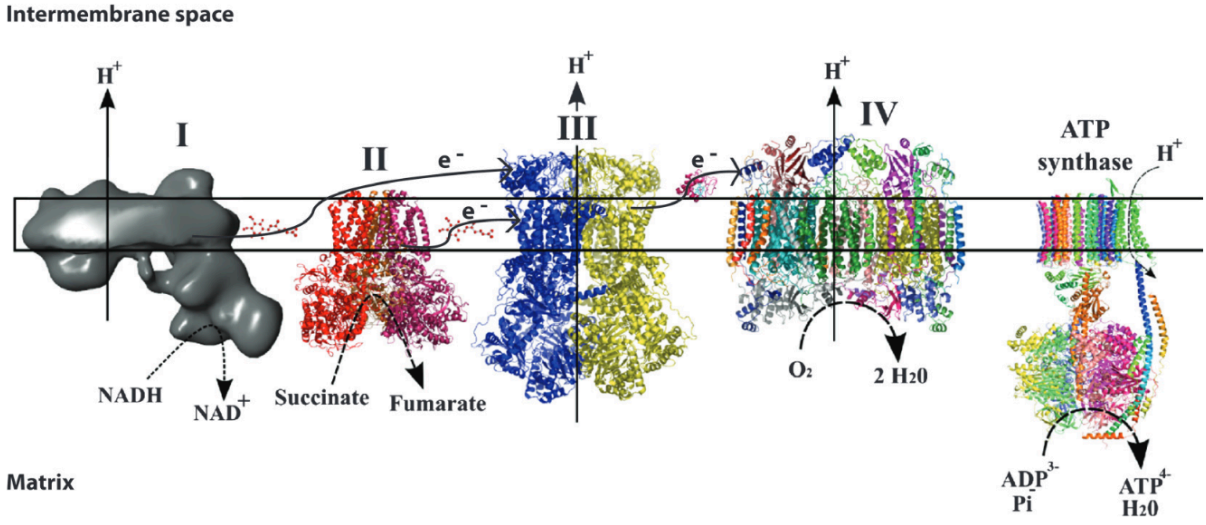

Figure 3. The OXPHOS system (32)

The MFRTA is built on several observations, including: A correlation between age and degree of oxidative damage, decrease in OXPHOS with aging, and the association of various age-related diseases with redox homeostasis imbalance. Additionally, the theory predicts that mitochondrial dysfunction leads to an increased ROS generation. While the theory, based on correlations, led to trials with the goal to reduce ROS levels for health benefits, there is evidence, reported in various studies (34,35), that contradicts the MFRTA. The results suggest that the increase in ROS is the consequence rather than the cause of aging (34). ROS, according to this perspective, are linked to aging as they act as mediators in the stress response to age-

related damage. Consistent with this hypothesis, ROS production gradually increases with age until it reaches a level at which its toxic effects do more harm than good (Figure 4) (32,34).

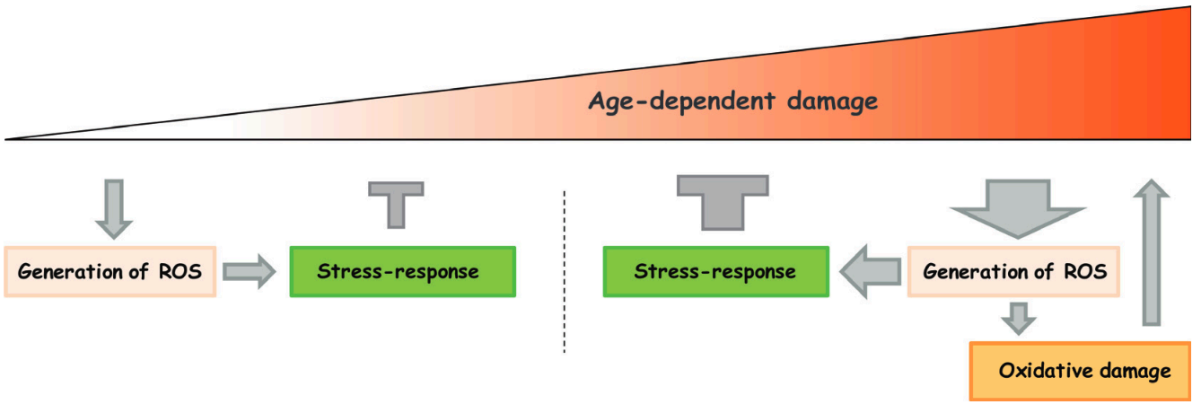

Figure 4. The gradual ROS response hypothesis (32)

One of the emerging theories challenging the traditional MFRTA is the gradual ROS response hypothesis (Figure 4) (34). This hypothesis suggests that ROS act as stress signals in response to damage related to aging, indicating that ROS aren't a cause of aging in the first place but rather a result of it. This new theory offers space for new perspectives on the interpretation of experimental results that contradicted the MFRTA before. It is essential to develop specific tools that can accurately measure ROS levels and oxidative damage. Further experiments are needed to better understand the correlations linking ROS to mtDNA mutations and aging. It would be beneficial to explore whether reducing levels of mutated mtDNA can extend lifespan in vivo models. To prevent age-related diseases, we must first gain a more comprehensive understanding of how ROS can be both harmful and beneficial, as they are simultaneously involved in various diseases related to oxidative stress (32).

3.1.1.5 Importance of autophagy in cellular health

ROS and RNS cause irreversible oxidation of DNA and other biomolecules, making them a major cause of cellular damage. Autophagy plays a crucial role in clearing cells of biomolecules, including DNA, proteins and lipids which have been oxidatively damaged. Therefore, autophagy can be considered as a part of both the antioxidant system as well as DNA repair mechanisms.

Autophagy can be classified into three types: (I) In macroautophagy / autophagy an autophagosome is created, which is responsible for enclosing damaged organelles and biomolecules; (II) microautophagy is characterized by direct lysosomal digestion of cytosolic

content; (III) chaperone-mediated autophagy involves the targeted delivery of specific proteins to lysosomes for degradation. The goal of autophagy is for cells to coordinate the availability of sources necessary for essential processes such as growth and proliferation. Therefore, cells activate autophagy when the required resources (e.g. ATP) for metabolic reactions are insufficient. This process allows cells to quickly break down old or damaged components, recycling the generated biomolecules for reuse (36).

Most intracellular ROS is produced by mitochondria in their RC. Particularly the first and third complex of the RC can release electrons. These electrons can then partially reduce oxygen to form O_2•, which quickly transforms into H_2O_2 either spontaneously or through the catalysis mediated by SOD. In certain situations, like when cells need more energy or as mitochondria may become less efficient (e.g., during aging), the flow of electrons through the mitochondrial RC can increase. Additionally, other factors like external sources (e.g., UVR) or internal sources of ROS can also contribute. This highlights that evolution has likely favored the development of a highly effective antioxidant response to safeguard and maintain cellular components as much as possible (37).

NO• is a very reactive gas-like radical that can dissolve in water and move through cell membranes. It's naturally produced by enzymes called NO synthases. Like ROS, NO• can create conditions that lead to oxidative damage. If the production of NO-derived oxidant species is too high and overwhelms the antioxidant response, it can cause irreversible harm to biomolecules (38).

ROS have been widely reported to be early triggers of autophagy when cells lack nutrients. According to a detailed study by Chen et al. (39), O_2• is suggested to be the main ROS involved in autophagy. However, some evidence points to H_2O_2 being produced right after starvation, while others simply propose that ROS are essential for autophagy. This is supported by observations where treating cells with antioxidants reverses the autophagy process (40).

In contrast, other data suggest that mitochondria are the main producers of the ROS needed to trigger autophagy, even if they aren’t located near the cell's outer membrane. A sudden lack of nutrients which leads to energy stress could be a potential explanation for this finding. This stress increases the demand for ATP, putting force on mitochondria to cope with unfavorable conditions. Consequently, the leakage of electrons and the production of ROS also go up (30).

Mitophagy is a form of autophagy in which mitochondria are selectively broken down. While mitophagy is essential, it's considered an extreme measure for a cell experiencing nutrient deprivation, and there are at least two primary reasons for this. (I) Mitochondria play

a crucial role in producing ATP, which is vital when there's a shortage of carbon sources; (II) mitochondria are relatively large organelles, and they need to be fragmented beforehand to be correctly identified and engulfed by autophagosomes. Both of these factors help explain why, in general, mitochondria are resistant to undergoing mitophagy unless they are severely damaged (41).

Recent findings over the past years indicate that NO• may also play a role in influencing autophagy. However, instead of acting as a positive factor promoting the process, it appears to function as an inhibitory molecule. This in turn contrasts with the previously discussed role of ROS, making understanding the complex relationship between oxidative stress and autophagy even more difficult.

Antioxidant response and autophagy seem to be activated at the same time in response to oxidative stress. Their coordinated action aims to lower the concentrations of ROS and RNS and minimize oxidative damage. This repair system is well-suited for cells to fight oxidative stress and regain redox homeostasis. While autophagy efficiently responds to oxidative stress by rapidly removing toxic oxidized molecules and damaged organelles, it differs from other cellular components like proteins and organelles, which exist in multiple copies. Autophagy cannot mediate the degradation of the cell nucleus as this would lead to a loss of genetic information as the nucleus contains the DNA which cannot be completely re-synthesized and replaced. The intactness must be preserved and carefully maintained, requiring accurate repair of any damage. It appears that autophagy is an important part of the DNA damage repair system, particularly in response to damage caused by ROS and RNS (36).

Free radicals are significant contributors to DNA damage, directly modifying DNA or causing different types of lesions, both of which can harm cell viability. NO• and certain RNA can cause nitrative stress and thus induce DNA damage. Moreover, ROS and RNS pose a threat to the integrity of mtDNA. This has a profound impact on the transcription of certain mtDNA-coded proteins, which are essential for synthesizing subunits of the complexes within the mitochondrial RC. This can lead to a vicious cycle in which dysfunctional mitochondria generate even higher levels of ROS, leading to even greater damage in the mitochondria. Once DNA damage becomes too severe, it ultimately leads to cell death (42).

Evidence shows that free radicals play a role in regulating autophagy, likely influencing different stages of the process. In this case, ROS and RNS target internal signaling that indicates the availability of nutrients outside the cell. Through this mechanism, the energy level is transmitted through negative feedback to the autophagosome, which then decides to restore balance while eliminating oxidative damage when the energy level is too low. In this way

autophagy becomes crucial for cells to cope with both nutrient deprivation and oxidative stress conditions simultaneously (36).

3.1.2 Oxidative skin damage

As our largest organ, the skin provides a barrier to the external world and therefore protects us from external factors. One significant aspect of skin health involves the cellular redox homeostasis. An imbalance of free radicals and available antioxidants leads to oxidative stress. This condition not only poses a risk to various skin disorders but also holds the potential to impact both the functional and aesthetic aspects of the skin. This includes the development of conditions such as skin cancer, acute or chronic inflammation as well as intrinsic and extrinsic aging-related manifestations such as blemishes, marks, expression lines, and other signs of aging (43).

3.1.2.1 Endogenous factors

The skin's intrinsic production of ROS involves various intracellular enzymes, inter alia, those within the mitochondrial RC, NADPH oxidase and cytochrome P450 (CYP450) family enzymes. They are distributed across key cellular components such as mitochondria, endoplasmic reticulum (ER), cell membrane, and cytosol, with mitochondria and ER playing crucial roles in ROS generation. Within the mitochondria, oxygen undergoes conversion to a O_2• by the first and third complex of the RC. This radical is then transformed into H_2O_2 through the action of SOD. Antioxidant enzymes within the mitochondria further break down H_2O_2 into water and oxygen. During redox imbalance, H_2O_2 can contribute to the generation of additional ROS (44). Notably, during the synthesis of nitric oxide synthase (NOS), the generation of NO• occurs as a by-product. Hence, endogenous ROS primarily arise as by-products of OXPHOS and enzymatic reactions integral to supporting aerobic metabolism. This complex process highlights the dynamic nature of ROS generation within the skin's cellular machinery (45).

3.1.2.2 Exogenous factors

Exogenous ROS primarily derive from environmental influences, with UVR standing out as a prominent contributor (Figure 5). UV light can be divided into three categories according to its

wavelength: UVA, UVB, UVC, from long to short wavelength, respectively. UVA has the highest penetrating power and reaches the dermal layer, causing skin tanning, while UVB with more moderate penetration can cause epidermal sunburn. Sunlight constitutes the primary source of UVR, with 95% being UVA and 5% UVB reaching the Earth's surface (46). Extended exposure of the skin to sunlight results in elevated ROS levels. Furthermore, various environmental factors, including radiation, chemicals, and thermal stimuli, can contribute to the generation of ROS, highlighting the multifaceted nature of external oxidative stress on the skin (Figure 5) (44).

Airborne pollutants are widely acknowledged for their detrimental impact on health. According to WHO, air pollutants can be divided into four main pollutants. (I) particulate matter; (II) ozone; (III) nitrogen dioxide; and (IV) sulfur dioxide. These pollutants can increase oxidative stress in the skin, primarily through peroxidation of lipids, thereby reducing the amount of antioxidants in the skin and impairing its barrier function. For instance, ozone has been linked to lipid peroxidation, a process that has been reported to contribute to increased skin wrinkling (47).

Both endogenous and exogenous factors play a role in stimulating the generation of ROS, leading to various adverse consequences for the skin (Figure 5). These consequences encompass cellular senescence, inflammation, and an increased susceptibility to skin cancer, underlining the interconnected nature of environmental and internal factors in influencing skin health (48).

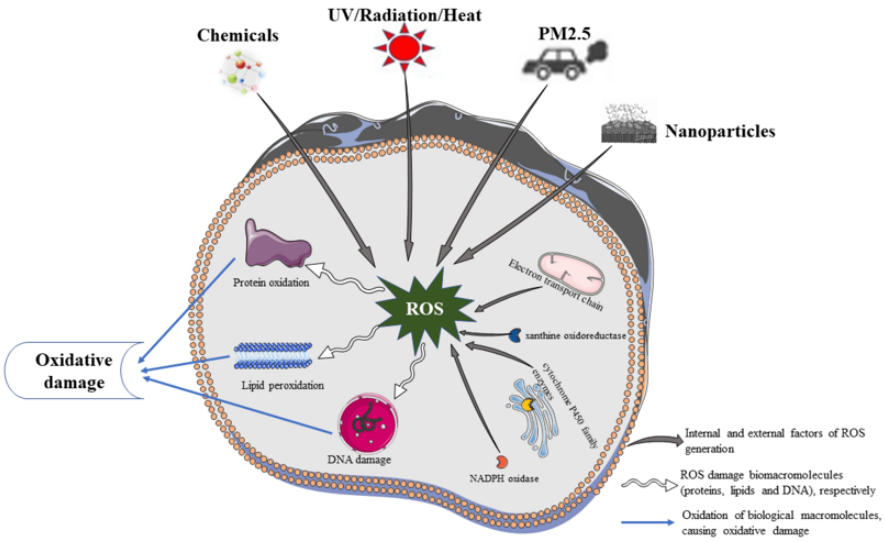

Figure 5. Oxidative damage of biological macromolecules (44)

3.1.2.3 Role of oxidative stress in the skin aging process

The skin undergoes considerable oxidative stress, primarily due to sun exposure and pollution. Environmental changes, such as heightened radiation resulting from ozone layer depletion and increased pollution levels, create a substantial demand for antioxidants in the body (49).

Aging is a natural process in our body that is based on physiological changes. Intrinsic aging affects all tissues and is accompanied by a decline in the functionality of our body, which makes us more susceptible to various diseases as we get older and years of exposure to environmental stress may also become noticeable. Notable skin changes associated with intrinsic aging include rough, drier skin with fine lines and wrinkles (1).

Extrinsic aging, however, results from various environmental factors like years of unprotected sunbathing, both under natural and artificial sunlight as well as air pollution. The skin's response to these factors varies from person to person and depends on the level of melanization, the individual's predisposition and how much UVR it has been exposed to throughout life. This type of aging is manifested by thickening of the skin, dryness, the development of wrinkles, telangiectatic changes and other precancerous or cancerous lesions. Interestingly, intrinsic aging is noticeable in areas with low radiation exposure, while extrinsic aging is particularly visible in areas that are highly exposed to the sun, such as the face, arms and legs, and neck. Photoaging, defined as the combination of chronic UVR exposure and extrinsic aging, is listed in many studies as an etiology for skin cancer (Figure 6). Skin type plays a large role in susceptibility to skin cancer development as it is primarily related to the skin's ability to produce melanin, a skin pigment that protects us from UVR damage. Therefore, darker skin types are generally better protected than lighter skin types. Excessive sunbathing, especially in summer, causes skin damage, which then becomes acutely noticeable as sunburn. Depending on the severity, the skin is red, feels hot, may be slightly swollen, tense, painful, itchy and burns; if you are severely sunburned, blisters form on the skin (2).

Studies have found that oxidative stress and the associated production of free radicals triggered by exposure to sunlight play a role in the skin aging process (Figure 6). ROS are mainly generated indirectly by enzymes (which are activated by solar radiation). The amount of antioxidants has been shown to be lower in aged skin compared to younger skin (Figure 6), which means that the skin's own cellular resilience weakens with age (50).

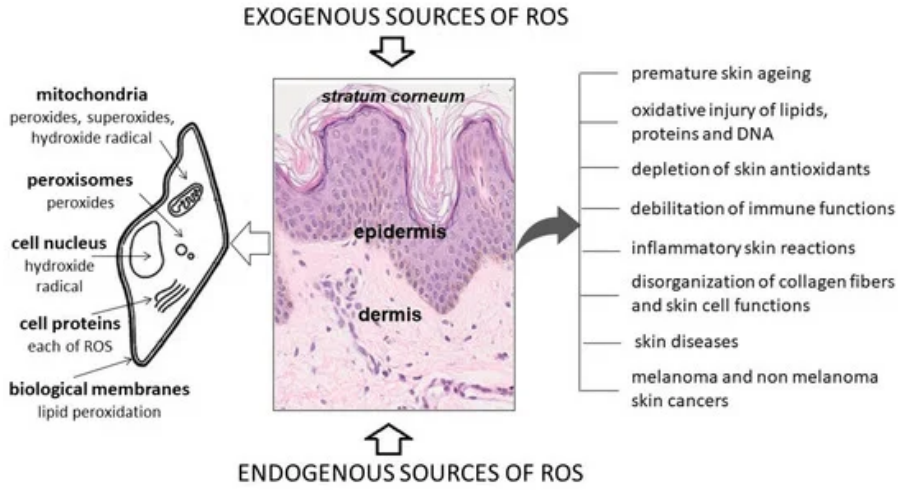

Figure 6. Cellular components attacked by ROS and the effect of oxidative stress on the skin (51)

3.2 Cutaneous antioxidants

Antioxidants form a crucial line of defense against oxidative stress by maintaining a delicate balance between neutralizing free radicals and regulating their own radical status. This complex process of electron exchange underlines the dynamic nature of antioxidant mechanisms, ensuring effective protection against cellular damage. As antioxidants engage with and neutralize reactive radicals, the resulting transformed radicals possess reduced reactivity and diminished potential for harm. This controlled process highlights the adaptive nature of antioxidants, emphasizing their role not only in direct radical neutralization but also in the regulation and termination of radical status through interactions with other antioxidants and complementary mechanisms.

The cutaneous antioxidant system comprises both enzymatic and non-enzymatic antioxidants. Glutathione peroxidase (GPx), catalase (CAT), and SOD are enzymatic antioxidants of the skin (52).

Moreover, non-enzymatic antioxidants play a vital role in preserving the cellular redox equilibrium. This category encompasses various antioxidants also including vitamins such as vitamin C, vitamin D, and vitamin E. These antioxidants collectively contribute to the defense mechanisms against oxidative stress, supporting the overall health and integrity of cells and tissues in the skin (53).

3.2.1 Enzymatic antioxidants

GPx is a widespread intracellular enzyme that facilitates the UV light-controlled decomposition of H_2O_2 into water and oxygen. This enzyme is present in both the cytosol and mitochondria, making it a versatile defender against oxidative stress. Notably, GPx's activity is minimally affected by UV exposure, highlighting its significance as a primary antioxidant defense system in the skin. In a mouse skin model exposed to acute UVB radiation, Fuchs et al. (3) observed a decrease in glutathione reductase and catalase activity, while SOD and GPx remained unaffected. This finding emphasizes the resilience of GPx in the face of UV exposure, contributing to its critical role in preserving the antioxidant defense system. However, if repeated UV exposures occur before the recovery of inactivated enzyme activities, significant cellular damage may result due to a compromised antioxidant defense system.

In various animal organs, catalase is typically located in peroxisomes, while GPx is distributed in both mitochondria and cytosol. These enzymes collaborate effectively in decomposing endogenous H_2O_2. Interestingly, their stability under UVB irradiation differs, with GPx exhibiting greater resilience compared to catalase in terms of activity after the same UV exposure. This shows the complementary roles of these enzymes in cellular resilience against UV-induced oxidative stress (54).

Researchers are actively seeking health products or drugs to fulfill the widespread desire for enhanced health and youthfulness. The effectiveness, mechanisms of action and anti-aging properties of SODs were researched and further developed. Core elements of the aging process, including mitochondrial ROS generation, oxidative stress, apoptosis and diminished functional capacity have been identified. Research found that SOD3 levels were higher in the aging skin of older people and mice compared to the skin of younger study participants. Interestingly, SOD1 and SOD2 were equally expressed regardless of the age of the participants (55). This observation has prompted further investigation into the role of SODs in combating aging. Notably, research findings indicate that SOD3 is indispensable in anti-aging efforts. Studies with SOD1-deficient mice have shown signs of premature aging. Similarly, increased mitochondrial oxidative damage and proton leakage were demonstrated in mice lacking mitochondrial MnSOD (SOD2), further supporting the essential role of SODs in anti-aging strategies. These insights contribute to the ongoing development of interventions aimed at promoting health and youthfulness (56).

3.2.2 Non-enzymatic antioxidants

Unlike their enzymatic counterparts, these antioxidants act independently of enzymes and serve as the primary defense against harmful free radicals. Among these defenders, vitamins stand out as essential players in maintaining the vitality and resilience of our skin.

3.2.2.1 Vitamin C

Vitamin C stands out as the most abundant antioxidant in the human skin, belonging to the category of non-enzymatic antioxidants crucial for shielding the skin from ROS. Vitamin C is an essential vitamin that is found and synthesized in vivo in various plants and animals. However, humans cannot synthesize it because they lack an enzyme needed for synthesis. That's why it's necessary for us humans to supplement it with foods rich in vitamin C, for example black currants, sea buckthorn and citrus fruits (57).

While the gut absorption of vitamin C is limited by an active transport mechanism, making oral dosage less effective, the bioavailability of orally administered vitamin C in the skin is insufficient. Therefore, dermatological practice often favors the use of topical ascorbic acid (57).

Vitamin C, with its hydrophilic properties, acts in the aqueous compartments of cells and is crucial for combating oxidative stress caused by ROS production following UVR exposure. Vitamin C shows its antioxidant effect by donating an electron to free radicals to neutralize them and is itself oxidized in this reaction. In the oxidized form, however, it is relatively non-reactive and stable. Enzymes like dehydroascorbic acid reductase, can convert oxidized forms back to vitamin C. UVR, however, can reduce the cutaneous vitamin C (58).

Unlike sunscreens, which only block 55% of ROS production caused by UVR, vitamin C is effective against both UVB and UVA. The combination of sunscreens with topical antioxidants like vitamin C optimizes UV protection. Vitamin C in the form of topical application has been shown to significantly reduce UVB-induced erythema and sunburned epidermal keratinocytes under laboratory conditions (59).

Vitamin C works synergistically with vitamin E, potentiating its action four-fold. Together, they protect the hydrophilic and lipophilic compartments of cells, limiting oxidative damage by reducing ROS formation. Vitamin C is also crucial for collagen biosynthesis, influencing both quantitative and qualitative aspects of collagen production. Clinical research

has demonstrated that topical application of vitamin C promotes collagen synthesis in the human skin across all age groups (57,59,60).

Various forms of vitamin C, such as creams, serums, and transdermal patches, are available in the market. Only active vitamin C in form of a serum, provides a persistent reservoir for effective photoprotection through regular applications. Dermatologists should be aware that not all vitamin C preparations are physiologically effective, and there is ongoing interest in finding efficient methods for the transepidermal delivery of stable active compounds (57,59).

Interestingly, application of vitamin C after microdermabrasion and certain resurfacing techniques enhances its transepidermal penetration. Vitamin C has also shown promising effects in preventing erythema by application both before and after dermatological laser treatments, particularly in those patients with low vitamin C levels, such as smokers or those with UV-damaged skin. Its effectiveness in combating smoking-related skin aging is being actively researched (57,58).

3.2.2.2 Vitamin D

Overexposure to solar UVR can accelerate the aging of the skin and may lead to the development of cutaneous cancer. However, paradoxically UVR also plays a positive role in regulating various skin functions. Despite UVB being implicated in the increased risk of precancerous and cancerous cutaneous lesions, it is essential for the production of vitamin D_3 in the skin, fulfilling more than 90% of the body's vitamin D_3 requirements. Vitamin D_3, in turn, is an important constituent for maintaining the epidermal barrier (61).

Upon UVB exposure to the skin, 7-dehydrocholesterol (7-DHC) undergoes a non-enzymatic transformation into vitamin D_3 facilitated by thermal energy. This reaction is influenced by the UVB dose and temperature. In the liver, vitamin D_3 is hydroxylated to 25-hydroxyvitamin D_3 ($25(OH)D_3$). In the skin, kidneys, and other tissues, it is further hydroxylated to generate 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the biologically active derivative of vitamin D_3 (61,62).

Vitamin D_3, especially its active forms, can provide protection against oxidative stress caused by UV radiation and pollution and therefore also has anti-aging effects. Studies have shown that oral intake of vitamin D_3 in high doses shortly after UVB exposure resulted in a reduction in skin damage caused by oxidative stress by triggering epidermal barrier repair mechanisms and reducing inflammation. There is evidence that, depending on the dosage, the active derivatives of vitamin D_3 develop antioxidant abilities that prevent the formation of ROS

and therefore the formation of sunburn cells. This, in turn, promotes cytoprotection and detoxification, diminishing the effects of photoaging (Figure 7). Consequently, these derivatives protect our skin cells against UVB-induced oxidative stress (63).

Chronic UVB and UVA exposure damages our DNA and leads to the generation of RNS, potentially causing premature skin aging and carcinogenesis (Figure 7). Hydroxyderivatives of vitamin D_3 such as $1,25(OH)_2D_3$ also share synergistic effects with p53, a so-called tumor suppressor protein, as it upregulates the phosphorylation and expression of p53 as well as its transport into the nucleus where it exerts photoprotective and repair properties. The p53 gene family emerges as a significant target for vitamin D in the context of prevention of oncogenesis and premature aging, given the shared mechanisms that drive both processes (64,65).

Moreover, $1,25(OH)_2D_3$ has the ability to reduce skin cell apoptosis and inhibit DNA damage caused by oxidative stress by inducing autophagy and mitophagy, which are considered energy-conserving processes. These actions contribute to the intrinsic photoprotection mechanism of the skin (63,66).

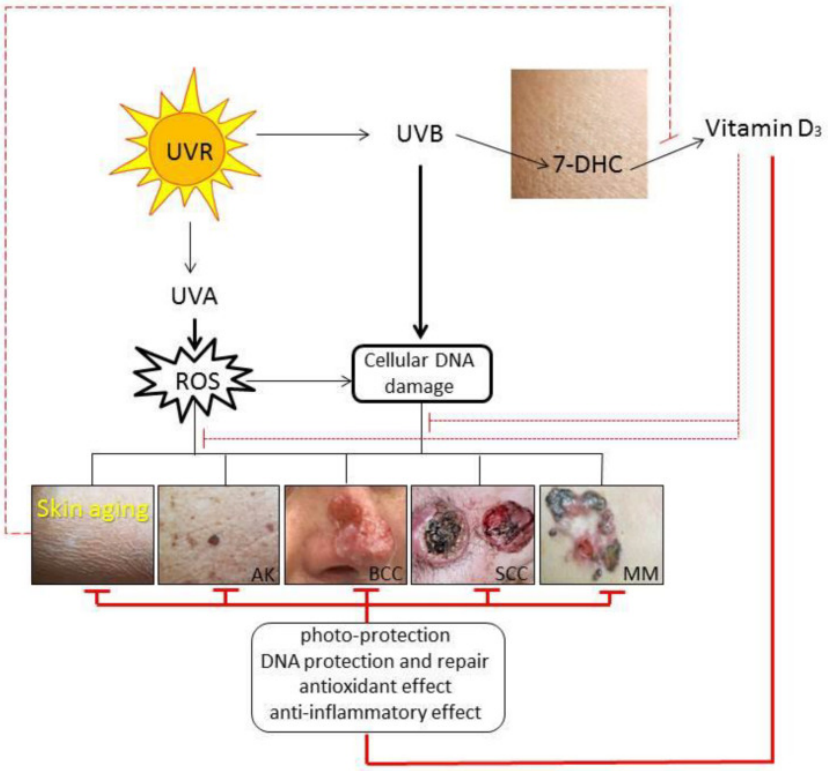

Figure 7. Photoprotective effects of vitamin D_3 in premature skin aging and cutaneous oncogenesis (66)

3.2.2.3 Vitamin E

It has long been known that vitamin C and E, as natural antioxidants, play a crucial role in skincare, exerting various effects such as photoprotection, enhanced antioxidant activity, and collagen synthesis. Vitamin E, being fat-soluble, is predominantly found in vegetable oils, especially sunflower oil and rapeseed oil, as well as in various nuts such as walnuts, peanuts, hazelnuts and especially almonds (67).

Vitamin E inhibits the free radical peroxidation of polyunsaturated fatty acids (RH) in lipoproteins and cell membranes. RH are highly susceptible to oxidative damage induced by free radicals, such as •OH. In the first step, RH transforms into an alkyl radical (R•) and reacts with oxygen (O_2), which leads to the formation of a peroxide radical (ROO•). The peroxide radical then reacts with a neighboring RH to produce a hydroperoxide radical (ROOH) and another R•, initiating an auto-oxidation cycle. Vitamin E works synergistically with vitamin C to break this chain. Vitamin E directly interacts with ROO•, forming a stable hydroxy fatty acid (ROH). By this process vitamin E itself becomes a free radical (vit E•), which is being neutralized by vitamin C, which, in turn, becomes a free radical (vit C•). These mechanisms highlight the complex network formed by antioxidants, emphasizing the need for a balance between them to disrupt the process of lipid peroxidation (68).

Vitamin E deficiency, in animal models, led to alterations in the cross-linking of collagen in the skin (69). Topical application of vitamin E has shown his antioxidant and photoprotective effects against UV-induced skin damage, reducing skin dryness, elasticity, facial lines and wrinkling very well (70).

A balanced diet rich in vitamins C and E has been shown to be important for maintaining skin health. Oral antioxidants are suggested to be good supplements for healthier skin, particularly in protecting the skin from UV-induced oxidative damage. Vitamin E in particular, along with other antioxidants, has been studied for its photoprotective effect on the skin. Direct topical application of vitamin E is considered the most effective route for skin protection due to its ability to inhibit lipid peroxidation. Oral use of high-dosage vitamin E exhibits photoprotection, and when combined with high doses of vitamin C, its effects are amplified (71).

3.2.3 Antioxidants in cosmetics

There has been a significant shift in the cosmetic formulation landscape towards ingredients that primarily contribute to the overall health of the skin and also have a positive impact on aesthetics. Today's skincare products go beyond the traditional roles of cleansing, protecting, and moisturizing, now encompassing the qualities of renewal, restoration, and rejuvenation. Very important is the incorporation of vitamins and antioxidants, a focal point generating significant interest within the cosmetic industry. Research has given substantial evidence supporting the benefits of integrating vitamins and antioxidants into skincare formulations. While the essential role of these elements in human health is well-established through dietary sources and supplements, the topical application of these potent compounds in cosmetics is gaining prominence. By doing so, it is believed that these formulations can offer an additional layer of defense against free radicals, potentially defending and correcting damage by neutralizing these oxidative stressors (72).

3.2.3.1 Sunscreen protection

Our skin has natural defense systems against damage, including enzymatic antioxidants like GPx and SOD, and non-enzymatic antioxidants like vitamin C and vitamin E. Applying antioxidants directly to the skin can strengthen these defense systems. Additionally, using antioxidants in sunscreens can improve their ability to protect against UV rays. Studies have shown that certain antioxidants in sunscreen can reduce redness and the development of sunburn cells. Antioxidant-rich cosmetic ingredients not only contribute to the stability of UV filters but also help prevent damage caused by free radicals. The cosmetic industry is continually searching for new antioxidant compounds to include in skincare products (73,74).

444 sunscreens sold in pharmacies in Portugal in 2021 from 43 different cosmetic brands have been analyzed in a study. The aim of the study was to identify the antioxidant properties of these sunscreens. The analysis revealed 38 different antioxidants in the sunscreens. Vitamin E and its derivatives were found most frequently (66.3%), followed by vitamin C and its derivatives in second place (12.9%) (Figure 8) (75).

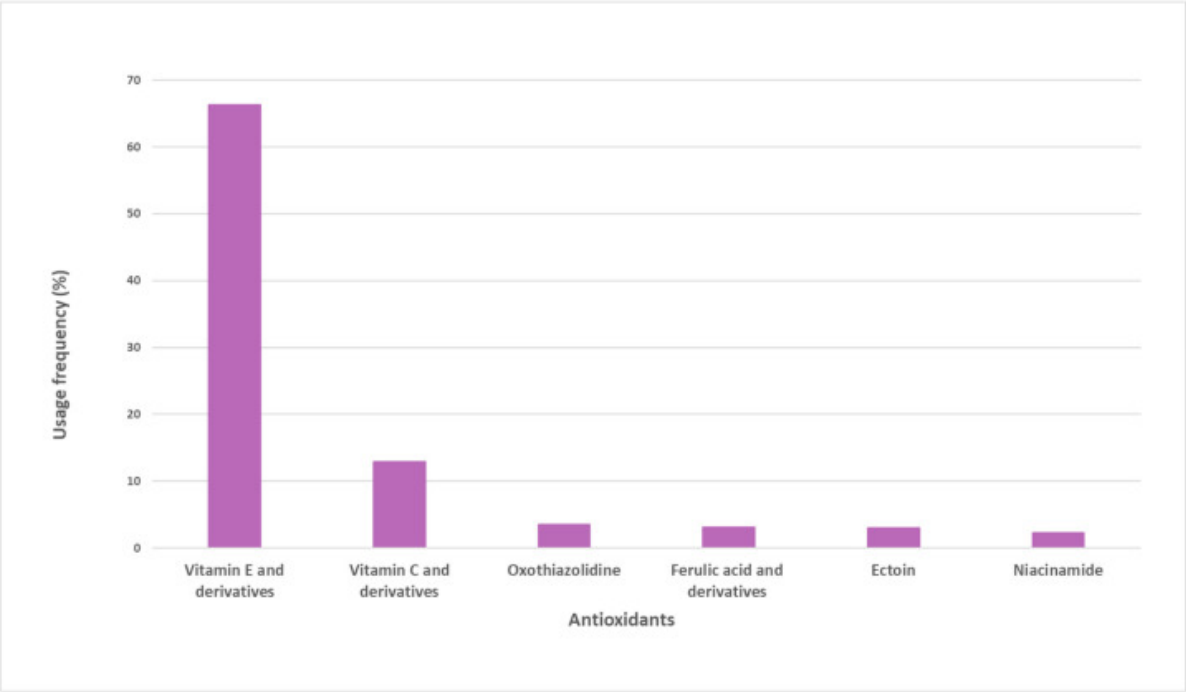

Figure 8. Abundance of the top six antioxidants used in sunscreens (75)

Interestingly, while vitamin E is used more frequently, vitamin C is also present in some sunscreens. The lower frequency of vitamin C usage might be due to its water-soluble nature, making it challenging to incorporate into cosmetic formulations compared to the fat-soluble vitamin E (75).

3.2.3.2 Anti-aging formulations

Over a seven-year period Silva and Ferreira et al. conducted an analysis of antioxidants in anti-aging formulations. Their results showed that vitamin E and its derivatives were the most commonly used antioxidants in anti-aging formulations. Vitamin C and its derivatives have also been commonly used in such products, however vitamin C derivatives (20%) are used almost twice as often as pure ascorbic acid (11%) because they are more easily absorbed by the skin. Interestingly, in sunscreens, these antioxidants were engaged in smaller proportions, potentially due to their additional benefits, such as promoting collagen synthesis, which is more relevant in anti-aging formulations.

The presence of antioxidants is evident in both anti-aging and sunscreen products, demonstrating a similarity in the use of these beneficial compounds. Vitamin E, vitamin C and their derivatives have become indispensable in both sun protection and anti-aging formulations due to their excellent antioxidant properties (75).

4 Conclusion

In conclusion, the complex web of oxidative stress and skin aging necessitates a comprehensive approach to cellular resilience. Endogenous and exogenous factors synergistically contribute to ROS generation, emphasizing the need for robust antioxidant defenses. The interplay between intrinsic and extrinsic aging underlines the importance of understanding both physiological changes and environmental influences. Cutaneous antioxidants, both enzymatic and non-enzymatic, play central roles in maintaining redox balance and protecting against oxidative stress-induced damage. The resilience of enzymes like GPx and the indispensability of SOD3 highlight potential routes for anti-aging interventions. Non-enzymatic antioxidants, particularly vitamins C, D, and E, demonstrate their efficacy in combating oxidative stress and promoting skin health. The cosmetic industry's integration of antioxidants into formulations reflects the growing recognition of their benefits in skincare. Sunscreens and anti-aging products enriched with antioxidants present promising strategies for enhancing skin protection and rejuvenation.

5 Summary

This thesis is a short review of the current knowledge in the field of cellular resilience, focusing on antioxidant approaches to combat skin stress. Environmental factors, especially UVR and pollutants, contribute significantly to the generation of ROS, leading to oxidative stress and skin aging. Intrinsic and extrinsic aging processes further exacerbate the impact on skin health, emphasizing the interconnected nature of internal and external factors. Examining the role of antioxidants, both enzymatic and non-enzymatic, reveals their complicate dance in maintaining redox balance and protecting against oxidative stress. The cosmetic industry's focus on incorporating antioxidants into sunscreens and anti-aging formulations reflects a paradigm shift towards comprehensive skincare. This thesis contributes to the understanding of cellular resilience and offers insights into potential strategies for promoting skin health and combating the effects of oxidative stress.

Key-words: cellular resilience, oxidative stress, free radicals, skin aging, antioxidants

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7 Curriculum vitae

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