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# The European Journal of Aesthetic Medicine and Dermatology

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# The European Journal of Aesthetic Medicine and Dermatology

Volume 6, Issue 1, 2016 - ISSN 2240-5046

## EDITORIAL

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## The world of Aesthetic Medicine and Dermatology is very rapidly changing because of the development of Biophysics, Chemistry, Biochemistry and Technology. On this background the present issue of EJAMED was aimed to develop some relevant concepts we anticipated in the previous Editorial

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In particular our four-monthly choice has focused on oxidative processes because redox reactions i) are the common basic denominator of all biological reactions that take place in our body, and ii) are variously affected in aesthetic and dermatological diseases. In order to have an idea about the scientific relevance of oxidative and reductive processes one should consider that the biggest worldwide medical/scientific database i. e. PUBMED-MEDLINE detected up to now more than 150,000 academic papers for the words “oxidative stress”. In the herein issue the first three articles follow an imaginary line which starting from the description of the basis of redox reactions mechanisms enter into the clinical practice by providing some relevant examples of the application of such knowledge in the cosmetic and dermatological fields.

The first article is an updated review on the so-called oxidative stress (OS). Often and unfortunately described in the past as a result of the unbalance between free radicals and antioxidants, OS nowadays is recognised as the effect of an impairment of redox homeostasis that leads to a deep disturbance of cell signalling, immune defences and detoxification processes. Indeed reactive oxidising species (ROS, including reactive oxygen/nitrogen/carbon/sulphur/chlorine species) are not merely damaging chemical species but highly evolutionary conserved, generally unstable and short-living chemical species which main role is to transiently, reversibly and slightly oxidising some specific organic substrates –often the thiol groups of biologically active proteins like enzymes or second messengers or transcriptional factors – thus regulating globally cell homeostasis and survival. Moreover ROS are involved in the phagocytosis



Eugenio Luigi Iorio MD,  
PhD

process that allows monocytes/macrophages as well polymorphonuclear leukocytes to destroy bacteria and bacteria by-products like endotoxins. Finally ROS production is often the unwanted effect of detoxifying processes taking place in the liver. Due to their well-known reactivity – that in turn derives from their tendency to extract one electron from their natural substrates – ROS are finely regulated by a wide class of compounds/activities commonly called “antioxidants”, but nowadays described as “physiological modulators of redox homeostasis”. These physiological modulators are designed to give just the missing electron to ROS thus avoiding any improper and/or excessive oxidation. In such way any excess of ROS is promptly “buffered” and the OS – i. e. the unwanted consequence of redox homeostasis impairment – is prevented. On such basis the first review article of this issue describes and analyses the physiological role of ROS and the biological basis of OS, including its diagnosis and management, by considering it as an emerging health risk factor for early aging and at least one hundred diseases among which are aesthetic and dermatological disorders including wrinkles, cellulite and so on.

The second article is focused on the healthy effects of delphinidin, an anthocyanin specie, extracted from maqui berry (*Aristotelia chilensis*), that acts as physiological modulator of redox homeostasis even in the skin. Indeed nutraceuticals are the most common way to modulate redox balance and to protect our body from OS by a physiological way.

The third article is related to the “ROS paradox” where such species can be used properly to stimulate tissue regeneration. Indeed in this well documented case report a combination of topic and systemic ozone, a powerful oxidant, was safely and successfully used in order to favour breast tissues regeneration after a nipple-areola complex necrosis.

Finally the fourth article is a technical report that describes an original surgery technique of rhinoplasty. The correction of anatomical disorders of nose and paranasal sinuses is very important in order to warrant an optimal body oxygenation. Indeed a impaired oxygen availability (e. g. due to sleep apnoea disorders) can be responsible of oxidative stress.

*Eugenio Luigi Iorio*

# Free radicals, antioxidants and oxidative stress in Aesthetic Medicine and Dermatology

Eugenio Luigi Iorio MD, PhD

*Eur. J. Aesth. Medicine and Dermatology. 2016;6(1):9-47*

## INTRODUCTION

Oxidation and reduction are opposite but reciprocal ubiquitous reactions that take part to most of biological processes in any organisms, from viruses to humans, including plants (1). In the course of such “redox” reactions one or more reducing equivalent units – where a reducing equivalent unit is an electron, alone or bound to a proton as hydrogen atom – are transferred from a chemical species, i. e. the reducing species, to another chemical species, i. e. the oxidising species (1). The transfer of a couple of reducing equivalents (more simply hereinafter referred to as “electrons”) is related to energy metabolism being: i) the oxidation linked to the catabolism and to ATP production, and ii) the reduction bound to the anabolism and to ATP expenditure (2). The transfer of an electron alone is related to the metabolism of the so-called reactive species that are involved in the pathophysiology of stress i. e. the global response of a living organism to internal/environmental de-

mands or pressures (stressors) (3). In Humans stress initiates the so-called “fight or flight” response, a complex reaction that involve neurological, endocrine and immune systems, through the production of specific mediators like ACTH, cortisol and adrenaline (4). At molecular level stress response implies also the production of reactive species which main role is to mediate the cell response to endogenous and/or exogenous physical, chemical and biological stressors through a fine-tuning of cell signalling/transduction and inflammatory/immune/toxic responses (5). After acting reactive species – that are conceptually analogous to hormones or other molecular messengers – are inactivated by the so-called antioxidant system which main role is not to fight such species but rather “to modulate” their actions and to avoid their unwanted side effects (6). By this way reactive species play a relevant role in cell homeostasis and survival, according to the modern concept of “oxidative eu-stress”, where the

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## DISCLOSURES

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prefix “eu” means “good” or “physiological” (7, 8). Therefore any disturbance of one-electron transfer reactions can lead to the improper oxidation of target molecules (including lipids, proteins and nuclei acids) and hence to the “oxidative di-stress” or “oxidative stress” as such, an emerging health risk factor that is related to early aging and at least one hundred different diseases (9).

Oxidative stress may play a key role in Aesthetics. Indeed the combined effects of physical agents (e. g. ultra-violet radiations), chemicals (e. g. drugs and xenobiotics) and biological factors (e. g. viruses, bacteria, toxins), often together with abnormal lifestyle (e. g. cigarette smoke, overweight, inadequate exercise, psycho-emotional stress) may enhance the generation of reactive species thus impairing – depending on genetic conditioning – the function and the structure of superficial organs, mainly the skin and its annexes, like hairs and nails, as well as all subcutaneous layers.

In particular, skin, as the largest human body organ, provides a major interface between the body and environment and is constantly exposed to an array of chemical and physical exogenous pollutants. In addition, a large number of dietary contaminants and drugs can manifest their toxicity in skin. These environmental toxicants and/or their metabolites are inherent oxidants and/or directly or indirectly drive the production of ROS. The subsequent cumulative oxidative damage incurred throughout lifetime was suggested to be related to classical “aesthetic” disorders such as wrinkling, sagging and actinic lentigo (11). Moreover reactive species may activate proliferative and cell survival signalling that can alter apoptotic pathways thus leading finally to photoaging, photosensitivity diseases and some types of malignancy (12). On the

other hand many of the above factors responsible of skin diseases may affect also subcutaneous layers where the so-called extracellular matrix (ECM) which plays a crucial role in the modulation of metabolic fluxes and molecular signalling between microcirculatory system and tissues, takes place (13). Evidence suggests that oxidative stress induces endothelial dysfunction and impairs the physiological balance between metalloproteinases and their inhibitors thus favouring the demolition of ECM (13). One of the most troubling consequence of these processes is the so-called “cellulite” that was shown to improve after antioxidant supplementation (14).

Therefore, any strategy aimed to prevent or slow-down aesthetic disorders should consider the specific impact of the oxidative stress. A deeper basic knowledge of such phenomena is of prominent interest for both Clinicians and Surgeons who are daily involved in Aesthetics practice in order to improve the success of their treatments in terms of efficacy and tolerability as well as of quality of life. In line with such statements this Editorial will revisit redox processes from biochemistry to clinical practice in order to provide to clinicians, surgeons and dermatologists a key to approach this new field of Biology and to transfer it in the daily professional activity.

## **OXIDATION AND REDUCTION. BASIC PRINCIPLES.**

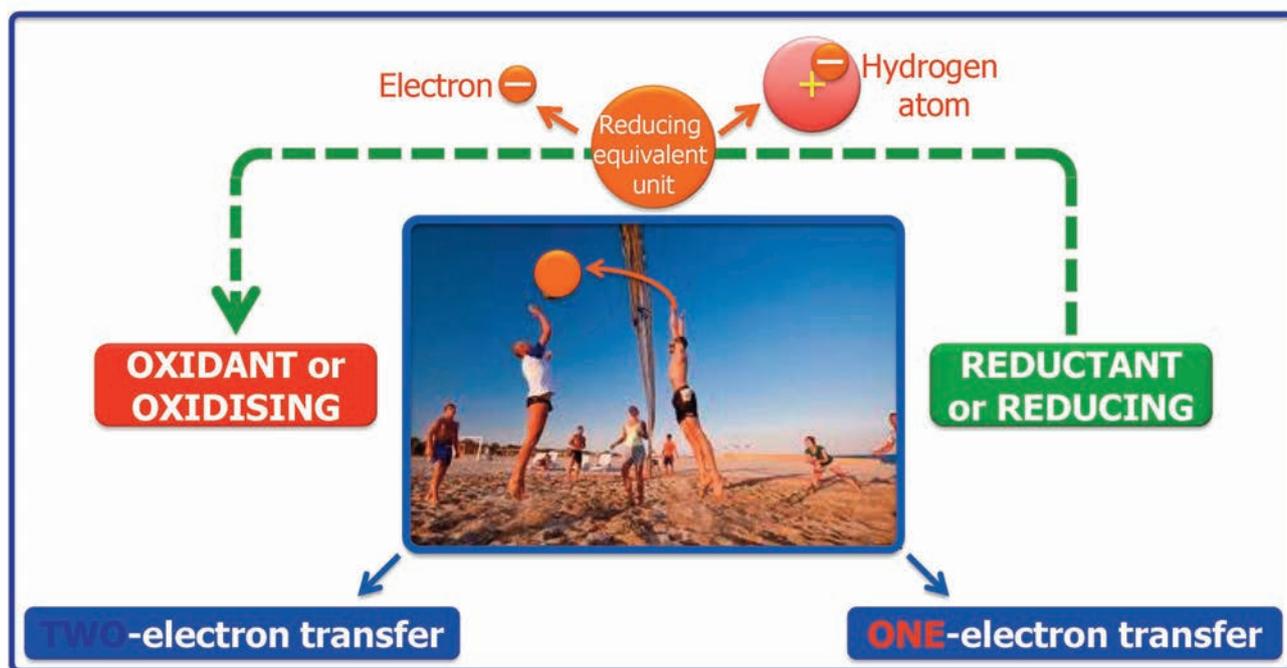
The oxidation and its reciprocal reaction i. e. the reduction (REDOX reaction) imply the transfer/exchange of one or more (for the purposes this review a couple) of entities that are called “reducing equivalent units”. In turn a reducing equivalent unit is defined as one electron alone or bound to a proton (the nucleus of the common element Hydrogen) as hydrogen

atom (one nuclear proton plus such extra-nuclear electron); however in the common language a reducing equivalent unit is practically an electron or a hydrogen atom (15) (Figure 1).

By simplifying the concept (that is more complex due to the possibility to transfer multiple electrons), in a classical redox reaction a “chemical oxidant/oxidising species” extracts one or a couple of reducing equivalent units from a “chemical reductant/reducing species”. Because this reaction the chemical oxidant species becomes reduced while the chemical reductant species becomes oxidised. The concept of oxidation and reduction is not absolute but relative because a chemical species can work either as oxidant against a reducing species or as reducing against an oxidant species the direction of the redox reaction being established on the basis of the so-called

reducing potential (as measured in mV, compared to the hydrogen that is assumed as reference); this latter drives the electron flow (i. e. the transfer of equivalent reducing units alone or in couple) from the chemical species showing the higher potential (i. e. more negative) to the chemical species showing the lower potential (i. e. more positive), respectively. The translation of a redox reaction into an equation where one or more reagents (lefts side) are transformed to one or more products (right side) allows to identify two couples of oxidising–reducing species by following the changes of the so-called “oxidation number” i. e. the difference between the sum of nuclear protons and the sum of extra-nuclear electrons: such number shifts against “negative” values for the oxidant species and against “positive” values for the reducing species, due to the acquisition

**FIGURE 1.** General schema of an oxidation-reduction (REDOX) reaction. In a REDOX reaction reducing equivalent units (one electron alone or bound to a proton as hydrogen atom) are transferred from a reductant/reducing to an oxidant/oxidizing chemical species. The most important REDOX reactions of biological interest are related to the transfer of two or one electron.



and the loss of negative charges (electrons), respectively (1, 15) (Figure 2).

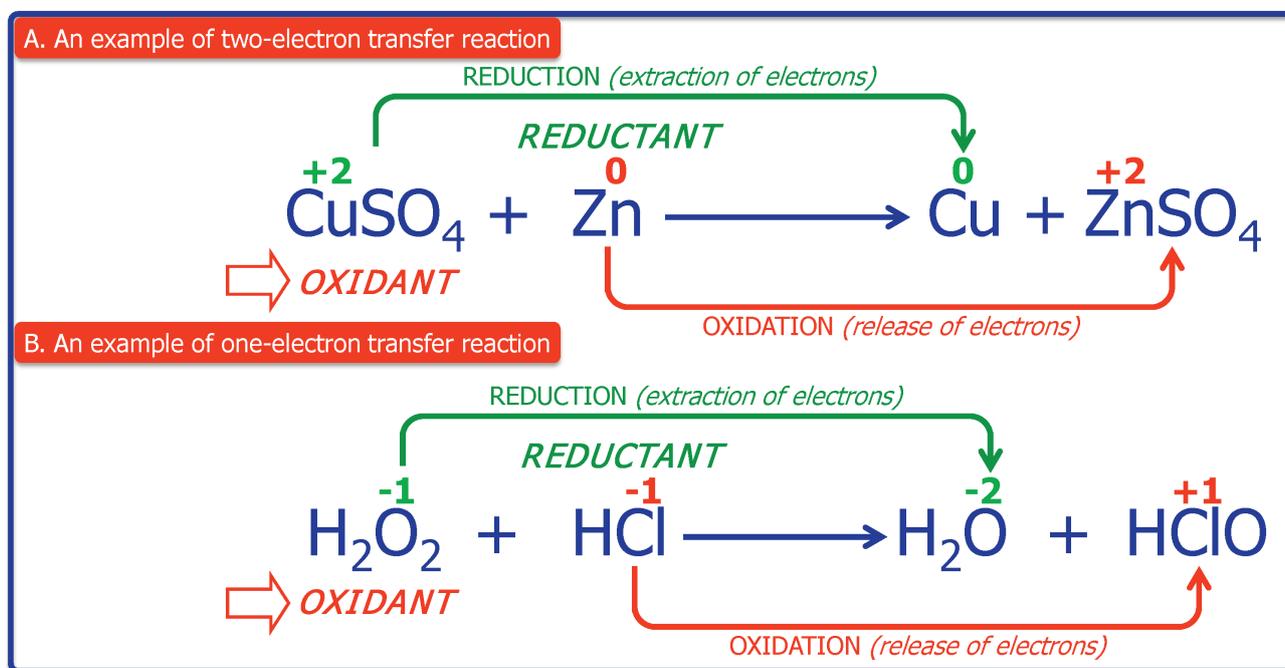
Examples of two-electron transfer reactions can be taken from inorganic chemistry as well as from organic/biological chemistry (Figure 3). In this latter field, two-electron transfer pathways are an essential part of energy cell metabolism. Indeed the oxidation of organic substrates by nicotinic and flavinic coenzymes ( $\text{NAD}^+$  and  $\text{FAD}$ , deriving from  $\text{B}_3$  and  $\text{B}_2$  activated vitamins, respectively) during catabolic processes like Krebs's cycle allows the transfer of couples of reducing units from carbohydrates, lipids and amino acids to molecular oxygen thus generating ATP in the mitochondrial respiratory chain (16). On the other side when the production of ATP is enough for cell need the excess of reducing equivalent couples is exploited in the pentose

shunt to reduce  $\text{NADP}^+$  to  $\text{NADPH} + \text{H}^+$ , i. e. the coenzyme that the cell requires for anabolic processes (e. g. biosynthesis of cholesterol and fatty acids). In other words two-electrons driven oxidative and reductive processes are closely related to catabolism and anabolism, respectively (16). On the contrary one-electron transfer reactions provide the molecular basis for biochemical pathways related to host defense, cell signalling and detoxification being such reactions linked to the metabolism of the so-called reactive species like reactive oxygen species (2) (Figure 3).

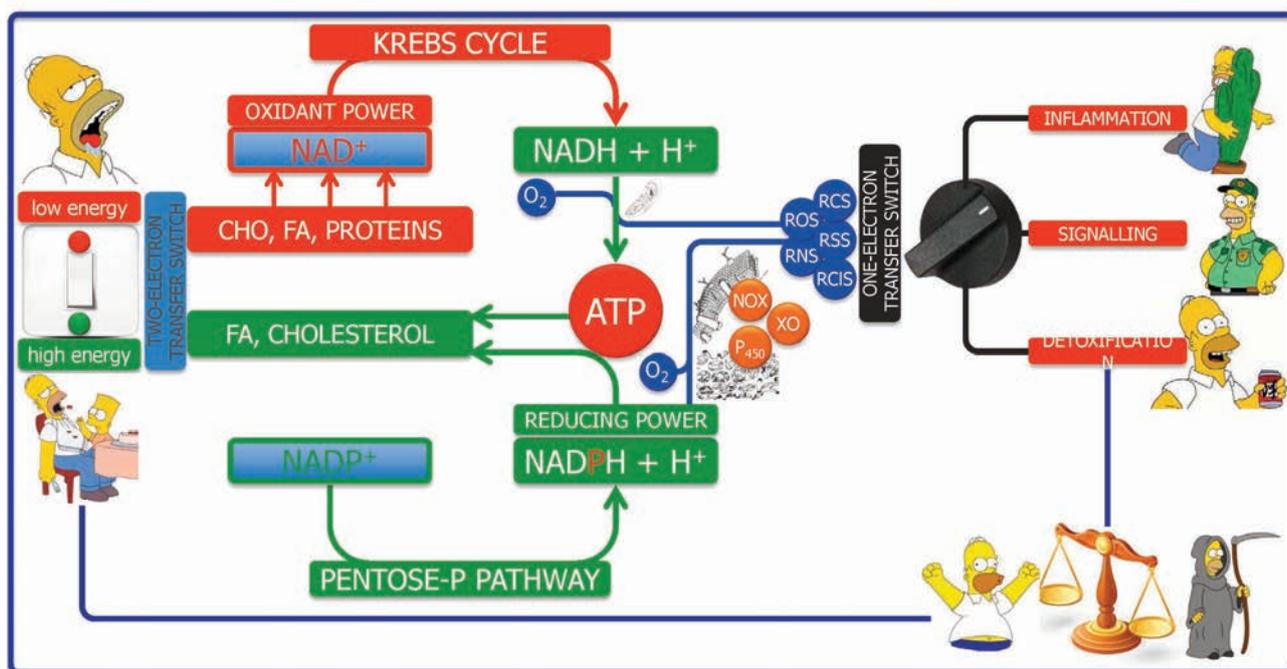
### REACTIVE OXIDISING/REDUCING CHEMICAL SPECIES. AN OVERVIEW.

In their continuous movement around the nucleus, the electrons generate three-di-

**FIGURE 2.** Paradigmatic examples of REDOX reactions. A. Two-electron transfer reaction: copper (as ionic ion) works as oxidizing species by extracting two electrons from zinc (element); indeed its oxidation number shift from +2 to 0. B. One-electron transfer reaction: oxygen (as peroxide) works as oxidizing species by extracting one electron from chlorine (as chloride ion); indeed its oxidation number shift from -1 to -2.



**FIGURE 3.** REDOX reactions and cell metabolism. *Left side. Two-electron transfer reactions through oxidation (i. e. dehydrogenation) drives cell metabolism towards ATP production (catabolism) while through reduction (i. e. hydrogenation) they allow to build big from small molecules (anabolism). Right side. One-electron transfer reactions are relate to the generation of reactive oxidant species that are involved in cell defense, signaling and detoxification. CHO, carbohydrates; FA, fatty acids; NAD, nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; NOX, NADPH oxidase; XO, xanthine oxidase; CytP450, cytochrome P450; ROS, reactive oxygen species; RNS, reactive nitrogen species; RCS, reactive carbon species; RSS, reactive sulfur species; RCIS, reactive chlorine species.*



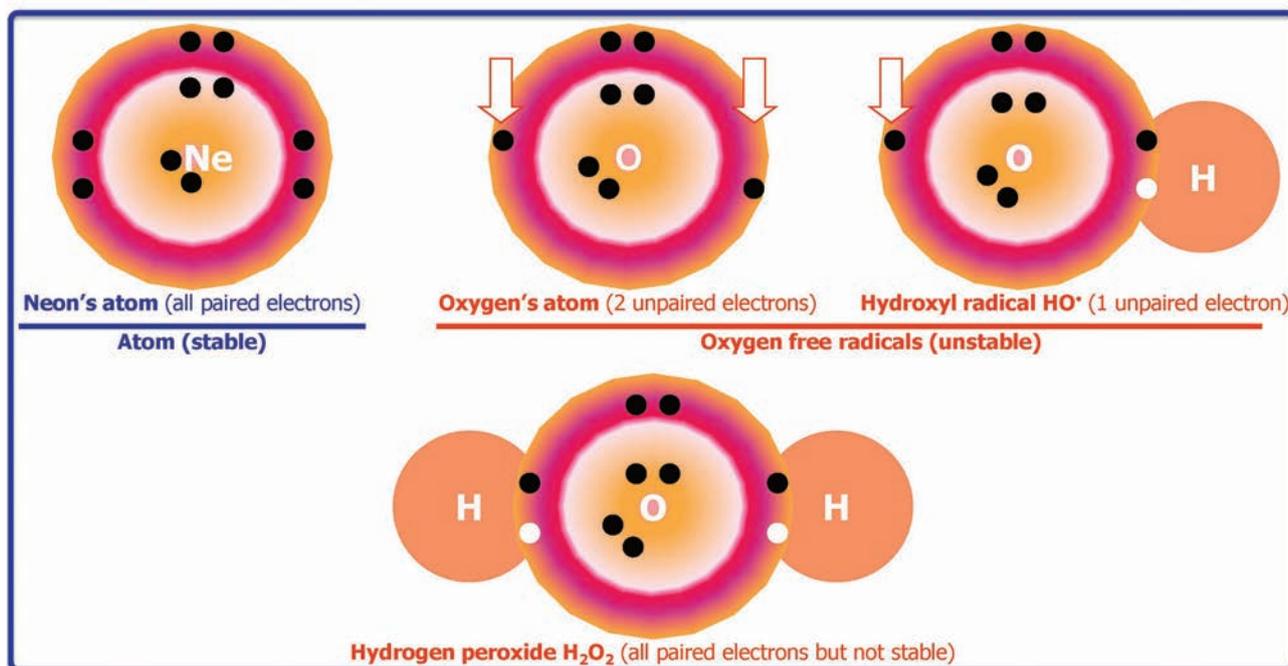
mensional areas of the space called orbitals; each orbital can contain a maximum of two electrons; the presence of one instead of two electrons in one or more external orbitals of an atom (alone or grouped, like a molecule) makes the correspondent chemical species unstable and therefore reactive; such reactivity pushes the chemical species to reach a condition of energetic stability where all the orbitals are filled by couples of electrons (1, 15).

In the most simply example, in order to become stable a reactive species can release such exceeding unpaired electron or extract a further electron from another chemical species: the reactive species is a reductant/reducing

species in the first case, an oxidant/oxidising species in the second one (1, 15). For instance all the atoms of elements belonging to the first group of the Periodic Table (e. g. lithium, sodium and potassium) show the behavior of reactive reducing species due to their trend to release rather than extract one electron from another chemical species. In the opposite side of the Periodic Table all the atoms of elements belonging to the seventh group (e. g. fluorine, chlorine, bromine, and iodine) work as reactive oxidising species (ROS) because they trend to extract rather than release one electron to another chemical species.

In the chemical language the reactive

**FIGURE 4.** Atoms and reactive chemical species. *Instead of noble elements (e. g. neon) the atoms of most elements showing at least one unpaired electron are radical species with reducing or oxidant capacity (top); among oxidizing species are non-radical species, like hydrogen peroxide (bottom).*



species containing one or more unpaired electrons are called free radicals or simply radicals (1, 15) (Figure 4). The radical nature of such reactive specie is indicated i) in their symbol or formula by adding a dot in the upper right in the exponent position and ii) in their name by adding the the suffix “-yl” (e. g. hydroxyl) (1, 15). A radical can be either an atom (e. g. the atom of hydrogen or oxygen) or a group of two or more atoms (e. g. the hydroxyl radical, HO•) independently from its inorganic or organic nature (1, 15).

Noticeably a radical can act both as an oxidant either by a reducing depending on its chemical nature/environmental conditions. In both the cases its reactivity is a direct function of its reducing potential (the highest reactivity being associated to the highest negative values) and to its ratio volume/surface (the highest

reactivity being associated to the highest ratios) (1). In other words both the behavior (oxidant/reducing) and the reactivity are not an intrinsic features of a radical in opposition to that widely reported in many papers: indeed many radicals of biological interest like tocopheryl are relatively stable.

Furthermore the oxidising capacity – as the capacity to extract one electron from another chemical species – is a not an exclusive feature of radicals being shared also with other “oxidising” species like hydrogen peroxide or hypochlorous acid (1, 3) (Figure 2).

Therefore the “oxidising reactive species” is a wide class of chemical species showing or not at least one unpaired electron but sharing the ability in opportune conditions to extract one electron from another chemical

species due to the presence in their context of a reactive atom of oxygen, carbon, nitrogen, sulfur or chlorine; on other words the so-called oxygen free radicals or reactive oxygen species are only a little part of all the known reactive oxidising species of biological interest. In a specular way we can easily identify also radical and non-radical reactive reducing species (see below).

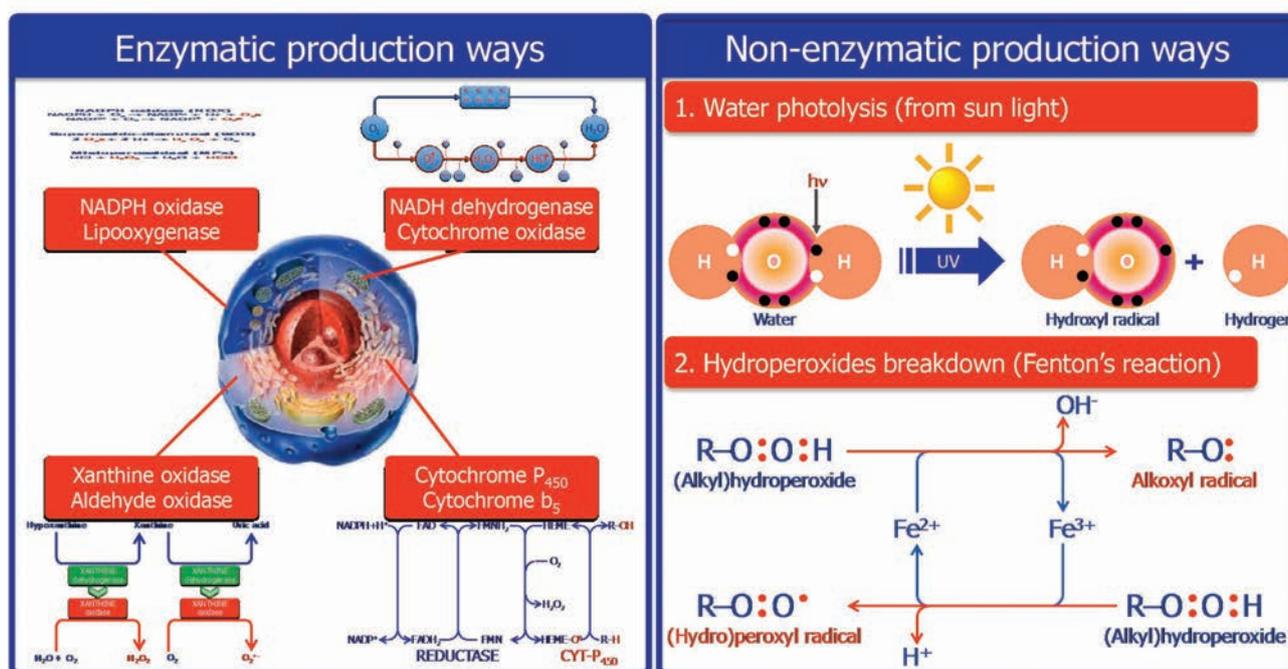
### REACTIVE OXIDISING SPECIES. METABOLISM AND BIOLOGICAL ACTIONS.

In living organisms reactive and other oxidising species are continuously and physiologically generated during the normal metabolism by means of two mechanisms: the first one is mediated by enzymes while the second one is triggered by physical agents or transition metals (Figure 5) (1).

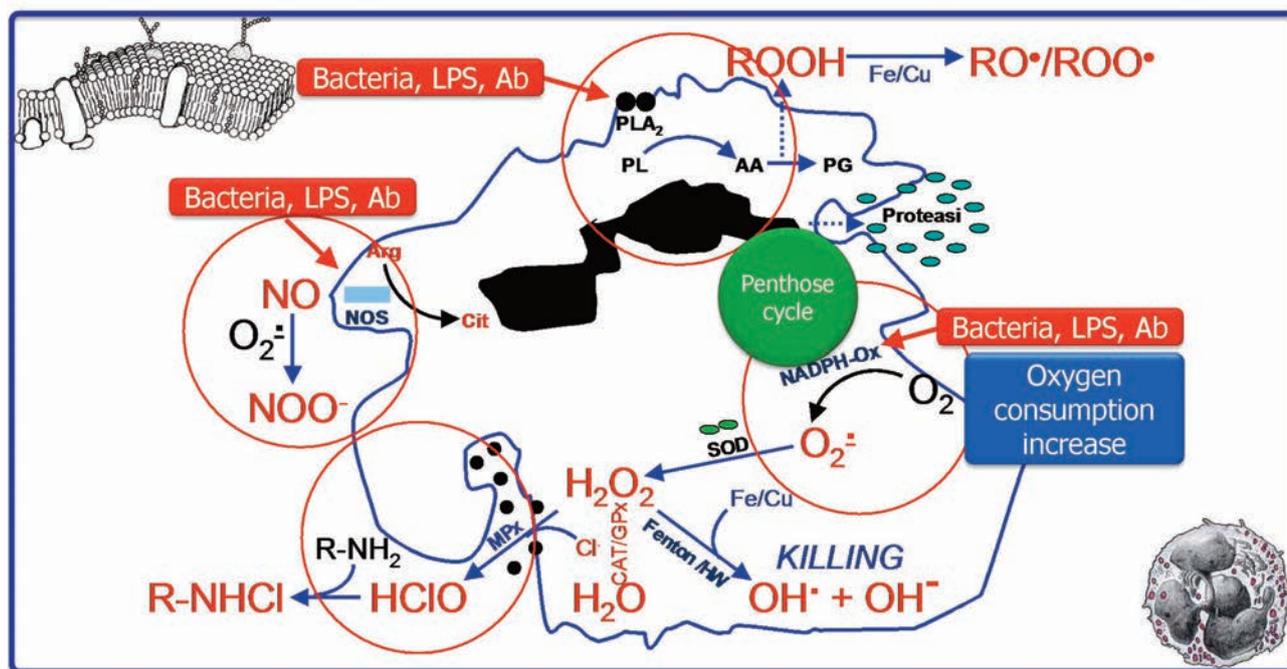
The enzyme-mediated production of reactive oxidizing species (ROS) is due to the activation of catalytic proteins that are located on the plasmamembrane, into the mitochondria, in the endoplasmic reticulum (microsomes), into the peroxisomes and in the cytosol (1, 3) (Figure 5).

A paradigmatic example of ROS production from plasmamembrane is provided by polymorphonuclear leukocytes (Figure 6) (17). In such model bacteria, endotoxins or antibodies trigger the so-called respiratory burst where the increased availability of  $\text{NADPH} + \text{H}^+$  from pentose cycle and oxygen from blood activates the enzyme NADPH oxidase; this latter reduces molecular oxygen to reactive superoxide anion that contributes to the bacteria damage or killing during the phagocytosis (17). On the other the activation of plasmamembrane

**FIGURE 5.** The generation of reactive oxidizing species (ROS) in biological systems. *In living organisms ROS can be produced through enzymatic (left) or non-enzymatic ways (right).*



**FIGURE 6.** The generation of reactive oxidizing species (ROS) polymorphonuclear leukocytes. *Leukocytes produce physiologically ROS in order to kill bacteria through phagocytosis. HO.*, hydroxyl radical; *ROO.*, any hydroperoxyl radical; *ROOH*, any hydroperoxide; *GPx*, glutathione peroxidase; *RO.*, any alkoxy radical; *NOO-*, peroxy nitrite; *OH-*, hydroxyl ion; *R-NHCl*, any organic chloroamine; *NO*, nitric oxide; *O<sub>2</sub><sup>-</sup>*, superoxide anion; *NOS*, nitric oxide synthase; *arg*, arginine; *cit*, citrulline; *PMN*, polymorphonuclear leukocyte; *LPS*, lipopolysaccharide; *Ab*, antibody; *PL*, phospholipid; *PLA<sub>2</sub>*, phospholipase A<sub>2</sub>; *AA*, arachidonic acid; *PG*, prostaglandins; *CAT*, catalase; *SOD*, superoxide dismutase; *MPx*, myeloperoxidase; *HW*, Haber-Weiss reaction.



lipooxygenase leads to the release of hydroperoxides that are potentially precursors of oxygen free radicals (see below, *Fenton reaction*). Such mechanisms are a relevant part of reactive processes (e. g. inflammation, pathological immunoreactions, and infectious diseases) (17).

Mitochondria are the primary metabolic source of ROS because in their “cristae” are located the respiratory chain enzymes responsible for oxidative phosphorylation (Figure 5, left side) (18). Indeed, the transfer of electrons ideally only results in the production of a molecule of water by means of the tetravalent reduction of molecular oxygen. In reality, this process is not perfect and small but significant amounts (1-2%) of electrons are shifted from respiratory

chain enzymes and coenzymes (i. e. flavoproteins, ubiquinone, cytochromes etc.) directly to molecular oxygen, thus generating superoxide anion and/or hydroxyl radical (univalent reduction) and/or hydrogen peroxide (bivalent reduction). Such physiological production of reactive oxygen species can increase after strenuous aerobic exercise and can reach pathological levels when metabolic rate increases (e. g. due to hyperthyroidism) (19).

In the smooth endoplasmic reticulum reactive species production occurs via cytochrome P<sub>450</sub>, which is well known as the system that plays a major role in the detoxification processes (Figure 5, left side) (20). Cytochrome P<sub>450</sub> acts as an “immediate” donor

of electrons in several hydroxylation reactions, such as the reactions that occurs into the hepatocytes in order to inactivate either hormones (e. g. steroids) or no-physiological compounds (xenobiotics, such as toxic agents, and hydrophobic drugs, that become more soluble and less toxic) (20). Cytochrome P<sub>450</sub> is an heme protein that acts a “trait-d’union” between the NADPH(H<sup>+</sup>) (the source of electrons) and the substrate to be hydroxylated. In this very complex reaction an “hydroxylable” substrate (SH) reacts with the NADPH(H<sup>+</sup>) and molecular oxygen (O<sub>2</sub>) to produce the correspondent hydroxylated compound (S-OH), together with NADP<sup>+</sup> and H<sub>2</sub>O (20).

Peroxisomes also produce free radicals. Indeed, inside these organelles fatty acids undergo a particular oxidative process that differs from the so-called oxidation (21). In the first step of such process, a flavoprotein extracts a couple of hydrogen atoms from a molecule of activated fatty acid (acyl-CoA). The two hydrogen atoms then are directly transferred to molecular oxygen, which is reduced to hydrogen peroxide (this latter is therefore inactivated by catalases because its toxicity) (21).

Remarkable amounts of reactive species are generated also by a number of other biochemical reactions into the cytosol, such as during the final steps of purine catabolism (AMP IMP inosine hypoxanthine xanthine uric acid) that is signed by the oxidation of hypoxanthine to xanthine and by the oxidation of xanthine to uric acid (Figure 5, left side). Both the reactions are catalyzed by xanthine dehydrogenase, a molybdenum enzyme (22). In some conditions, such as in the ischemia-reperfusion damage, xanthine dehydrogenase is converted to xanthine oxidase

(may be by means of a calcium-dependent proteolytic cleavage). Xanthine oxidase, in turn, by utilizing as final “acceptor” directly molecular oxygen, generates not only hydrogen peroxide but also superoxide anion, by hypoxanthine and xanthine, respectively (2).

Other reactions which generates free radicals by enzymatic way are described in the biosynthesis of catecholamines (23).

Besides of the enzymatic way living cells can produce free radicals by means of physical or chemical agents (Figure 5, right side, top) (1, 3). The most common mechanism is the homolytic breakdown where the administration of energy breaks a covalent bond of the target molecule thus generating two distinct radical species both showing one unpaired electron. If the administered energy derives from a radiant source the breakdown is called photolysis while the administration of heat is responsible for pyrolysis. A biologically relevant example of homolytic breakdown is the water breakdown triggered either by X-ray (radiolysis) or UV-ray (photolysis) that generates a hydrogen atom and the hydroxyl.

However free radicals can be generated not only by homolytic cleavage but also by the interaction of some compounds (i. e. peroxides) with a transition metal in ionic form (e. g. iron or copper) (1, 3, 5) (Figure 5, right side, bottom). By means of this mechanism, for example, iron (Fe<sup>2+</sup>/Fe<sup>3+</sup>) or copper (Cu<sup>+</sup>/Cu<sup>2+</sup>) act as catalysts in a sequence of reduction-oxidation reactions to generate alkoxy (RO<sup>·</sup>) and peroxy (R-O-O<sup>·</sup>) radicals from peroxides (R-O-O-R). In the simplest case – firstly described by Fenton – a ferrous ion (Fe<sup>2+</sup>), by oxidizing itself to ferric ion (Fe<sup>3+</sup>), give its electron to a hydrogen peroxide molecule (H<sub>2</sub>O<sub>2</sub>), thus generating a free radical

(hydroxyl radical,  $\text{HO}\cdot$ ) and an anion (oxydyl ion,  $\text{OH}^-$ ). In turns, ferric ion ( $\text{Fe}^{3+}$ ) is reduced – thus regenerating itself as all catalysts – to ferrous ion ( $\text{Fe}^{2+}$ ), by extracting an electron from a second molecule of hydrogen peroxide, that is broken in to a free radical (the perhydroxyl radical,  $\text{HOO}\cdot$ ), and a cation (an hydrogen ion,  $\text{H}^+$ ). Analogously, hydroperoxides also are broken, by means of the iron-mediated catalysis, in to alkoxy ( $\text{RO}\cdot$ ) and hydroperoxy ( $\text{ROO}\cdot$ ) radicals.

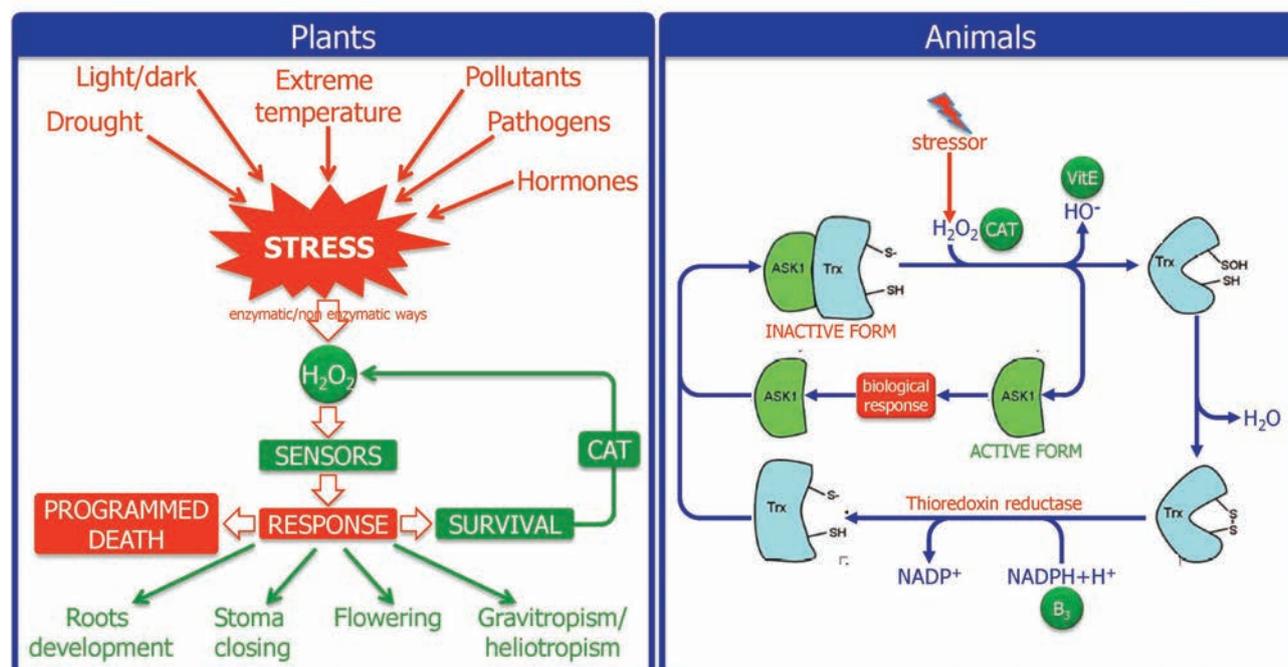
Therefore it is clear that ROS are almost “forced” intermediates of cell metabolism. Since their production is close related to life, rightly ROS has been defined as “almost irreplaceable journey companions” to living cells.

The main action of ROS is to extract one electron to another chemical species. Such species can be any organic compound but generally speaking the preferential targets of oxidative processes are all molecules contain-

ing a double bond because the second bond is a relatively free couple of electrons (1, 3). On this basis a descendent scale of susceptibility to oxidation has been proposed for the most common biomolecules: unsaturated fatty acids, amino acids/proteins, nucleic acids and carbohydrates. Selective targets of oxidation are also reduced thiol groups ( $-\text{SH}$ ) (e. g. from cysteine) that can be reversibly or irreversibly oxidized (9).

In any case by extracting one electron ROS can change not only the structure but also the function of the target biomolecule with final effects that can result positive, negative or apparently neutral, depending on the environmental conditions. By a physiological point of view such reactions being highly conserved during the evolution seems to have been planned to allow the adaptation of living organisms either to external or internal stressors (24). In other words exogenous and/or endogenous stimuli

**FIGURE 7.** The adaptive physiological role of reactive oxidizing species (ROS) in living organisms. An example of adaptation to environmental changes in Plants (left side) and in Animals (right side).

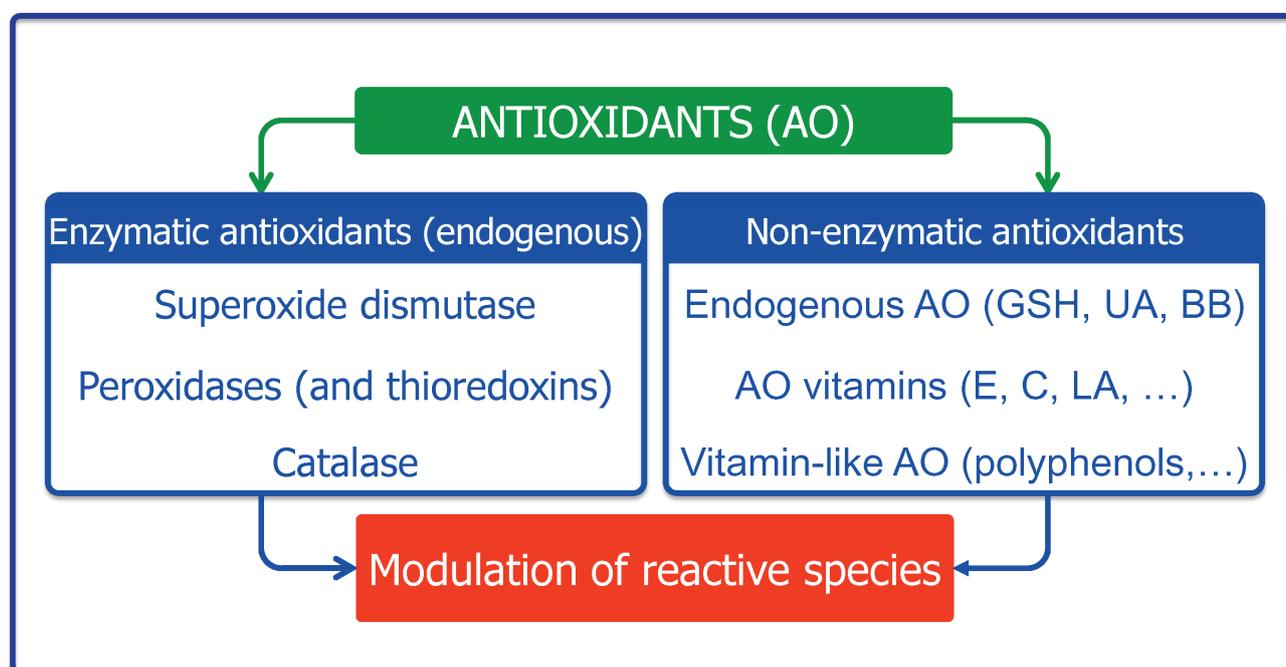


may trigger the production of ROS (among which the so called reactive oxygen species are only a small part) that activate/inhibit specific biochemical pathways inside the cells thus allowing them to face environmental changes. For instance plants can adapt to changing weather conditions thanks to the production of hydrogen peroxide and other ROS (25, 26) (Figure 7, left side). Similarly animals seems to exploit analogous mechanisms of adaptation by inducing reversible oxidative change of protein thiols thus modulating key processes involved in cell homeostasis and survival (Figure 7, right side) (8, 9); a specific example of ROS-mediated mechanism of adaptation is provided by the production of oxidants (e. g. hydrogen peroxide and hypochlorous acid) by inflammatory cells in order to protect the tissues against bacterial infections (Figure 6) (17). Another relevant example of physiological mod-

ulation by reactive oxidising species is provided by nitric oxide pathway (26, 27).

The generally low energy expenditure required, the fast and easy way of production, the high diffusibility, together with the very short half-life make really these mechanisms essential for survival especially in a context of autacid modulation. The success of such mechanism is closely related to the efficacy of its restoring machinery. As with the neurotransmission mechanisms, where the mediator after acting must be destroyed or inactivated, even ROS must be neutralized, after having successfully reached their target molecules (25-27). For this reason, in the course of millions of years of evolution, the living species have developed, in parallel with the reactive oxidising species, a complex modulation system represented, commonly called “antioxidants” but that in fact act

**FIGURE 8.** The antioxidant network. *The antioxidant network includes either enzymatic or non-enzymatic systems which main role is to modulate in a physiological way the activities and the functions of reactive oxidizing species. AO: antioxidants; GSH, glutathione; UA, uric acid; BB, bilirubin; LA, lipoic acid.*



as “physiological modulators” of oxidizing reactive species (28, 29).

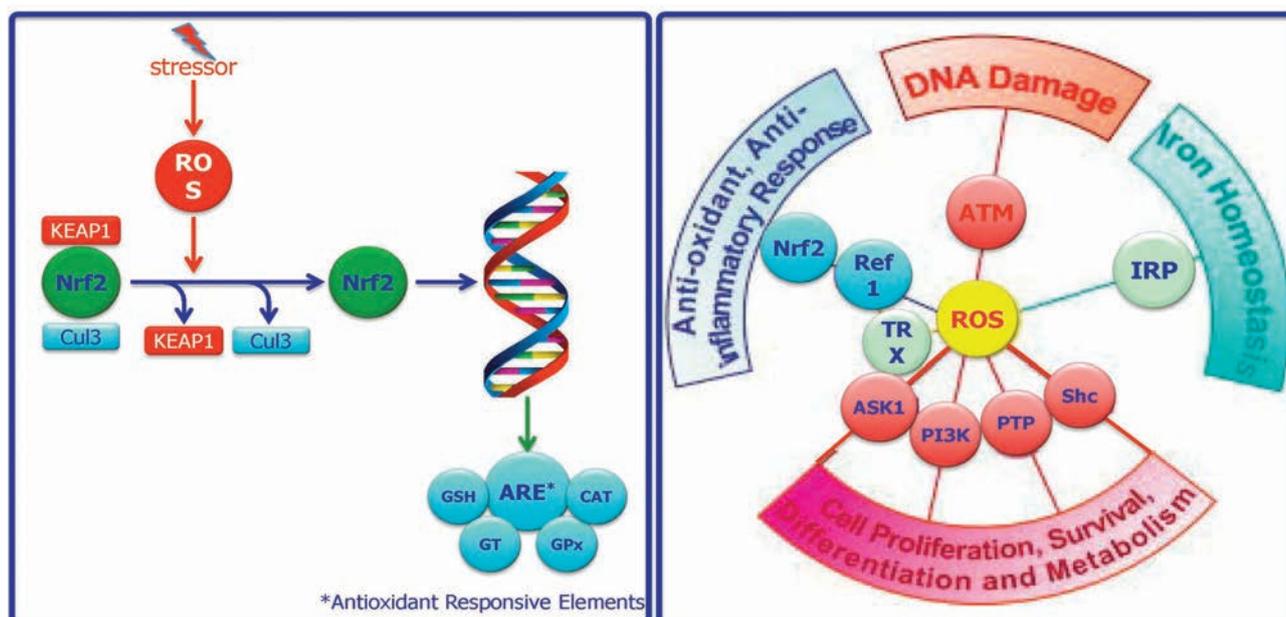
Such antioxidants can be distinguished on the basis of their origin (exogenous and endogenous), their chemical nature (enzymatic and non-enzymatic) or by their solubility (hydrophilic and hydrophobic) (Figure 8) (1, 30). They include a number of enzymes (e. g. superoxide dismutase, catalase and peroxidase) and exogenous compounds (vitamins and vitamin-like antioxidant compounds, such as polyphenols, oligoelements, ecc.) (1, 30). However, to better understand the pathogenesis and the therapy of oxidative stress (see *below*) it is useful to classify the oxidants by their modality of action: preventive antioxidants, radical scavengers, repair agents and adaptation agents. Specifically, preventive antioxidants include some agents that by means of several mechanisms, such as the chelation of transition metals, prevent the reactive oxidising species generation. Radical scavengers, that also act through several mechanisms, include either hydrophilic (e. g. ascorbate, uric acid, bilirubin, albumin) or hydrophobic compounds (e. g. carotenoids, tocopherols, ubiquinol). Repair agents include only enzymes that intervene after the establishment of ROS damage. Their action – often sequential – implicates first the identification and then the leaving of the oxidized molecular fragment and, finally, the synthesis and the insertion of a novel fragment instead of that damaged. Repair agents include hydrolases (e. g. glycosidases, lipases, and proteases), transferases and polymerases. All these enzymes are responsible for the repair of damage induced by free radicals on important cell compounds or structures (e. g. DNA, plasmamembrane etc.). Finally, adaptation agents

include all the compounds or techniques or procedures able to potentiate the physiological antioxidant system of a living organism. For example, a correct exercise or the adoption of an adequate and equilibrate diet have the potential of to control the oxidative metabolism by means of a reduction of the production of reactive species and the induction of antioxidant enzymes (31).

Antioxidant system is regularly distributed inside a living organism either at extracellular or intracellular level (28). In the extracellular compartment and, particularly, in the blood plasma, all the compounds potentially able to “give” a reducing equivalent (as hydrogen atoms or as electrons) “to satisfy” the “electron avidity” of free radicals constitute the antioxidant plasma barrier. This latter includes plasma proteins (e. g. albumin), bilirubin, uric acid, cholesterol and all the exogenous, dietary or pharmacological, antioxidants (e. g. ascorbate, tocopherol, polyphenols, bioflavonoids etc) (32-34). In this context, thiol compounds play a crucial role in ROS modulation (see *below*). Inside the cell the antioxidant defense system is well distributed in several compartments. Because the majority of free radicals are generated in lipid layers where are the enzymes necessary to catalyze the radical-producing reactions, the lipophilic antioxidants (i. e. ubiquinol, vitamin E, and beta-carotene) located in biomembranes constitute the first defense line against ROS. Later defense line includes the water-soluble vitamin C, several members of the vitamin B group, etc. (35).

The production of antioxidants can be also directly stimulated by ROS themselves as evidenced by Nrf-2 system that provide an excellent example of signal transduction whit the

**FIGURE 9.** The Modulating action of REDOX systems. Reactive oxidizing species (ROS) activate Nrf-2 machinery that is deputed to stimulate the production of Antioxidant Responsive Elements (ARE), that include glutathione (GSH), glutathione transferase (GT), glutathione peroxidase (GPx), and catalase (CAT) (left side); through this and other signaling pathways (ATM, IRP, Trx and so on) ROS regulate homeostasis and cell survival in living organisms (right side).



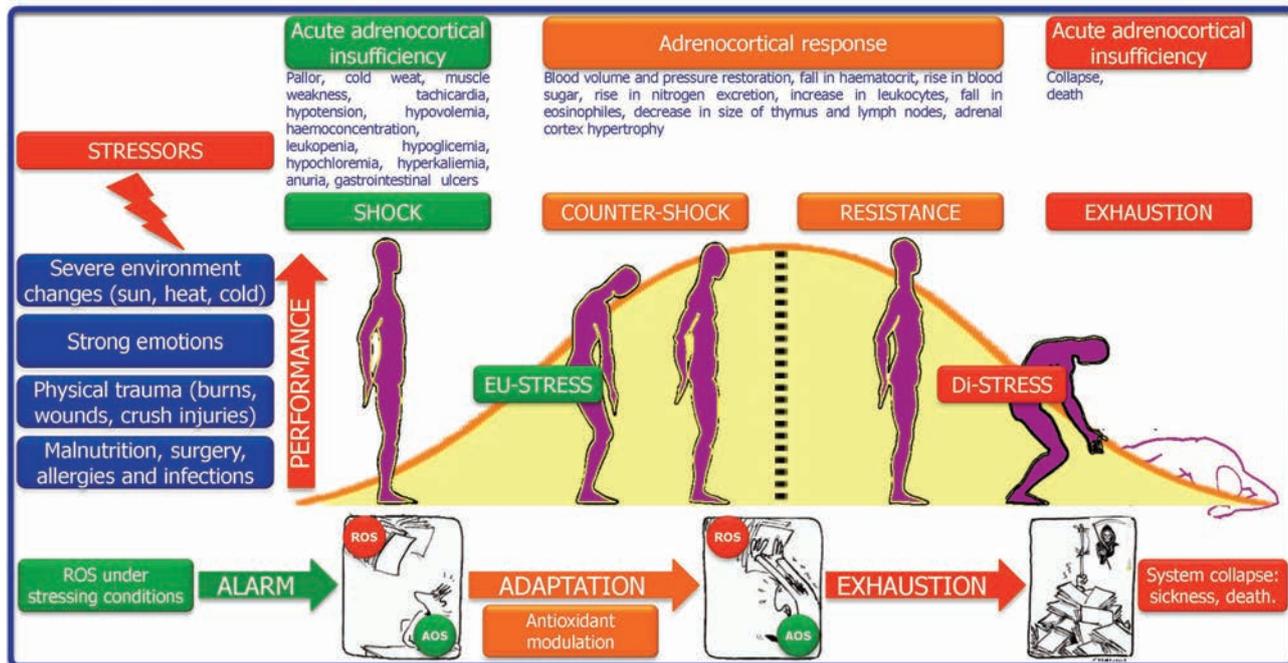
The physiological modulation by the commonly called “antioxidants” is crucial because a ROS as opposed to a neurotransmitter (or a hormone), acting in a non-specific way, especially if in excess, can also involve molecules different from those targets like unsaturated fatty acids or nucleic acid (28). This can lead to irreversible oxidative reactions or to unwanted side effects potentially responsible for intracellular or extracellular damage (e. g. peroxidation of lipids, DNA mutations and so on) (1). To define these phenomena from the pathophysiological point of view the term “oxidative stress” has been coined (1, 10).

## FROM OXIDATIVE EU-STRESS TO OXIDATIVE DI-STRESS.

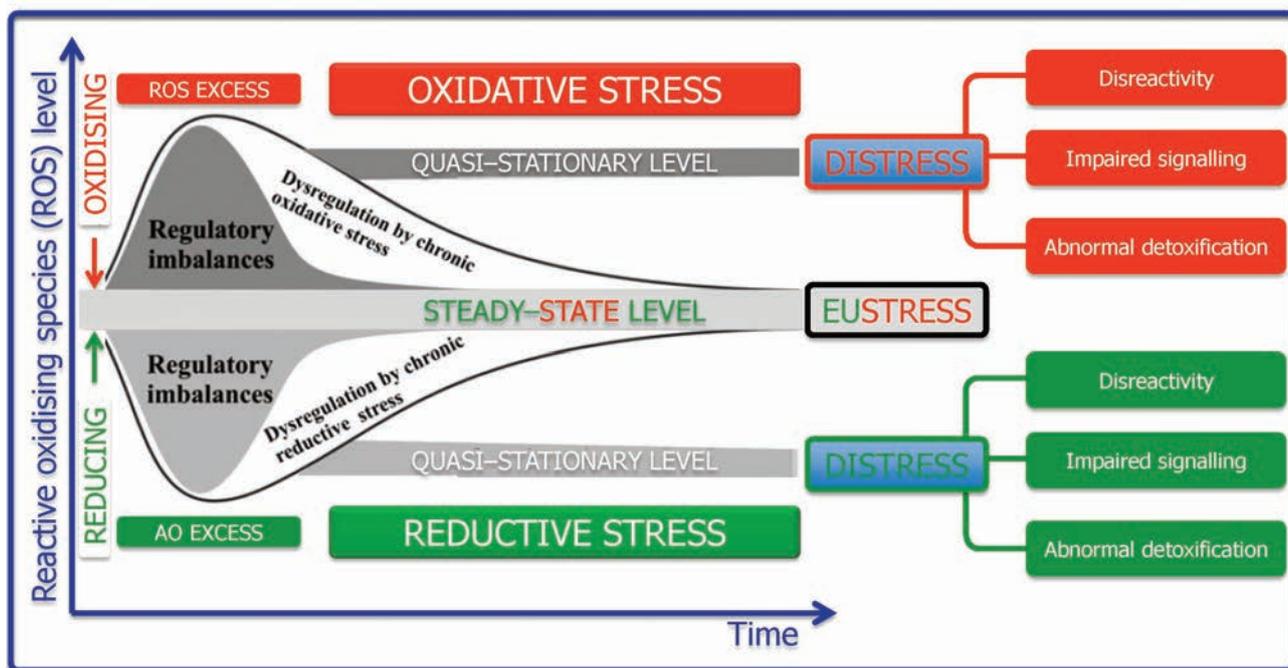
Oxidative stress is often but improperly

oxidants and antioxidants (1). However it is must be considered as a necessary mechanism of homeostasis like “emotional stress” (4). Indeed oxidative stress and emotional stress share many features and in some way the first one provides a solid biochemical basis for the second one (5-7). Furthermore it seems that the evolution of living organisms and their metabolic, energetic and reproductive changes during the last billion of years was driven by redox changes like (e. g. increased levels of oxygen in the atmosphere, increased level of cysteine in the proteome) according to a “redox code”, in turn based on the NAD/NADH and SH/S ratios (24). Therefore the so-called “oxidative stress” is by itself a “positive” adaptive mechanism, of course when it allows through an appropriate oxidation the living organism to successfully respond to an environmental

**FIGURE 10.** From the emotional stress to the oxidative stress. Oxidative stress may provide some biochemical basis to the classical stress. Indeed antioxidant (AO) response to reactive oxidizing species (ROS) is conceptually overlapping to the stress response in all living organisms.



**FIGURE 11.** The novel paradigm of oxidative/reductive stress. Depending on the level of reactive oxidizing specie (ROS) and their lifespan living organism can react physiologically (eu-stress) or pathologically (di-stress).



change (stimulus or stressor) (Figure 10) (24). In this case we use the correct term of “eu-stress” that means “good or favourable stress”. However when the host’s biochemical system is not able to manage the radical chain triggered by the stimulus because the reactive species are in excess and/or the physiological systems of modulation are ineffective a condition of oxidative di-stress derives (Figure 11) (37).

The activation of polymorphonuclear leukocytes provides a clear example of oxidative di-stress (Figure 6) (38). Indeed in such cell the contact with bacteria or endotoxins or antibodies activates the enzyme NADPH oxidase that generates the superoxide anion. This latter in turn contributes to the bacteria destruction/killing thus supporting the physiological process of phagocytosis (see above). However superoxide anion can be harmful for

leukocyte itself and/or for surrounding tissues so the cell activates the enzyme superoxide dismutase which role is to convert the superoxide anion to the less harmful hydrogen peroxide, finally responsible for bacteria killing. Because hydrogen peroxide too still can damage cells and tissues the leukocyte enables a second line of defence by activating the enzyme catalase that converts hydrogen peroxide to water; for other peroxides like lipoperoxides, which derive from fatty acid membrane oxidation, the leukocyte activates the enzyme glutathione peroxidase that converts such compounds to harmless organic alcohols. By means of such physiological mechanism – oxidative eu-stress – a stressor (e. g. a bacterium) triggers the production of ROS which after facing the “aggressor” are neutralised by the endogenous antioxidant systems. Unfortunately

**FIGURE 12.** Oxidative stress is an emerging health risk factor. *Oxidative stress is related not only to early aging but also to at least one hundred diseases most of them related to lifestyle (red).*

Aceruloplasminemia	Down's syndrome	<b>Parkinson's disease</b>
Acute/chronic alcoholic liver disease	Eclampsia	<b>Periodontal disease</b>
Acute autoimmune myocarditis	End-stage renal disease	Peritoneal dialysis
Acute chest syndrome of sickle cell disease	<b>Erectile dysfunction</b>	<b>Photoaging</b>
Acute pancreatitis	Friedreich ataxia	Preeclampsia
Acute Respiratory Distress Syndrome	Heart failure	Primary biliary cirrhosis
<b>Alcoholic liver disease</b>	Helicobacter pylori infection/inflammation	Professional bronchopulmonary diseases
Alzheimer's disease	Hemodialysis	Progeria
Amiotrophic lateral sclerosis	Hepatic cirrhosis	Psoriasis
<b>Arterial/systemic hypertension</b>	Human Immunodeficiency Virus infection	Psoriatic arthritis
Asbestosis	Huntington disease	Pulmonary hypertension
Asthma	Hyperbaric diseases	Radio-therapy side effects
Ataxia telangiectasia	<b>Hypercholesterolemia</b>	Reactive arthritis
<b>Atherosclerosis</b>	<b>Hyperhomocysteinemia</b>	Renal cell carcinoma
Atopic dermatitis	<b>Hyperlipidemia</b>	Respiratory distress syndrome
<b>Brain ischemia</b>	Juvenile chronic arthritis	Retinopathy of prematurity
Bronchopulmonary dysplasia	Kidney transplantation	<b>Retrolenticular fibroplasia (neonatal cataract)</b>
Burns	Idiopathic pulmonary fibrosis	Rheumatic disease
<b>Cancer (several kinds)</b>	Interstitial lung disease	Rheumatoid arthritis
Cardiopulmonary bypass	<b>Ischemia/Reperfusion injury</b>	Sarcoidosis
<b>Cardiovascular diseases</b>	<b>Leukaemia</b>	Sepsis
<b>Cataract</b>	<b>Lung cancer</b>	Sickle cell disease
Cellulitis	Lung injury	<b>Sleep apnoea</b>
<b>Chemotherapy side-effect</b>	Macular degeneration	Spheroctosis
Chronic fatigue syndrome	<b>Male infertility</b>	Spinal cord injury
Chronic hepatitis C	Ménière's syndrome	<b>Stroke</b>
Chronic kidney disease	Meningitis	Synucleinopathies
Chronic Obstructive Pulmonary Disease	Mild cognitive impairment	Systemic amyloidosis
Chronic renal failure	<b>Multiple sclerosis</b>	Systemic lupus erythematosus
<b>Colitis</b>	Myelodysplastic syndromes	Systemic sclerosis (scleroderma)
<b>Coronary artery disease</b>	<b>Myocardial infarction</b>	Thrombophily
Creutzfeldt-Jakob disease	<b>Obesity</b>	Tauopathies
<b>Crohn disease</b>	Osteoarthritis	Tuberculosis
Cutaneous leishmaniasis	Osteoporosis	Unstable angina
Cystic fibrosis	Pancreatitis	Uremia
Diabetes mellitus type 1	Parkinsonisms	<b>Venous insufficiency</b>
<b>Diabetes mellitus type 2</b>	Myocarditis	Werner syndrome
<b>Distlipidemia</b>	Neonatal bronchopulmonary dysplasia	Zellweger syndrome

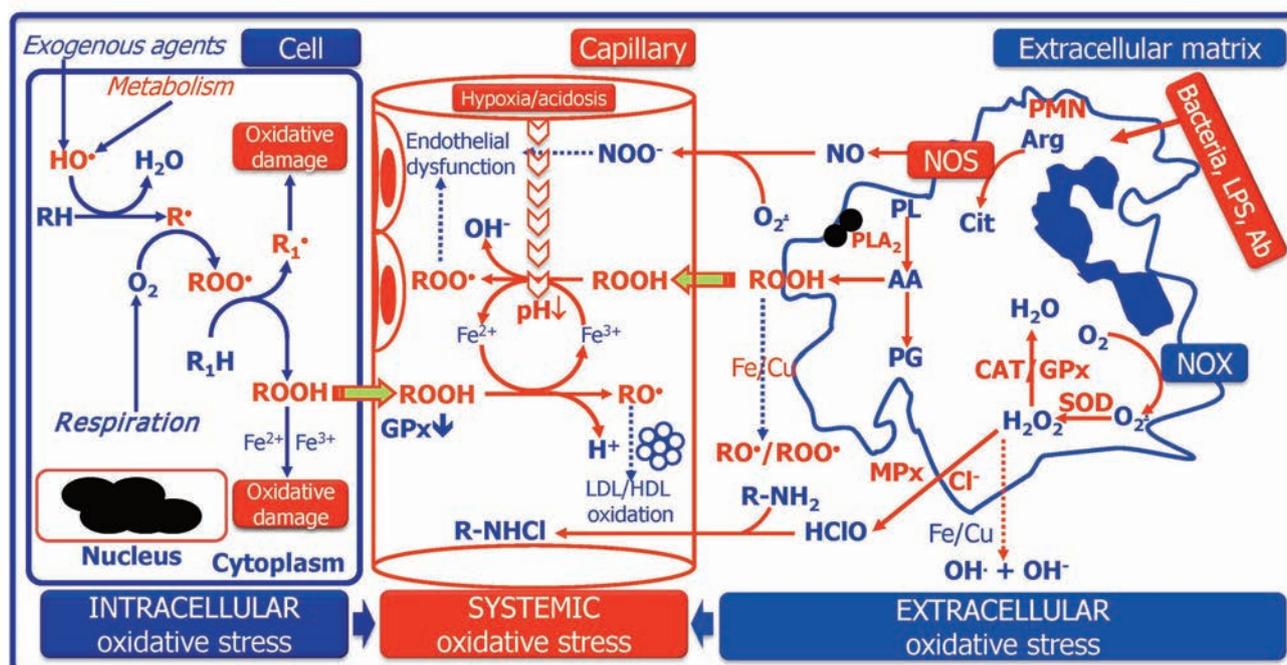
if the bacterium is particularly aggressive and/or its load is high from one side and/or the enzymes responsible of ROS inactivation are defective the leukocyte try to dispose of the excess of hydrogen peroxide activating secondary metabolic pathways like the myeloperoxidase system. This latter converts hydrogen peroxide to the powerful oxidant hypochlorous acid that by oxidising every amine group can destroy potentially every cell and tissue. Moreover the unprocessed hydrogen peroxide can undergo to the so-called Fenton reaction this generating the most harmful ROS, i. e. the hydroxyl radical, that enhance the tissue damage of hypochlorous acid. By

this complex mechanism a condition of oxidative eu-stress can switch to a condition of oxidative di-stress (37, 38). Human pathology shows many examples of such situation among which periodontitis is the most relevant (39, 40).

An additional example of oxidative distress is provided by the inactivation of nitric oxide to peroxynitrite by superoxide anion in cardiovascular diseases (41).

Oxidative di-stress or oxidative stress, as commonly indicated, is generally recognized to play a pathogenic role in early aging and in several inflammatory and/or degenerative diseases including atherosclerosis and hypertension (and their consequences, such as stroke

**FIGURE 13.** Biochemical and cellular basis of oxidative stress. Nucleate cells produce reactive oxidizing species (ROS) that trigger radical chain reactions which end-products, hydroperoxides (ROOH), become again reactive under hypoxia/acidosis conditions, thus leading to endothelial dysfunction and systemic oxidative stress. HO·, hydroxyl radical; RH/RH1, any organic molecule; R·/R1·, any organic radical; ROO·, any hydroperoxyl radical; ROOH, any hydroperoxide; GPx, glutathione peroxidase; RO·, any alkoxyl radical; HDL, high density lipoprotein; LDL, low density lipoprotein; NOO·, peroxy-nitrite; OH·, hydroxyl ion; R-NHCl, any organic chloroamine; NO, nitric oxide; O<sub>2</sub><sup>-</sup>, superoxide anion; NOS, nitric oxide synthase; arg, arginine; cit, citrulline; PMN, polymorphonuclear leukocyte; LPS, lipopolysaccharide; Ab, antibody; PL, phospholipid; PLA<sub>2</sub>, phospholipase A<sub>2</sub>; AA, arachidonic acid; PG, prostaglandins; CAT, catalase; SOD, superoxide dismutase; MPx, myeloperoxidase; NOX, NADPH oxidase.



and myocardial infarction), Alzheimer's disease, Parkinson's disease, and cancer (Figure 12) (42).

Oxidative stress is not a "disease" in the traditional sense of the word. It is the unwanted effect of a biochemical dysfunction related to redox systems. Therefore it can impact, often deceitfully, upon the onset and/or course of several basic diseases. As it is not a classical disease, oxidative stress does not exhibit a specific clinical picture but hides itself behind the symptoms and signs of the basic disease. Therefore, oxidative stress can be found only if the clinician refers the patient to specific biochemical tests (42, 43).

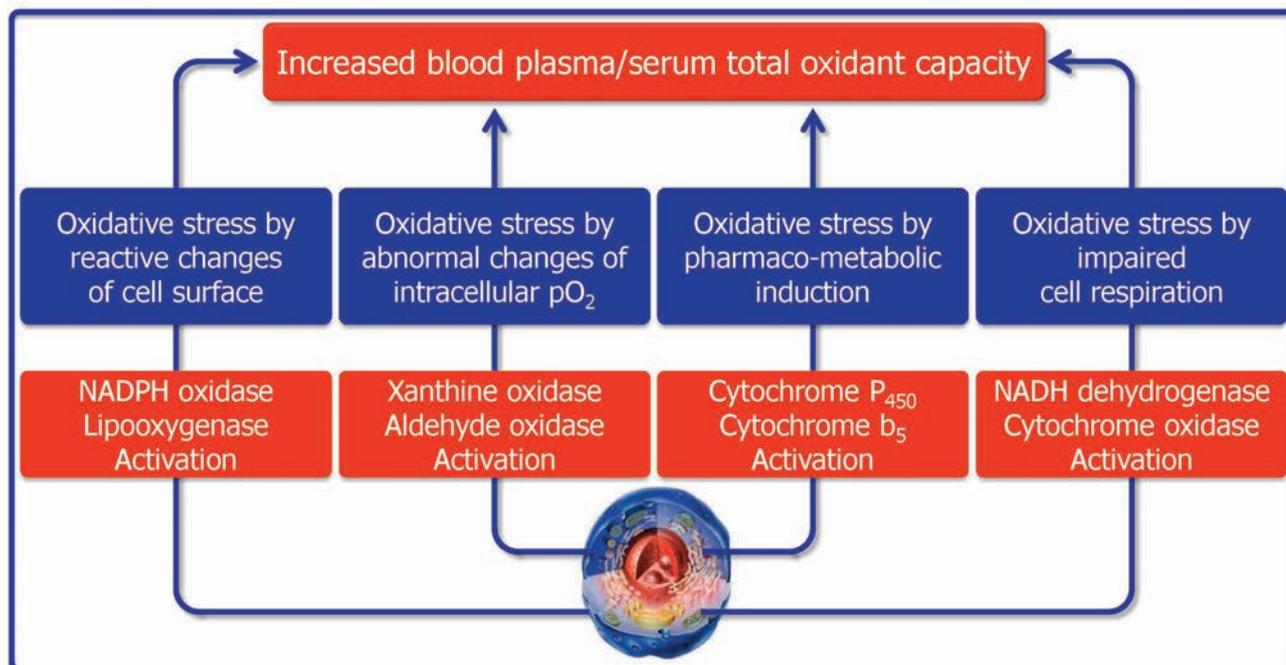
At long last, research now offers to health professionals the opportunity to identify and quantify many markers of oxidative stress which are currently used with the general purpose of preventing oxidative damage, diagnosing and monitoring oxidative stress, and evaluating the indications and effectiveness of antioxidant supplementations and/or therapeutic interventions. Some of these markers have even been proposed as being predictive of disease (44-46). Their potential usefulness in aesthetic medicine and dermatology is increasing (47).

## **PATHOPHYSIOLOGY OF OXIDATIVE STRESS.**

The impact of oxidative stress on the structure and the functions of cells can be exemplified by the peroxidative process (Figure 13) (1, 3, 5, 48, 49). In this pathophysiological model – due to exogenous stressors (physical chemical and biological agents) and/or to its metabolic activity (particularly into the plasma membrane, the mitochondria, the endoplasmic reticulum and cytosol) – the cell starts to produce increasing amounts of free radicals,

among which there is the very powerful hydroxyl radical (HO). After acting on its target molecules its excess is normally scavenged by vitamin E. However being one of the most potentially dangerous ROS, hydroxyl radicals can "hit" every kind of molecule (including carbohydrates, lipids, amino acids, peptides, proteins, nucleotides, nucleic acids and so on). As the consequence of this action, the hit molecule loses an electron and becomes, in turn, a radical. Therefore a radical chain reaction starts, leading – if molecular oxygen (by respiration) is present – to the generation of hydroperoxides. In normal conditions hydroperoxides are neutralised to organic alcohols by the enzyme glutathione peroxidase that glutathione as coenzyme and selenium as cofactor. Although hydroperoxides are relatively stable chemical species, they have the potential to generate again free radicals and to oxidize other molecular targets. For this reason hydroperoxides especially if in excess are partially released in the external environment, i. e. in the extracellular matrix and finally in the extracellular fluids, including blood, cerebrospinal fluid, pleural fluid and so on, in order to undergo their catabolism through extracellular glutathione peroxidase. When a condition of ischemia is induced due to prolonged vasoconstriction or partial thrombus, the reduced availability of oxygen inside the micro-circulation (hypoxia) compels the cell to activate anaerobic metabolism with the releasing into the small blood vessels of acidic metabolites, including lactate. The consequent lowering of pH may induce a conformational change of transition metal-carrier protein, including transferrin and ceruloplasmin. In turn, the low-pH induced conformational change of transferrin triggers

**FIGURE 14.** Pathophysiology of oxidative stress. Four different cellular mechanisms generate reactive oxidizing species (ROS) thus allowing identify four main pathophysiological and clinical patterns of oxidative stress in clinical practice.  $pO_2$ , oxygen partial pressure.



the release from the carrier of iron, which finally acts as a catalyst in the so-called Fenton's reaction, where hydroperoxides are broken into alkoxy (RO) and hydroperoxy (ROO) radicals. Both radicals if in excess and not adequately neutralised by the circulating antioxidant systems are able to oxidize either the endothelium surface or the circulating lipids and cholesterol, thus favouring the atherosclerosis. In any case, it is evident that hydroperoxides are not only the witnesses or markers of oxidative stress (due to their origin from the cell) but also potential amplifiers of the initial damage to the whole body (because their ability to circulate in the extracellular fluids). The inflammation of extracellular matrix can amplify tissue damage.

Keeping in the mind this general model (Figure 13) a deeper analysis allows to recognize at least 4 pathophysiological patterns of

oxidative stress on the basis of the main cell site involved in ROS production: oxidative stress by reactive changes of cell surface (plasmamembrane), oxidative stress by reduced efficacy of cellular respiration (mitochondria), oxidative stress by pharmaco-metabolic induction (microsomes), and oxidative stress by changes in the intracellular oxygen pressure (cytosol) (Figure 14) (50).

Oxidative stress mainly related to reactive changes of cell surface is induced by the activation of plasmamembrane, where are several enzymatic activities which are able to generate ROS (17, 38). It is peculiar of reactive processes, such as infections (e. g. bacterial infections like in periodontitis) (39, 40) and inflammations (e. g. rheumatoid arthritis) (51).

Oxidative stress by reduced effectiveness of cell respiration is induced by an impair-

ment of mitochondrial function, in turn responsible of an unbalanced production of ROS (18). This condition is related to an increased metabolic activity, as observed in after strenuous exercise (52) or hypernutrition (53), as well as in thyroid hyperactivity (19). Alternatively, exaggerate amounts of ROS can be produced either by a primary disease of mitochondria or by the activation of a “vicious circle” (metabolic activation @ ROS production by electronic shunt @ mitochondrial dysfunction @ reduced respiratory effectiveness @ further production of ROS by electronic shunt) (54).

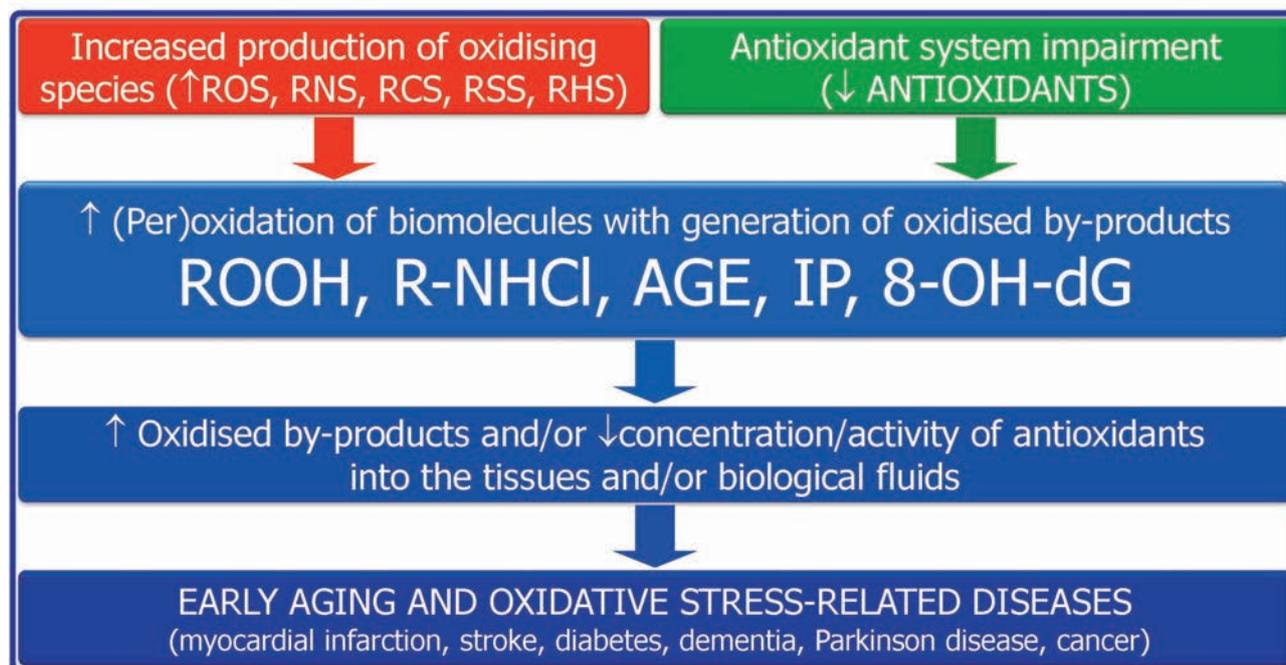
Oxidative stress by pharmaco-metabolic induction is associated to the activation of cytochrome P<sub>450</sub> hydroxylation system which have a detoxifying function (20). This kind of oxidative stress is primarily related to

alcohol abuse and to xenobiotics exposure. In this condition variably centered reactive species can be observed (e. g. the radical of acetaminophen, a common antipyretic drug) (55). Oral contraceptives also may stimulate ROS production (56).

Oxidative stress by intracellular pO<sub>2</sub> changes is primarily related to ischemia-reperfusion damage and it can be observed in myocardial infarction, during surgical bypass and after transplantation (22). In such conditions xanthine oxidase activation seem to play an important role in the generation of hydrogen peroxide and superoxide anion, as above discussed.

In some cases oxidative stress can be related to multiple mechanisms. This happens after exposition to cigarette smoke, pollutants, ionizing or UV radiations, and toxic agents or

**FIGURE 15.** Rationale for oxidative stress measurement. Oxidative and/or peroxidative processes lead to an increase of oxidized/peroxidised by-products which level in tissues, fluids and secretions reflects the degree of oxidative unbalance in vivo. ROS, reactive oxygen species; RNS, reactive nitrogen species; RCS, reactive carbon species; RSS, reactive sulfur species; RHS, reactive halogen species; ROOH, hydroperoxides; R-NHCl, organic chloroamines; AGE, advanced glycosylated end-products; IP, isoprostanes; 8-OH-dG, 8-hydroxy-2'-deoxyguanosine.



xenobiotics (1, 3, 5).

It is obvious that this outline is an oversimplification of the problem, because the biochemical situation in the cell and tissues is more complex and several mechanisms are concomitantly involved in oxidative tissue damage (50). Indeed, as above discussed about polymorphonuclear plasmamembrane and muscle mitochondria, in all the reactive conditions, such as the infections, some processes, e. g. the fever, are strictly related to an increased metabolic activity and, vice versa, chronic muscular efforts can induce tissue inflammation, responsible for skeletal muscle injuries. In other words it is not always possible to distinguish whether oxidative stress is induced only by a plasmamembrane activation or only by a reduced respiratory effectiveness. Moreover, in chronic muscular efforts oxidative tissue lesions are strictly related also to the ischemia-reperfusion damage, so that in such condition at least three mechanisms can be responsible for oxidative stress (i. e. reduced effectiveness of cell respiration, plasmamembrane activation and reduced intracellular  $pO_2$ ) according to the schema above discussed. For these reasons is right to indicate the different kind of oxidative stress with term “mainly”, e. g. oxidative stress *mainly* induced by plasmamembrane activation. However, despite these limitations, such classification of oxidative stress is useful for the clinician in order to make a correct diagnosis and to orientate antioxidant therapy (50).

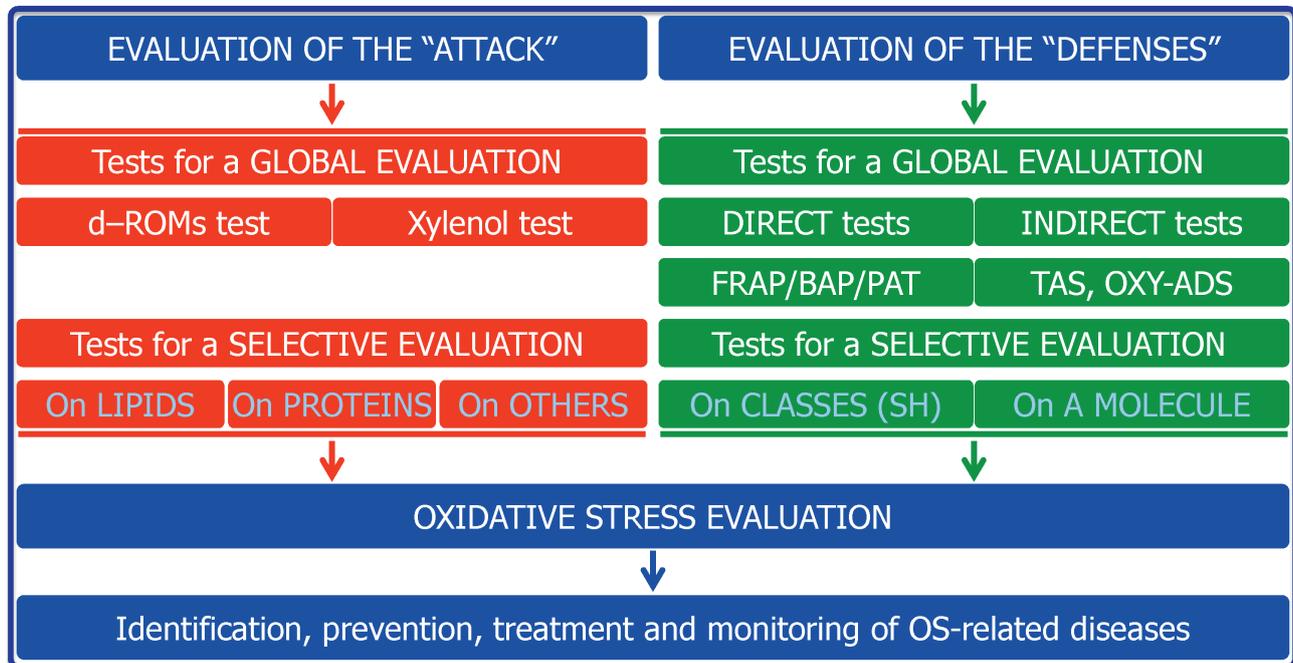
## **EVALUATION OF OXIDATIVE STRESS. THE EMERGING FIELD OF REDOXOMICS.**

According to the generally accepted

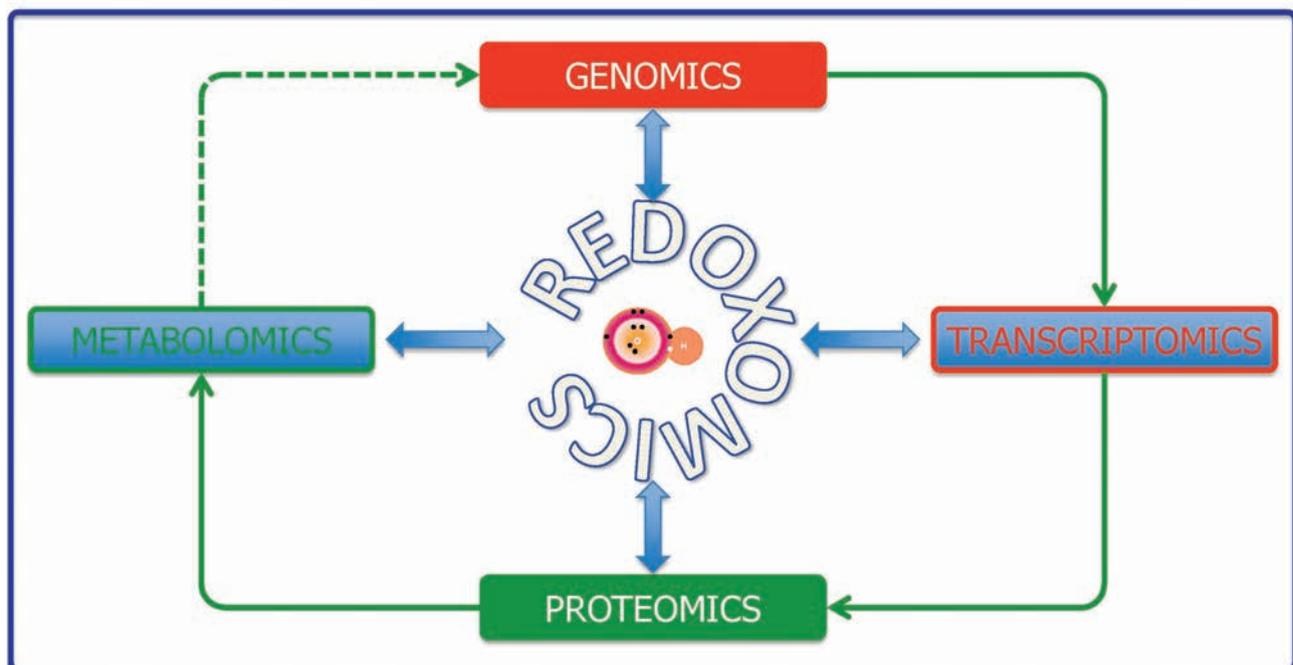
definition of oxidative stress given above, a dysfunction of the redox system due to the inability of antioxidants to modulate ROS activities inside or outside the cells may lead to the (per)oxidation of a number of biomolecules with generation of (per)oxidized by-products (e. g., hydroperoxides, chloramines, advanced glycosylation end products, isoprostanes, 8-hydroxy-deoxyguanosine) (57) (Figure 15). This may be followed by an increase in (per)oxidized by-products and/or a reduced concentration/activity of antioxidants either in tissues or extracellular fluids, which will represent the optimal specimens in which to evaluate the oxidative stress (57).

The first analytical approach therefore involves the direct measurement of the oxidant(s) in a biological specimen (57). This goal can be achieved by using electron spin resonance for radical ROS like hydroxyl or peroxy radicals, or other photometric/fluorescent methods for non-radical OCS like hydrogen peroxide. When direct measurement of ROS is not possible, different methods, referred to as fingerprinting, must be applied. According to this approach, a radical is inferred from the molecular nature of the damage it causes to biological molecules. When the oxidative stress is great enough to overcome the antioxidant defence, ROS can theoretically damage every component of the cell, including lipids, amino acids, proteins, and nucleic acids, thus generating oxidized by-products (1, 57). These damaged molecules – or the products resulting from their breakdown – are the “fingerprinting” (57). In other words, oxidative damage is presumed to happen in vivo when it generates identifiable and quantifiable specific by-products in vitro. (57) These by-products are as-

**FIGURE 16.** Oxidative stress measurement. Oxidative unbalances can be detected by laboratory test able to evaluate either the deficiency of reactive oxidizing species (ROS) or the impairment of antioxidant systems.



**FIGURE 17.** The novel field of redoxomics. Compared to classical "OMICS" REDOXOMICS appears as a novel multidisciplinary and transversal approach for health and diseases.



sumed to be biomarkers of oxidative status. Notably, some of these “biomarkers”, like hydroperoxides, can also act as “amplifiers” of oxidative damage, which underscores the importance of detecting these molecules in order to reduce not only the effect but also the cause of oxidative stress (57).

The evaluation of antioxidant defenses – which is apparently easier than the quantification of OCS – is generally possible by direct methods evaluating the activity of enzymes (e. g. superoxide dismutase, catalases and peroxidases) or water/lipid-soluble antioxidants (e. g. vitamin C and E) by means of photometry or fluorescence. For the evaluation of oxidant and antioxidant capacities, some tests provide a global idea of the oxidant or antioxidant status (e. g. d-ROMs test and Total Antioxidant Status, respectively), while others provide the quantification of a specific enzymatic activity or concentration (e. g. measurement of glutathione peroxidase activity or serum levels of tocopherols, respectively) (42, 57).

On this basis we chose to classify the most commonly available methods for oxidative stress assessment into two main categories: tests to evaluate the oxidative capacity/potential and tests to evaluate the antioxidant capacity/potential (Figure 16). In each category we can further distinguish, when adequate, direct from indirect methods and global from selective methods. Further classifications can be made depending on the biological source (e. g. plasma, exhaled breath, seminal fluids, and so on) (57).

In this scenario, the systematic evaluation in biological samples (tissues or fluids) of primary oxidant chemical species and their derivatives, like hydroperoxides, as well as the

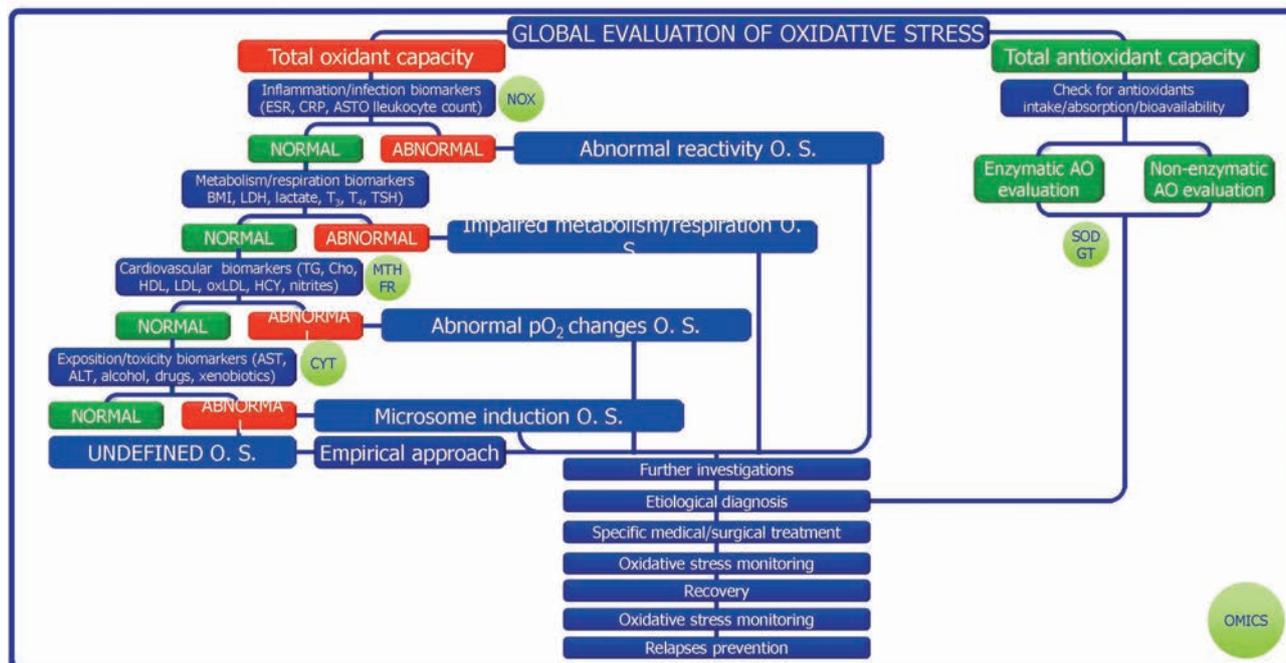
dosing of antioxidant compounds/activities, like selenium and glutathione peroxidase, respectively, are not a terminal “ring” in the diagnostic chain on informational flow in biological systems (DNA PROTEINS METABOLITES) but can take a “central” place compared to genomics, transcriptomics, proteomics and metabolomics (58-62). For this reason very recently we introduced the novel concept of “redoxomics” (a term previously and ambiguously used to identify only some oxidised by-products in the field of proteomics) (Figure 17) (63).

Redoxomics is a novel branch of “applied biochemistry” and “molecular diagnostics” having the following aims:

- to analyse the structure, the physiological role and the distribution of OCS and antioxidant systems in a living organism;
- to identify the reciprocal interactions of oxidant and antioxidant systems – in the general flow of information – in a biological system (cell, tissue, organ, apparatus, system, whole organism) in a defined step of its development, in basic conditions as well as after potentially stressful stimuli;
- to evaluate the implications of these findings by the view-point of epidemiology, pathophysiology, clinics, pharmacology and so on (64).

The ambitious goal of redoxomics (as well as for other “-omics” in other fields) is “to map” dynamically – by means of all the available and sophisticated analytical techniques, from electron spin resonance to imaging – the whole oxidative-antioxidant repertoire, i. e. the “redoxoma” of a living unit in different conditions. This “integrated” approach by allowing to monitor every qualitative/quantitative changes of oxidative balance can help the clinicians to find the optimal and the “personalized” solution to correct any eventual

**FIGURE 18.** An original algorithm for oxidative stress management. Depending on the results of oxidative stress evaluation done by measuring both total oxidant and total antioxidant capacity the clinician should try to identify the mechanism responsible of an eventual redox unbalance by means of specific laboratory tests, and then to play and monitor the treatment. For unrecognized oxidative stress an empirical approach is mandatory. O.S., oxidative stress; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ASO, anti-streptolysin O; NOX, NADPH oxidase; BMI, body mass index; LDH, lactate dehydrogenase; T3, triiodothyronine; T4, tetraiodothyronine; TSH, thyroid stimulating hormone; HDL, high density lipoprotein; LDL, low density lipoprotein; oxLDL, oxidized low density lipoprotein (oxidized cholesterol); HCY, homocysteine; MTHF, methylene-tetrahydrofolate reductase; AST, aspartate transaminase; ALT, alanine transaminase; Cyt, cytochrome P450; AO, antioxidants; SOD, superoxide dismutase; GT, glutathione transferase; OMICS, genomics and other omics.



abnormality of redox status associated to human or animal disease (65).

## THE MANAGEMENT OF OXIDATIVE STRESS IN CLINICAL PRACTICE.

The starting point of oxidative stress management is always the clinical suspicion that is generated, in turn, by the knowledge of the problem. If the clinician doesn't know oxidative stress he will not be able to formulate the correct questions aimed to evidence it. From this simple concept it becomes obvious the importance of the clinical history that will

lead to search the existence of risk factors for oxidative stress, including age, physiological status (pregnancy, lactation, menopause), overweight/obesity, abnormal caloric intake, minerals and vitamins deficiency in the diet, alcohol abuse, cigarette smoke, inadequate exercise, psycho-emotional stress, significant exposure to UV radiations, significant exposure to electromagnetic radiations, significant exposure to environment pollutants, current intake of estrogen-progesterone combination (especially as contraceptive pill), current chemotherapy, current radiotherapy, current dialysis, current cortisone treatments and so on (1, 10, 57, 65).

The task of the clinician will be easier where the patient suffers from a known disease. In fact the clinician will have to search only the current disease among the known diseases associated to the oxidative stress. On this subject, all the following conditions are generally associated to an oxidative imbalance: recent trauma, recent viral infection, recent bacterial infection, infectious disease from other agents, recent inflammatory non infective disease, thyroid hyper-function, arterial hypertension, clinical signs of atherosclerosis, dyslipidemia, complicated diabetes mellitus, liver dysfunction, neoplasms, malabsorption diseases, and so on (1, 65). In each of the above cases a careful clinical visit will confirm the suspicion of any eventual disregarded but hypothesized disease on the basis of the clinical history.

The first step of the clinical routine will end with the biochemical analysis of the oxidative stress by means of at least a couple of tests, the first one measuring the oxidant capacity (e. g. d-ROMs test) and the second one measuring the antioxidant capacity (e. g. Total Antioxidant Status) on a sample of blood serum or plasma. On the basis of the results the clinician will examine all possible combinations and will interpret each clinical situation (57).

In the evident case of oxidative stress (increased oxidant capacity and/or decreased antioxidant capacity test), on the model of a specific original algorithm, the clinician will try to identify the possible cause(s) and the relative mechanism(s) responsible for the impaired oxidative balance (*Figure 18*) (65). Practically the clinician should try to establish, with the aid of adequate laboratory/instrumental analyses (leukocytes count, ESV, CRP, AST, BMI, fat

mass/muscle mass ratio, thyroid biomarkers, serum lipid pattern, homocysteine, tumour markers and so on) whether the main mechanism responsible is one or more of those proposed (inflammation, impairment of mitochondrial respiratory function, ischemia-reperfusion damage and pharmaco-metabolic induction) (17-22). On the basis of the prevalent mechanism, the clinician will be able to prescribe, in the single clinical case, a specific treatment able to reduce the increased oxidant capacity (causative or etiological therapy) and/or to strengthen the antioxidant defenses (supplementation) (57, 65).

The prevention and/or the treatment of the diseases associated with the oxidative stress requires, besides specific options depending on the prevalent involved mechanism, an integrated approach that Cooper (Dallas, Texas, US) defined some years ago as the “antioxidant revolution” (66). In such a context it is very important, after undergoing the tests, to ameliorate the life style, by adopting a healthy nutritional model like “Mediterranean Diet” or “Okinawan Diet” that include exercise, good social relationships and spiritual/meditation thinks (67, 68).

The American Guide Lines for Food Intake, some of which are followed by Oncologists for the prevention of tumors, clearly suggest take everyday from 5 to 8 portions of fruits and vegetables, preferably fresh and in season (69). However, some Researchers prefer to this “empiric” suggestion more objective criteria, like the one based on the ORAC score (70). This system is able to quantify the “in vitro” antioxidant capacity of all common fruits and vegetables in “Oxygen Radical Adsorbent Capacity” unities. For instance, 100 g of dried

prunes allows an intake of 5770 ORAC UNITS. Alternatively, the clinician can exploit the nutritional requirement found in RDA tables (recommended dietary allowances) and LARN tables (minimal levels of recommended nutrients), which vary depending on the geographic area, the age and the gender (71).

However, we cannot exclude that the level of food nutrients, as expected on the basis of the above tables, is exactly the real level of the same nutrients we take when we eat a fruit or a vegetable. Indeed, the impoverishment of the soil (due to abnormal exploitation of the soil itself, acidic rains, increasing desertification, pollution and so on), the often uncontrolled use of pesticides, the processes of refinement of vegetables, the processes of transformation, storage, and even the cooking of foods can variably affect the original, as described in the above tables, antioxidant content of fruits and vegetables (72). Therefore, as a precaution, many nutritionists today suggest the indiscriminate use of antioxidants. However, the use of antioxidant supplements should be limited only to the documented cases of oxidative stress, as biochemically detected by specific tests (57, 64, 73).

In this background, before suggesting any supplementation, every clinician should try to identify and to remove the possible cause responsible for the increased production of free radicals. In particular, reduced levels of antioxidant capacity suggest the real need of an antioxidant supplement and the clinicians should follow some general criteria, which take into account the chemical characteristics and the amount of the micronutrients to be proposed, the possible onset of unwanted side effects, the route of administration, the clinical condi-

tions of the patient, the concomitant administration of other drugs and so on (73).

Generally speaking, the wide variety of oxidants responsible for oxidative stress and their ubiquitous distribution into the body implies the necessity to have a formula with a wide and complete spectrum of actions. Unfortunately a unique formula able to fit the above criteria is not available. Moreover because a unique antioxidant is only partially effective, it is indispensable that the clinician considers a cocktail of antioxidants, e. g. a formula containing multiple antioxidants with a wide range of activity (73). After stating that the combined antioxidants are more effective than one antioxidant alone, the main problem to be solved is the relative dosing.

Unfortunately, again, the opinions of researchers diverge one from another according to two main trends. The first one is the American opinion, according to which we should use a very large amount of antioxidants to prevent and to treat the oxidative stress, although this approach can be dangerous for our health (74). The second one, prevalent in Europe and conceptually linked to the homeopathy, suggests the use of low doses of supplements (75). After dosing has been established, the next major problem is the pharmaceutical formula. On this subject it has been established, that a fluid formula is more effective than a "solid" formula (e. g. tablets, powder and so on) (76). A specific role is also played by the route of administration: for instance many active principles taken by oral route can be neutralized or affected during transit to the bowel, where variable amounts are "sequestered" by the liver, so that the "bio-availability" of the original supplement for other tissues/organs is reduced (76). This is



the case of the reduced glutathione (77). On the other hand some clinical conditions, such as celiac disease, by involving the small intestine can affect the absorption of micronutrients (78). In these cases the clinician should consider the parenteral route (e. g. intravenous or intra muscular route). More recently spray oral formulas for sublingual absorption have been developed (79). These spray formulas theoretically warrant a quick and easy gain of the circulating blood by the active principle, avoiding also transmission through the liver. In all the remaining cases when the intravenous route is not accepted or contra-indicated, the clinician should consider the administration of metabolic precursors of the antioxidant. For instance, the reduced glutathione is rapidly oxidised in the plasma and should be administered for intravenous route; in this case the clinician can consider the opportunity to administer some cysteine-enriched peptides able to reconstitute the glutathione into the cells (80). Independently of the effectiveness of the antioxidant formula, a crucial aspect to be considered is the eventual toxicity. Indeed some antioxidants, including the vitamin C, can exhibit oxidant properties (81) while other supplements such as carotenes can increase the risk of accumulation into the fat deposits, due to their affinity for lipids, and/or increase oxidative stress (82). Finally, when the patient presents some co-morbidities which require specific drugs, the clinician should consider the possible risk of the interaction between such drugs and the antioxidant supplements. This is the case of *Ginkgo biloba* extracts, which active principles can bind itself to the plasma protein and release anticoagulant in the blood, thus increasing the risk of haemorrhagic syndromes in a

patient with thrombophilic conditions (83).

## OXIDATIVE STRESS AND SKIN DISEASES.

The skin being the largest organ (1.5 to 2.0 square meters) of the integumentary systems acts not only as a protective wall but, rather, as a perm-selective two-ways interface between the body and the environment (84).

By considering the flow of information and molecules from outside to inside, skin is sensitive to many environmental physical, chemical and biological stressors, and on behalf of neurological, endocrine and immune responses it allows our body to adapt to different conditions in order to maintain a right homeostasis (84). At a molecular level most of such responses are mediated by the redox system which dysfunction may cause or promote skin aging and/or carcinogenesis (*Figure 19*) (85). Indeed skin is one of the major targets of ROS attack since it is exposed to UV radiation and a variety of environmental pollutants, high pressure of molecular oxygen and, in addition, is rich in polyunsaturated fatty acids (86). A classical example of ROS-induced skin damage is provided by wrinkles which formation is representative of aging process where the decreased skin elasticity is associated to a degeneration of the extracellular matrix (*Figure 20*) (87).

Focusing on physical agents that are able to induce oxidative stress in the skin layers incident UV radiations – particularly UVB at 280–315 nm, and UVA at 315–400 nm – play the prominent role in the so-called skin photoaging (changes from other factors that contribute to aging, such as metabolic or hormonal, are termed “chronologic” or “intrinsic” aging) and cancer. It has been reported

that UVB rays make up only 5% of the UV radiation that reaches the earth surface and have little penetrance, but they display great biological activity, while UVA rays make up the remaining 95% of incident light and is more penetrating, promoting photo aging and carcinogenesis (although to a lower extent than UVB). All the main effects of acute and chronic exposure to UV radiation – i. e. DNA damage, inflammation and immunosuppression – are directly and/or indirectly related to a dysfunction of redox systems that leads to an uncontrolled production of ROS mainly triggered by photolysis (*see above*) (86, 88).

The predominant redox-sensitive pathways activated by UV radiations are: i) the mitogen-activated protein kinase (MAPK), iii) the signal transduction and activation of transcription factor (JAK/STAT; iii) the nuclear factor-kappa beta (NF- B)/p65; and iv) the nuclear factor erythroid 2-related factor 2 (Nrf2) (*Figure 20*) (86).

The activation of MAP kinase pathway, through the receptor tyrosine kinase, results in the activation of transcription factor activator protein-1 (AP-1) – that includes extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun-N-terminal- kinase (JNK) and p38 proteins – with subsequent expression of matrix-metalloproteinases (MMPs); in turn JNK and p38 pathways play a major role; in the UV radiation mediated increase in AP-1 and cyclooxygenase-2 (COX-2) expression, and are targets for chemoprevention of skin cancer.

Ultraviolet radiations through ROS stimulate also the NF- B pathway which first step is the activation of cytoplasmic I- B kinase; this latter phosphorylates and degrades I- B, the inhibitor of NF- B transcription factor; the release of NF- B from its inhibitor (I- B), results in the

translocation of active NF- B to the nucleus to activate the inflammatory cytokines and prostaglandins. Interestingly, the inhibition of NF- B by use of antioxidants, proteasome inhibitors, prevention of Ikb phosphorylation or expression of overactivated, mutant (Ikb) may mitigate UV-induced damage.

Instead of NF-kB the transcription factor Nrf2 acts as a protective pathway. In its inactive form, such factor is a protein consisting of three subunits (Keap1, Cul3 and Nrf2). UV-induced ROS by removing Keap1 and Cul3 activates the factor that translocates from cytoplasm to the nucleus thus binding directly to DNA and stimulating the transcription of antioxidant-response element (ARE), that includes glutathione, glutathione transferase and so on. A down-regulation of Nrf2 and its target genes is associated to many diseases and cancers.

In any way UV-generated ROS are particularly harmful because promoting radical chain reactions they can destabilize and damage rapidly other biomolecules thus resulting finally responsible of membrane degradation, mitochondrial dysfunction, structural/functional changes in enzymatic activities, DNA damage and telomere shortening in all skin tissues especially in epidermis. Moreover UV-induced ROS participates in the three stages of carcinogenesis. During initiation, they produce genetic damage through direct effect on the DNA or by activating other factors. In the promotion stage, they favour the proliferation of malignant cells by inhibiting the mechanisms of immune controls and by promoting genomic instability. Finally, ROS also enhance progression and dissemination of cancers by promoting protease release and angiogenesis (*see below*) (84-87).

The UV-induced ROS production may

affect not only the epidermis but also dermis i. e. the connective layer of skin where are located together with a number of different cells (fibroblasts, macrophages, lymphocytes and so on) and lymphatic/blood vessels, the extracellular matrix (ECM) and their main components, including proteoglycans, collagen, elastin, and MMPs/elastases. Evidence shows that UV radiations cause a loss of elastin fibres and deplete the microfibrillar network in the epidermal-dermal layer and the dermis thus contributing to aberrant elastic fibers. Moreover UV as well as pollutants and aging processes may increase the physiological level of the proteolytic enzyme MMPs from epidermal keratinocytes, resulting in the fragmentation of collagen and elastin fibers, both responsible of ECM remodelling but also of cancer spreading (Figure 19) (84, 87).

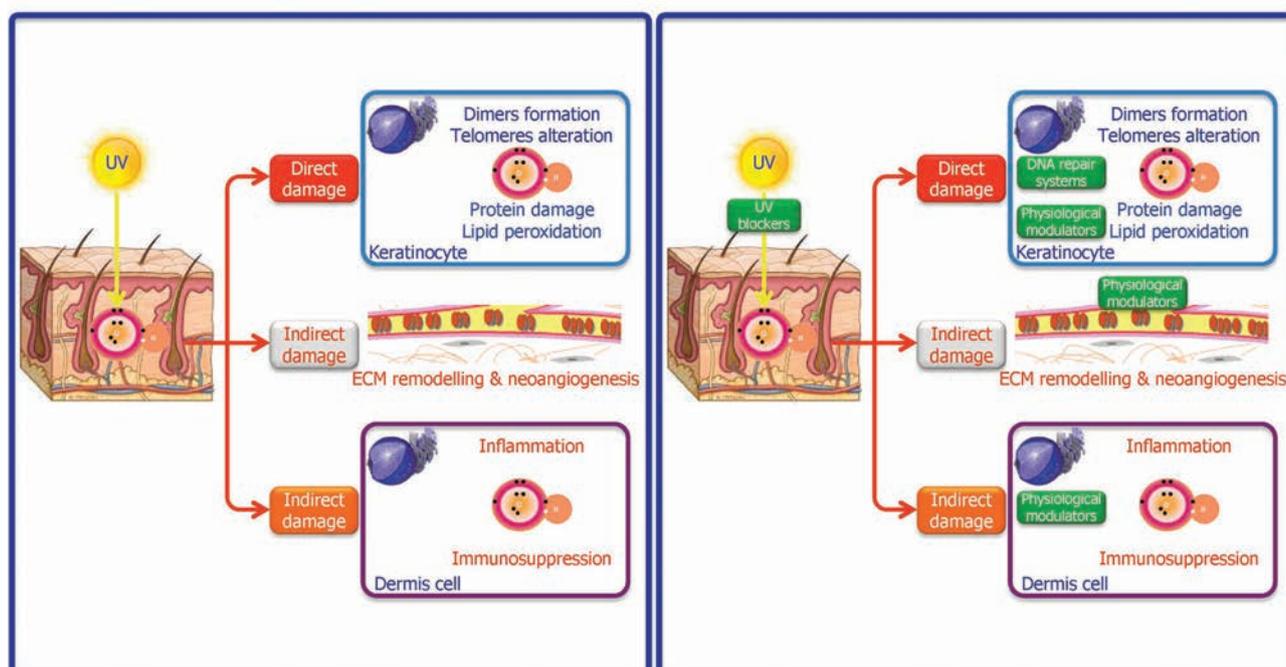
In any way induced (e. g. UV, pollutants,

virus, bacteria and so on), any dysfunction of the redox system through an excess of uncontrolled amounts of ROS may trigger or worsen a variety of skin diseases including erythema, oedema, heat, pain, photo-allergic reactions, autoimmune diseases, porphyrias, psoriasis, neutrophilic disorders (e. g. acne/rosacea), and ischemia-reperfusion injuries.

Although the exact mechanism that links a dysfunction of the redox system to such disorders is still under investigation it seems that ROS, produced either directly or indirectly by polymorphonuclear leucocytes (PMNs) and macrophages into inflamed areas, may mediate the activation of various cell signalling pathways that initiate or promote many skin diseases.

In this scenario ROS may play a relevant role in the pathophysiology of rosacea, a chronic inflammatory skin disease affecting the

**FIGURE 21.** Pathophysiology of UV-related oxidative stress in the skin and possible interventions. *Reactive oxidizing species (ROS) that can affect skin integrity (left side) can be modulated by endogenous as well as exogenous mechanisms driven by physiological modulators, normally called antioxidants, that are potentially able to control inflammation, immune function and extracellular matrix (ECM) remodelling (right side).*



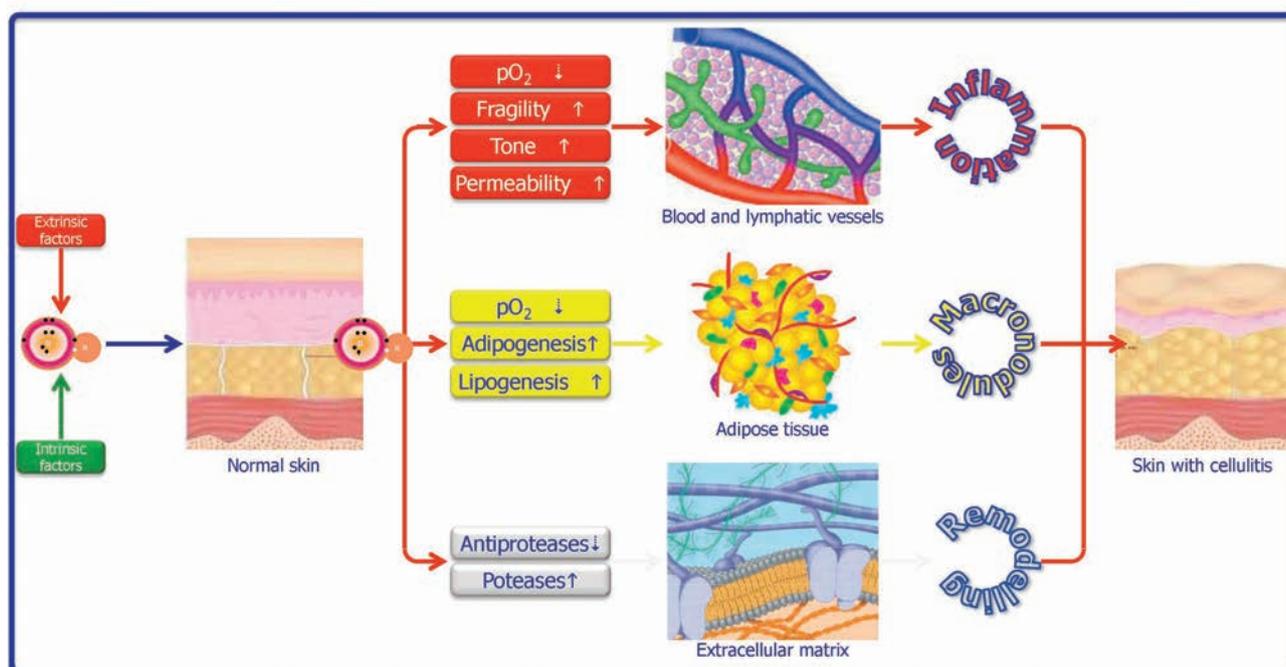
central part of the face which main signs are erythema, telangiectasia, papules and pustules (89, 90). Evidence shows that the level of ROS in skin biopsy samples from rosacea patients is higher than in samples from healthy individuals. Moreover, bacteriostatic drugs showing ancillary anti-inflammatory properties, like tetracyclines, inhibit the production of ROS and pro-inflammatory cytokines and block MMPs activities. Furthermore photosensitized reactions may increase oxidative stress level thus stimulating sebaceous gland function and sebum secretion as well as peroxidative processes; for example, in acne vulgaris, a Gram-positive anaerobic bacterium forms coproporphyrin which participates in type II reaction as a sensitizer, thus playing a key role in the inflammatory lesions of acne. As a whole

such data are in agreement with the potential role of oxidative stress in the pathogenesis of rosacea/acne.

Oxidative stress may affect also the keratinization process and pigmentation. Keratinocytes adjacent to melanocytes induce melanogenesis by up-regulation of tyrosine gene in melanocytes. In turn, vitiligo, the skin diseases characterized by depigmentation, is caused by melanocyte degeneration by ROS (91). Despite a large body of knowledge on cell peroxidation and antioxidant mechanisms, the mechanisms of altered keratinization are not well known. In general, UV-induced inflammation in the skin exhibits generation of cytokines, alteration of expression of adhesion molecules and the loss of antigen function.

In recent years, a direct relationship be-

**FIGURE 22.** Possible role of oxidative stress in the pathophysiology of cellulite. *Extrinsic factors* (e. g. as cigarette smoke, unbalanced diets and so on) as well as *intrinsic factors* (e. g. genetic predisposition, metabolic diseases, and so on) may trigger the production of reactive oxidizing species (ROS) by skin and subcutaneous tissues thus leading through changes in the physiology of fat tissue, blood/lymphatic vessels and extracellular matrix, to the typical clinical picture of cellulite (orange skin).



tween chronic, like atopic dermatitis (92), urticarial (93), and psoriasis (94), linked to the ROS formation, may arise in drug-induced photosensitivity.

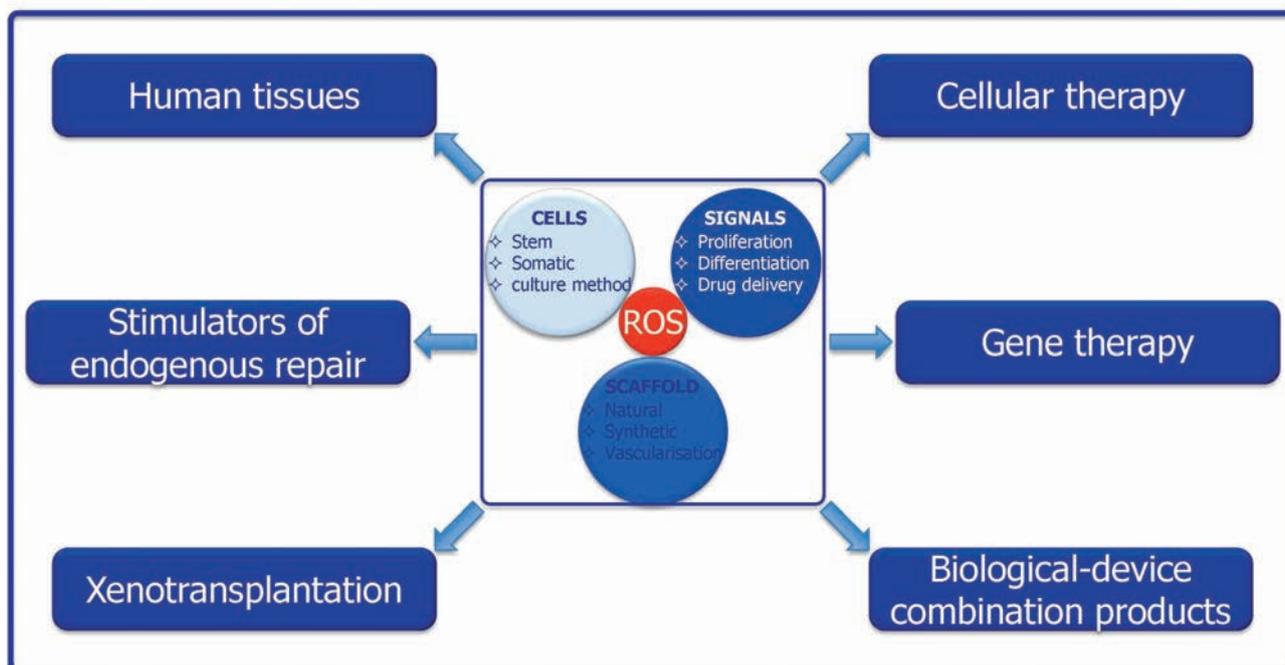
Such knowledge has been provided the basis for the development of many photo-protective strategies aimed to prevent and/or repair the deleterious effect of UV radiation leading to photoaging and photocarcinogenesis (95). They include the direct blockade of UV photons or the counteracting of direct or indirect effects of UV radiation through DNA repair systems and antioxidants/anti-inflammatory supplements/drugs (Figure 21) (95).

#### OXIDATIVE STRESS AND CELLULITE.

Cellulite is a multifactorial disease affecting skin and subcutaneous tissues and often described

by its appearance to that of the surface of an orange or an orange peel or with a look resembling cottage cheese look (96). It is most commonly found on the thighs and buttocks of women, but can also be seen in other areas, such as the abdomen, breasts, and arms. Despite numerous published works on this subject, the etiology as well as pathogenesis of cellulite still remains unknown and many hypotheses have been made over the time. However a recent review of the literature allowed to interpret cellulite as a chronic-inflammatory disease that derives from a process of *drifting in a like-visceral direction* of the morphological and functional properties of female gluteal-femoral adipose tissue (96). According to this hypothesis even in individuals with normal BMI, critical episodes, characterized by periods, albeit brief, of a calorie intake increase too fast

**FIGURE 23.** The emerging field of Regenerative Medicine. *Regenerative Medicine deals with the process of replacing, engineering or regenerating human cells, tissues or organs to restore or establish normal function. Reactive oxidising species may play a relevant role.*



and intense to allow carry out of a sufficient hyperplastic response, could compel the gluteal-femoral adipocytes to annex the lipid material through a growth of cell size, capable of triggering hypoxic conditions (96). In turn hypoxia would realize, in subcutaneous tissue, a complex process of tissue remodelling, characterized (as it happens in visceral fat), by the infiltration of macrophages and by a slight new collagen apposition around adipocyte clusters. Due to the activation of this very complex network of inter-cellular signalling, in which the fat cells and their dysfunction would play a central role, women affected by cellulite would present, in lower body fat, inflammatory phenomena similar to those typical of visceral adipose tissue in obese subjects (96). In this background the very close relationships between hypoxia, inflammation and oxidative stress make reasonable a direct involvement of redox-oma in the pathophysiology of oxidative stress (*Figure 22*). Indeed cellulite was shown to be associated to increased levels of biomarkers of oxidative stress (97) that decreased by combining medical treatment with antioxidant supplementation (98, 99).

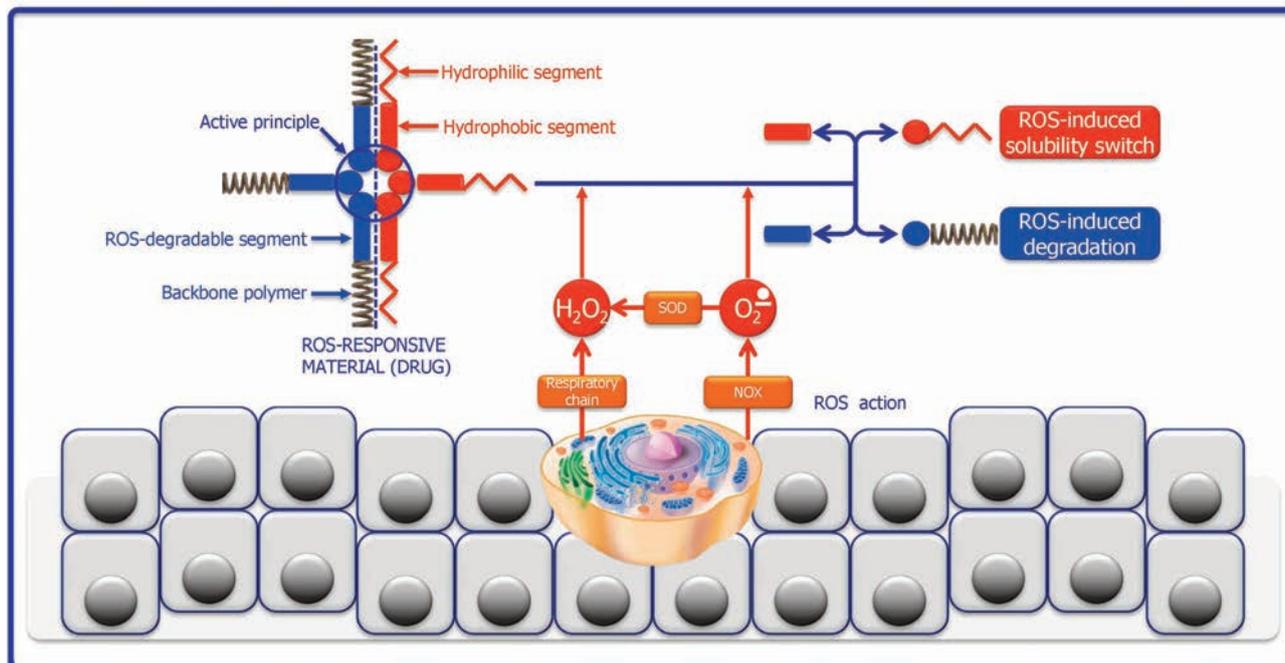
## **OXIDATIVE STRESS AND REGENERATIVE MEDICINE.**

Regenerative medicine is a branch of translational research in tissue engineering and molecular biology that deals with the “process of replacing, engineering or regenerating human cells, tissues or organs to restore or establish normal function” (*Figure 23*) (100, 101). This field holds the promise of engineering damaged tissues and organs via stimulating the body’s own repair mechanisms to function-

ally heal previously irreparable tissues or organs (100). Regenerative medicine also includes the possibility of growing tissues and organs in the laboratory and safely to implant them when the body cannot heal itself. If a regenerated organ’s cells would be derived from the patient’s own tissue or cells, this would potentially solve the problem of the shortage of organs available for donation, and the problem of organ transplant rejection (100, 101).

Inflammation play a relevant role in tissue regeneration as evidenced by studies aimed to promote bone regeneration in the craniofacial bone system (102). Most of these interventions utilize implantable materials or devices. Infections resulting from colonization of these implants may result in local tissue destruction in a manner analogous to periodontitis. This destruction is mediated via the expression of various inflammatory mediators and tissue-destructive enzymes. Given the well-documented association among microbial biofilms, inflammatory mediators, and tissue destruction, it seems reasonable to assume that inflammation may interfere with bone healing and regeneration. Paradoxically, recent evidence also suggests that the presence of certain pro-inflammatory mediators is actually required for bone healing. Bone injury (e.g., subsequent to a fracture or surgical intervention) is followed by a cascade of events, some of which are dependent upon the presence of pro-inflammatory mediators. If inflammation resolves promptly, then proper bone healing may occur. However, if inflammation persists (which might occur in the presence of an infected implant or graft material), then the continued inflammatory response may result in suboptimal bone formation. Thus, the effect of a given me-

**FIGURE 24.** Reactive oxygen species(ROS)-responsive materials for biomedical applications. *A generalized schematic diagram of ROS-responsive drug delivery and drug release via solubility switch and degradation mechanisms, respectively.*



diator is dependent upon the temporal context in which it is expressed. Better understanding of this temporal sequence may be used to optimize regenerative outcomes (102).

Due to the close relationships between inflammation and oxidative stress (38) it can be assumed that redox system too can modulate regenerative processes starting from the wound healing (103). This latter has been shown to require a fine modulation of ROS. A balanced ROS response will debride and disinfect a tissue and stimulate healthy tissue turnover; suppressed ROS will result in infection and an elevation in ROS will destroy otherwise healthy stromal tissue (104). Therefore understanding and anticipating the ROS niche within a tissue will greatly enhance the potential to exogenously augment and manipulate healing. On such basis modern tissue engineering

solutions to augment successful healing and remodelling of wounded or diseased tissue rely on a controlled balance between the constructive and destructive capacity of the leukocyte secretome, including ROS (105–107). Leukocyte derived ROS in tissue repair can be also a target of surgical intervention with inclusion of a biomaterial.

In this scenario the selection of predictive biomarker of oxidative stress for implant success is a critical point. In dental models nitric oxide, myeloperoxidase, 8-hydroxydeoxyguanosine, ROS from polymorphonuclear leukocytes and total antioxidant capacity showed very promising (108–112).

The evaluation of such biomarkers can be useful also in order to predict the success of platelet-rich plasma procedure (PRP): in fact abnormal levels of oxidative stress biomarkers

were found in smokers-derived PRP; unfortunately antioxidant like resveratrol may reduce platelet activation *ex vivo*.(113–114).

On the other hand many trials are in progress in order to modify the surface of some biomaterial through redox changes aimed to improve antioxidant power; for instance anodically oxidised titanium was shown to exhibit osteogenic and antioxidant properties (115) while N-acetylcysteine-loaded titanium nanotubes was able to enhance osteointegration (116).

The latest new derives form studies on ROS-responsive biomaterials (*Figure 24*) (117). Such “stimuli-sensitive” biomaterials appear as a new therapeutic approach to interact with dynamic physiological conditions. Because ROS are often overproduced locally in diseased cells and tissues, and they individually and synchronously contribute to many of the abnormalities associated with local pathogenesis, the advantages of developing ROS-responsive materials extend beyond site-specific targeting of therapeutic delivery, and potentially include navigating, sensing, and repairing the cellular damages via programmed changes in material properties. The mechanism and development of biomaterials with ROS-induced solubility switch or degradation, as well as their performance and potential for future biomedical applications are emerging areas of research (117–123). For instance a ROS-degradable poly(thioketal)-urethane tissue engineering scaffolds showed significant advantages over analogous polyester-based biomaterials and provided a robust, cell-degradable substrate for guiding new tissue formation (124). Moreover nanofiber membranes loaded with epigallocatechin-3-O-gallate (125) was able to prevent postsurgical adhesions while a vanillin-

scaffold reduced inflammatory response and enhanced extracellular matrix formation (127).

## CONCLUDING REMARKS

Reactive oxidising species play a crucial role in the maintenance and in the promotion of wellness of all tissues and organs including skin and subcutaneous being related to all basic processes of life i. e. the flow energy and information. Their activities are under the control of a network of physiological modulators – often but improperly called antioxidants – that prevents the unwanted side effects of a disturbed oxidative balance. Indeed oxidative stress – an emerging health risk factor – is co-responsible not only of early aging but also of at least one hundred diseases including cardiovascular diseases, neurodegenerative disorders and cancer. Oxidative stress is also involved in the pathophysiology of aesthetic as well as dermatological diseases like photoaging, wrinkles, and cellulite. Unfortunately oxidative stress does not show any specific clinical picture but can be diagnosed only by means of specific biochemical tests on biological fluids. This approach led to the development of new branch of applied biochemistry and molecular diagnostics called Redoxomics. On the basis of a Redoxomics profile the clinicians as well as the surgeons can identify early this new health risk factor and to fight it by using not only more properly the conventional strategies but also new approach based on lifestyle changes (127), nutraceuticals (73), bio-compatible biomaterials (e. g. threads) (128, 129), gases (e. g. oxygen infusion/propulsion, carboxy therapy, ozone therapy) (130) which

action mode is related to ROS. Indeed the maintenance of a optimal oxidative balance is becoming one of the true pre-requisite “to be beautiful on the outside and on the inside”.

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# Maqui (*Aristotelia chilensis*) berry and its major polyphenol delphinidin: Relevance for skin photo-protection and anti-aging

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## ABSTRACT

Healthy ageing involves the interaction between genes, the environment, and lifestyle factors, particularly diet. Diet-regulated genes play a crucial role in the onset and progression of several chronic disorders including skin diseases and dietary interventions can be used to mitigate these diseases but also to maintain health. The nutrient regulation of key genes involved in ageing may prevent oxidation and inflammation, reducing cellular damages to proteins, membranes, and mitochondria. In recent years there has been a growing interest, supported by a large number of experimental and epidemiological studies, about the beneficial effects of some commonly used food-derived products, including macro- and micronutrients and dietary phytochemicals. In particular, fruits, spices and herbs often contain active phenolic substances endowed with potent antioxidant and chemo-preventive properties. Among polyphenols, anthocyanins, a subfamily belonging to the flavonoid group, have shown to modulate a variety of biochemical/signalling pathways involved in promoting organism physiology and general health status, including vasculo-protective effects, cognitive process enhancement, anti-cancer activity and skin photo-protection. Among the anthocyanin species, delphinidin [2-(3,4,5-trihydroxyphenyl)chromenylium-3,5,7-triol] represent the most potent antioxidant. The richest known natural source of delphinidin is the maqui berry (*Aristotelia chilensis*), a super-fruit indigenous to Chile. We have recently performed a randomized double-blind nutritional trial, and demonstrated the *in vivo* ability of maqui polyphenols to protect lipids from oxidative damages. This and other studies have begun to provide a basis for considering the use of maqui and delphinidin in the development of novel nutritional interventional strategies for health management and against specific age-associated diseases. In this review we will provide an overview of the current literature emphasizing antioxidant and anti-inflammatory pathways modulated by maqui berry and its polyphenolic components, mostly delphinidin. Moreover, we will focus on experimental studies showing that delphinidin of maqui may have a positive impact on skin health.

**KEYWORDS:** Maqui; *Aristotelia chilensis*; Polyphenols; Delphinidin; antioxidants.

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## DISCLOSURES

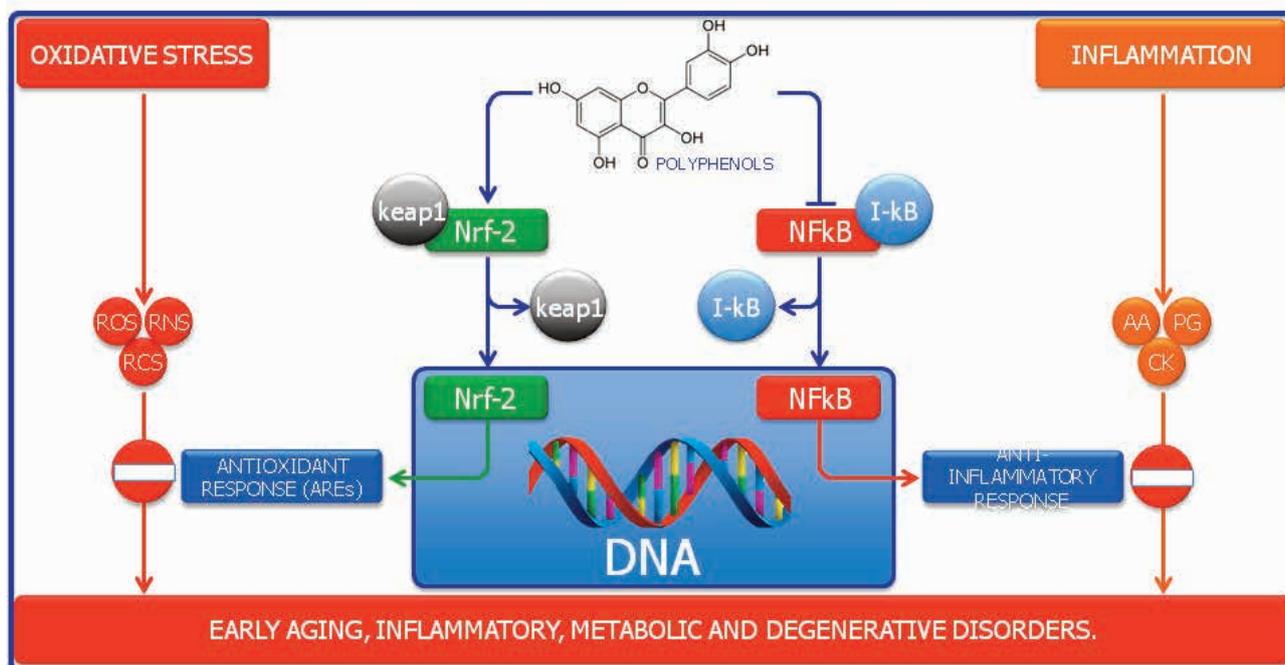
**Conflict of Interest:** None.

## INTRODUCTION

Proper nutrition is a direct factor affecting wellbeing, health and proper skin condition. Beyond the nutritional value, nutraceuticals and functional foods contain health-promoting components with specific beneficial effects on skin. The human skin is subject to constant change, which is why dietary supplements can complement the normal diet by providing properly balanced nutrients. A number of efficient micronutrients are capable of contributing to the prevention of UV damage in humans and a growing body of scientific evidence is becoming available to support that food derived compounds with antioxidants and anti-inflammatory activities contribute to en-

dogenous photo protection and are crucial for the maintenance of skin health. Spices and herbs often contain active phenolic substances endowed with potent antioxidative and chemopreventive properties (1). All of these compounds appear to have a number of different molecular targets, impinging on several signalling pathways, and showing pleiotropic activity on cells and tissues. A possible general mechanism of polyphenols healing activity, relate to their ability to overexpress highly protective inducible genes, involved in the cellular stress response. Several data from our and other laboratories, have previously shown that different classes of polyphenols, such as an-

**FIGURE 1.** Schematic drawing, showing the two major transcriptional factors involved in cellular stress response, nuclear factor erythroid 2-related factor 2 (Nrf2) and Nuclear Factor kB (NFkB), respectively, modulated by polyphenols. Polyphenols like delphinidin activate Nrf2 pathway thus leading to the enhancement of antioxidant response via antioxidant responsive elements (AREs) that include glutathione, glutathione peroxidase, catalase and so on. On the other hand polyphenols inhibit NFkB pathway thus blocking inflammatory response. The final effect of such concerted action on DNA is the control of oxidative stress and inflammation that can be very useful in the prevention of early aging and many diseases.



tochyanins, epicatechins and curcuminoids, strongly induce heme-oxygenase-1 (HO-1) expression and activity in skin cells via the activation of heterodimers of nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant responsive element (ARE) pathway (2). Many studies clearly demonstrate that activation of Nrf2 target genes, and particularly HO-1, is strongly protective against inflammation, oxidative damage, and cell death, in skin and in several tissues (3). Furthermore, most of these compounds, have been shown to efficiently inhibit the activation of Nuclear Factor  $\kappa$ B (NF $\kappa$ B), the master regulator of cellular pro-inflammatory events (4). This double pathways interference by polyphenols-Nrf2 activation/-NF $\kappa$ B inhibition, induce an over expression of endogenous antioxidants, and inhibit the production or expression of pro-inflammatory mediators including cytokines, chemokines and matrix metalloproteinases

(Figure 1). These studies have begun to provide a basis for considering the use of such polyphenols in the development of novel nutritional interventional strategies for skin health management and against specific age-associated diseases, including photo carcinogenesis. In this review we have examined the nutritional value of a super fruit, the berries of *Aristotelia chilensis* ([Molina], Stuntz), also known as maqui (Figure 2), and its promising effects against skin aging and skin inflammatory diseases, with a special focus on delphinidin[2-(3,4,5-trihydroxyphenyl)chromenylium-3,5,7-triol], a specific polyphenol highly contained in this fruit (Figure 3).

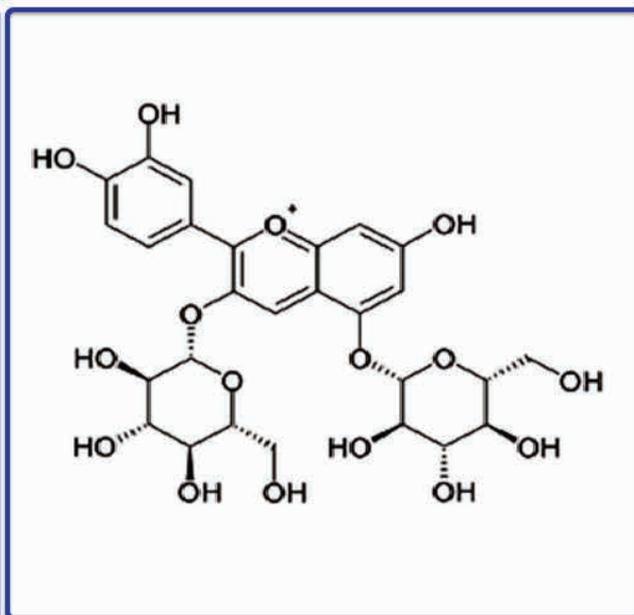
#### MAQUI: FROM TRADITIONAL USE TO PHYTOCHEMICAL CHARACTERISTICS.

Maqui belongs to the family of Elaeo-

FIGURE 2. Maqui berries (photo by the Author).



FIGURE 3. The chemical structure of delphinidin [2-(3,4,5-trihydroxyphenyl)chromenylium-3,5,7-triol] belonging to anthocyanidin species.



carpaceae, with 10 genera and about 400 species, is a plant native to the Valdivian temperate rainforests of Chile. Maqui berries, very similar to blueberries, are rich in anthocyanins, in particular delphinidin, antioxidants responsible for their purple coloration and, in all likelihood, for many of the medicinal properties attributed to it. Maqui's therapeutic qualities have been known for centuries to the Mapuche indigenous people who have traditionally lived in the southern part of Chile. According to the conquistadors the Mapuche warriors ate very little solid food and drank both a fresh and a fermented beverage called "chicha" made from maqui berry which might have contributed to the strength and stamina that the warriors exhibited. The Mapuche Indians have used maqui's berry leaves, stems, fruits and wine medicinally for thousands of years. Traditionally, it is believed to heal wounds, relieve sore throats and as analgesic. Today, maqui berry is regarded as "super fruit" due to its superior antioxidant properties. Currently berries maqui are marketed in the form of juices and infusions, and supplements are also derived from the maqui.

Phytochemical screening of maqui extract (fruits or leaves) revealed the presence of anthocyanins and other flavonoids, alkaloids, cinnamic and benzoic acid derivatives, other bioactive molecules, and mineral elements. There are several reports concerning the anthocyanins chemical composition of *A. chilensis* indicating relatively high anthocyanin content (~135 mg for 100 g fresh weight). Anthocyanins are glycosides of anthocyanidins, and are widely distributed in colored fruits and berries. Anthocyanins are water soluble and non-toxic pigment, divided in five major groups: malvidin, delphinidin, petunidin, cyanidin, peonidin. Del-

phinidin, that contains 3 hydroxylations in the B ring, posses the highest antioxidant activity. The total anthocyanin content in the maqui berry extracts (MBE) was ~35%, of which the anthocyanin proposition is ~80% of delphinidin, and malvidin, petunidin, cyanidin, peonidin derivatives being the rest (5). Recently Delphinol® (trademark owned by MNL Chile) an high polyphenols standardized extract of maqui berries, bearing  $\geq 25\%$  delphinidin, has been introduced in the European and Japanese supplement market (5).

## MAQUI BIOLOGICAL ACTIVITIES.

Regarding biological activity, maqui shows good responses in terms of antioxidant, anti-inflammatory anti-diabetic, anti-photo aging, etc. The broad range of activities of the fruits indicates that multiple mechanisms are responsible for its biological healing properties, linked to their characteristic phenolic content, and suggesting a very attractive potential for skin photo-aging and photo carcinogenesis.

### *Antioxidant activity*

In vitro antioxidant potential of maqui berries have been widely explored. Maqui fruits represent a rich source of antioxidant compounds, considering that they show high activity with respect to the DPPH. decoloration assay. This is due to their high anthocyanins content as demonstrated by the positive and direct correlation between DPPH. and total anthocyanins content (TAC). Maqui fruits show higher oxygen radical absorbance capacity (ORAC) values than over 100 different kinds of foods, including fruits, vegetables, nuts, dried fruits, spices and cereals (20 times stronger

than lemon, 3.5 times stronger than blackcurrant, and 2.9 times stronger than wild blueberry).

The effect of anthocyanins on lipid peroxidation was examined *in vitro* (using artificial membrane lipid bilayer model). Results showed that anthocyanins strongly inhibited lipid peroxidation by Fe<sup>2+</sup> ion, particularly, delphinidin demonstrates powerful inhibitory effect.

Hydrogen peroxide is the simplest peroxide with powerful oxidizing capacity, hence a highly reactive oxygen species. The effect of anthocyanins on hydrogen peroxide was examined on membrane lipids (using rat brain homogenate). Delphinidin exhibits strongest inhibitory effect on hydrogen peroxidation of membrane lipids with lowest ID50.

The antioxidant effects of *A. chilensis*, with its exceptionally high content of phenolics, have been studied in different cellular models. Maqui extract has been shown to protect both LDL from oxidation and endothelial cells from intracellular oxidative stress (6), suggesting that it could have anti-atherogenic properties (7), being atherosclerosis a possible consequence of oxidative stress on LDL cholesterol in the vascular wall. Oxidized LDL support foam cells formation and represent a potent inducer of inflammatory molecules, which leads to apoptosis of vascular endothelial cells, thus to progression of atherosclerosis.

The majority of *in vitro* and *in vivo* studies conducted so far have attributed the protective effect of bioactive polyphenols to their chemical reactivity toward free radicals and their capacity to prevent the oxidation of important intracellular components. However, observations from our and other laboratories, reveal a potential novel aspect in the mode of

action of polyphenols, that is the activation of Nrf2 transcription factor, and by this, the upregulation of inducible genes characterized by antioxidant responsive element (ARE) in their promoter region. Unprecedented data from our laboratory have shown that maqui berries extract Delphinol®, strongly induce hemoxygenase-1 (HO-1) expression and activity in endothelial cells via the activation of Nrf2/ pathway (unpublished data). Many studies clearly demonstrate that activation of Nrf2 target genes, and particularly HO-1, is strongly protective against inflammation, oxidative damage, and cell death.

Antioxidant activity has been also proposed as one of the possible mechanism of the strong neuroprotective activity of maqui anthocyanions, in hippocampal cultured neurons exposed to soluble oligomers of beta-amyloid 1-40 (8).

*In vivo* studies have also confirmed the ability of maqui berry to reduce oxidative stress in different tissues. Orally administered maqui berry extracts (MBE) suppress reactive oxygen species formation from lacrimal gland tissue, preserve and restore tear secretion capacity in dry eye. This effect is associated with the modulation of the lacrimal gland secretory system stimulated by MBE containing the anthocyanin delphinidin 3,5-O-diglucoside (9). We have recently investigated the effects of oral administration of Maqui Berry extract, titled in delphinidin, Delphinol®, on lipid peroxidation in healthy smokers subjects, aged 50-70 years (10). A randomized placebo-controlled, double-blind study (n=43) was conducted, during which anthocyanins from Maqui Berry (~300 mg/day) or placebo were orally administered to 43 healthy smokers

subjects once daily for 4 weeks. Basic biochemical and hematological parameters were determined throughout the trial. Oxidative damage to lipids was assessed by measuring plasma-circulating oxidized LDL (immunoenzymatic assay) urine total F2-isoprostanes (HPLC with tandem MS). Efficacy was defined as the change from baseline and after oral administration of berry anthocyanins, oxidative stress indicators in the supplemented group were better than in the placebo. Indeed, a statistically significant reduction in oxidised LDL and total F2-isoprostanes, was observed.

#### *Anti-inflammatory effect*

The anti-inflammatory effect of anthocyanins was evaluated using mouse macrophage cells (RAW 264.7). Upon addition of LPS (lipopolysaccharides, inflammation inducer) to macrophage cells RAW264.7, the expression of cyclo-oxygenase-2 (COX-2) markedly up-regulated in response to activation of inflammatory cascades. However, in sample treated with delphinidin, up-regulation of COX-2 is inhibited. Meanwhile, the expression of COX-1 is not affected indicating that delphinidin is a COX-2 selective anti-inflammatory agent. COX-1 is important in the healthy maintenance of physiological functions. Upon UVB-irradiation on the skin, inflammatory cascade is activated with up-regulation of COX-2 and release of pro-inflammatory prostaglandins E2 (PGE2) (11, 12).

Dichloromethane and methanol extracts, from both leaves and fruits of *Aristotelia chilensis*, show similar effects against 12-deoxyphorbol-13-decanoate (TPA)-induced inflammation (63.9 and 66.0%, respectively). On the other hand, aqueous extract show an high effect

(56.2%) against arachidonic acid induced inflammation, more than the reference drug nimesulide, reaching almost double the effect exhibit for hexane and dichloromethane extracts (30.0 and 31.5%, respectively). The topical anti-inflammatory effect of methanol extract (20%) is not significant. Tests carried out with a mixture of alkaloids extracted from the same plant allow to exclude the possibility that these are the cause of these effects (13, 14).

The topical anti-inflammatory effect in the TPA and arachidonic acid assays and the analgesic activity of dichloromethane extract may be partly caused by the mixture of the pentacyclic triterpenoids, ursolic acid and friedelin, with quercetin 5,3'-O-dimethyl ether. This flavonoid has greater anti-inflammatory activity than the positive control mefenamic acid. Reports suggest that the topical anti-inflammatory activity of plant extracts is due to the presence of these compounds, mostly to the high content of ursolic acid. Quercetin 3-O--D-glucoside and kaempferol in methanol extract may be responsible for the inhibition of both topical TPA-induced inflammation and analgesic activity. *In vivo* assays show that kaempferol, in particular, has a significant dose-dependent anti-inflammatory and analgesic activity. *Aristotelia chilensis* extracts proved to be more efficient in relieving pain than inflammation in all the pharmacological models in mice, more potent than the maximum effect of the reference drug naproxen sodium (54%).

Considering the good bioavailability and the human nutritional efficacy demonstrated in clinical studies (15), we should consider maqui extract, with high levels of

delphinidin, a promising helpful dietary complement to counteract alterations in cellular redox status, in several physiological and pathologic conditions, included skin ageing and photo carcinogenesis.

## DELPHINIDIN FOR FOR THE MAINTENANCE OF SKIN HEALTH.

Exposure of solar UV radiation, particularly its UVB component, to humans causes many adverse effects that include erythema, hyperpigmentation, hyperplasia, immune suppression, photaging, and skin cancer. UVB exposure to skin cells results in several types of DNA damage such as the formation of cyclobutane pyrimidine dimers (CPDs), pyrimidine (6-4) pyrimidone. These effects of UVB radiation resulting in damaged DNA can initiate photocarcinogenesis. Experimental and epidemiologic studies have suggested that polyphenols protect the skin from the adverse effects of UV radiation, and they have gained considerable attention as photochemopreventive agents for human use. Among the phytochemicals with antioxidant/anti-inflammatory potent activity, delphinidin deserves particular consideration. The low molecular size of delphinidin, the high bioavailability and the expected good skin tissue distribution makes this anthocyanidin a promising candidate for skin-ageing protection (16).

First evidences for delphinidin ability to protect skin cells from UVB-mediated, have been demonstrated by an in vitro model using cultured human keratinocytes (HaCaT cells) (17). This study have shown that pretreatment of cells with delphinidin inhibited UVB-mediated

apoptosis as determined by flow cytometry, confocal microscopy, and PARP cleavage. Delphinidin treatment of HaCaT cells prior to UVB irradiation resulted in a significant decrease in Bax with concomitant increase in Bcl-2 resulting in a shift in Bax/Bcl-2 ratio that does not favor apoptosis. Moreover, it has been found that topical application of delphinidin (both pretreatment and post-treatment) inhibited UVB-induced apoptosis in SKH-1 hairless mouse skin. Another study, conducted on both in vitro and in vivo model, has highlighted the strong antiinflammatory effects of delphinidin against UVB induced inflammation (18). Treatment of JB6 P++ mouse epidermal cells with delphinidin suppressed UVB induced COX-2 expression and PGE2 production, and this was also shown in vivo on mouse skin exposed to UVB. This activity has been associated with the suppression of AP-1 and NF- $\kappa$ B transcriptional activities, and the phosphorylation of JNKs, p38 and Akt. The study also revealed that delphinidin binds directly with MAPKK4 and PI3K in an adenosine triphosphate-competitive manner. In another study, delphinidin treatment significantly inhibited UVB-induced MMP-1 expression in primary cultured human dermal fibroblasts (HDF), and significantly inhibited UVB-induced ROS production and NOX activity (19).

Delphinidin has been also shown to induces differentiation in human epidermal keratinocytes both in submerged cultures and 3D EEs model resulting in increased expression in caspase-14 expression, a protease tightly regulated during keratinization and down-regulated in hyperproliferative skin disorders including psoriasis (20). In another study, appli-

cation of delphinidin to flaky skin mice abrogated the histological characteristics of psoriasisiform lesions and greatly reduced infiltration of inflammatory cells such as neutrophils and macrophages (21).

Accordingly with the previous evidences, the positive effect of maqui berry, containing high amounts of delphinidin, has been demonstrated. Maqui extract has been experimented in fibroblasts cells exposed to UVB-irradiation. Results showed that maqui effectively inhibit UVB-induced cell damage of fibroblasts cells. Furthermore, MMP-1 gene up-regulation by UV, is actively downregulated by maqui, that offered a protection the degradation of collagen (22).

These promising biological effects coupled with the relatively low cost and toxicity of natural agents makes maqui and delphinidin, promising agents for the treatment of photo-aging and also inflammatory skin disorders such as psoriasis (23).

## CONCLUSIONS

Growing evidence shows that nutrition through the intake of fruits and vegetable can contribute to prevent and to treat almost all diseases including early aging. Indeed fruits and vegetables contain several active molecules like anthocyanins that are able to dial directly with DNA by genetic and/or epigenetic mechanisms. On this background delphinidin from *maqui berry (Aristotelia chilensis)*, a super-fruit indigenous to Chile, showed powerful antioxidant and anti-inflammatory properties either in vitro or in vivo that can be very useful in aesthetic medicine and in dermatology.

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# Post-surgery necrosis of the nipple-areola complex treated with intensive oxygen-ozone therapy. A case report.

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## ABSTRACT

The partial or total necrosis of the nipple-areola complex (NAC) is a not rare complication of nipple-sparing mastectomy (NSM) after breast cancer surgical treatment. In order to repair the defects dressing changes, debridement, skin grafting or implants have been proposed. On this background “biologically active oxygen” administrated either locally or systemically as hyperbaric oxygen or oxygen-ozone therapy appears an attractive, safe and potentially useful approach to improve the results of conventional treatments. Indeed “biologically active oxygen” was shown to favour healing processes due to its ability to control infections and to stimulate regenerative processes. Based on our personal experience in the field as well as on the available scientific literature we describe herein a successful case report of NAC necrosis that occurred in a 50-years old women three days after NSM. We treated the patient with a combined, local and systemic, approach of oxygen-ozone therapy. The healing was completed after 16 weeks. The procedure was safe and well tolerated. Although some evident limitations the study supports the potentially usefulness of oxygen-ozone therapy in NAC necrosis treatment.

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## BACKGROUND

The nipple is one of the key defining visual features of a breast: the surgical removal of the nipple-areola complex (NAC) in a women due to radical mastectomy for cancer treatment causes the loss of the point in the profile at which the most natural convexity of breast

occurs thus rising psychological and relationship problems (1). In order to prevent such unwanted side effect without compromising the surgical intervention efficacy more than 30 years ago nipple-sparing mastectomy (NSM) with immediate breast reconstruction allowed replace conventional radical surgery for the treatment of selected patients suffering from

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## DISCLOSURES

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breast cancer (2) thus improving their satisfaction (3). Indeed preserving the NAC not only helps to prevent the above reported problems but also eliminates the need for staged nipple reconstruction and areola tattooing, after which there can be loss of projection and fading over time, respectively (4). However NSM still remains a difficult technique because after dissection, the remaining breast skin and nipple-areola complex (NAC) must be thin enough to be free of neoplastic tissue and thick enough to preserve tissue perfusion (5). Therefore one of the most common complications of NSM – accounting for 0 to 20% of all cases (6) – is the partial or total NAC necrosis, a condition that negatively affects cosmetic results (7, 8). Superficial skin loss can recover spontaneously but full-thickness skin loss can result in infection and eventual prosthesis loss (6). Obesity, cigarette smoking, incision type, flap thickness, and preoperative irradiation are the most common risk factors for loss of breast skin (9) while pre-operative vascular assessment by nuclear magnetic imaging can be helpful in the risk assessment (10).

Once NAC necrosis occurs after NSM, surgical procedures are often performed to repair the defects that can be resolved with dressing changes, debridement, skin grafting or implants (7, 11, 12).

In such context it is reasonable that – despite the absence of clear clinical evidence – an additional and safe procedure aimed to favour the regeneration of soft tissues can be of support to the conventional treatment of NAC necrosis as optimisation of cosmetic result and/or fastness of recovery.

Among such procedures are hyperbaric

oxygen therapy and oxygen-ozone therapy. This latter (as such or in the form of ozonised oil/water or other devices) due to its general safety, tolerability and efficacy in tissue repair and regeneration has been applied – although its exact biochemical mechanism is not completely clear (13) – either locally or systemically (intravenously e. g. as major ozonated autohemotherapy or by rectal insufflation) in breast diseases with different purposes (14, 15) thus mimicking some favourable previously demonstrated effects of hyperbaric oxygen therapy on NSM (16-18). On this basis we applied a specific protocol of oxygen-ozone therapy in a case of NAC necrosis.

## CASE PRESENTATION

In December 2014, a 50-year-old woman was admitted to our medical centre with a diagnosis of NAC necrosis following breast surgery. Three days before the patient had undergone surgery for breast augmentation. During the intervention, aimed by aesthetic purposes, the surgeon had found in the context of breast tissues a lipoma showing the size of a tangerine located into the inferior-medial quadrant of the left breast. He had decided right away to remove the mass. Following the appearance of NAC necrosis the surgeon decided to send the patient to our observation just three days after the surgical intervention. Our team confirmed the diagnosis of NAC necrosis. Indeed the interested area showed a blackish appearance and resulted insensitive to the needle pricking (Figure 1). The woman had not significant history for diseases but was on overweight and smoker. After collecting clin-

**FIGURE 1.** Nipple-areola complex necrosis. *The lesion occurred in a 50-years old women, after three days after nipple-sparing mastectomy. The interested area showed a blackish appearance and resulted insensitive to the needle pricking.*



ical history data we visited the patients who undergo to conventional chest radiography, electrocardiography, and common blood/urine laboratory analyses in order to exclude any possible contra-indication to the planned treatment. According to the pooled data the patient was considered as good candidate to our protocol of oxygen-ozone therapy.

### THERAPEUTIC APPROACH

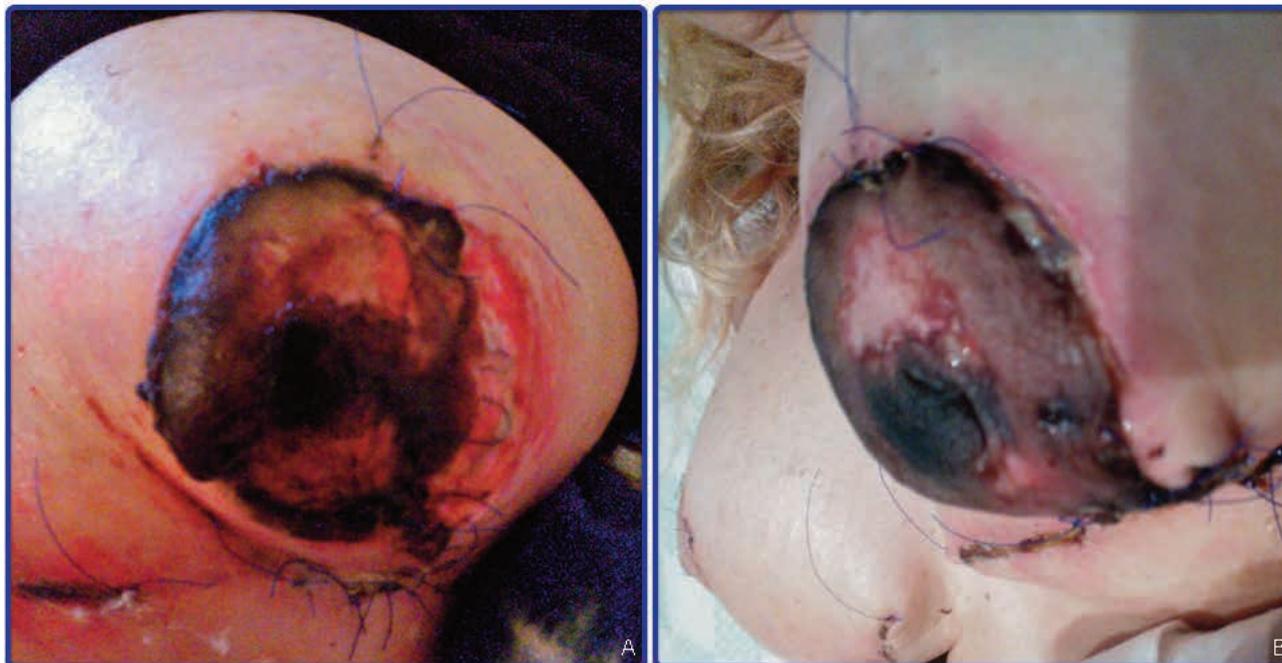
After obtained the informed consensus we performed on the patient to an original protocol based on the combination of local and systemic oxygen-ozone treatment (Medozon Compact, Herrmann Apparatebau, Elsenfeld, Germany). Local oxygen-ozone administration was done as mesotherapy by injecting 20 mL of a 10% oxygen-ozone mixture

in saline solution in the nipple's area. Then the wound was medicated daily with 10% iodopovidone (Betadine™, Meda Pharma S.p.A, Milan, Italy) and sterile gauze. Systemic oxygen-ozone was performed injecting intravenously 250 mL of a 30% oxygen-ozone mixture in saline solution (8 mL per minute). At the end of therapeutic seat an association of amoxicillin with clavulanic acid was prescribed daily (1 g three times a day) for 5 days in order to prevent any eventual bacterial complication. The protocol was repeated two times for week in the first six weeks and then once weekly in the next four weeks.

### RESULTS

In the period of treatment the healing process was slow but constant: the fourth ther-

**FIGURE 2.** Nipple-areola complex necrosis after local and systemic oxygen-ozone therapy. *Appearance of the lesion at the end of second week (A) and at the end of third week (B).*



**FIGURE 3.** Nipple-areola complex necrosis after local and systemic oxygen-ozone therapy. *Appearance of the lesion at the end of twelfth week (A) and at the end of sixteenth week (B).*



apeutic seat (i. e. at the end of second week) the nipple started to bleed and the patient experienced a partial recovery of the sensitivity to needle pricking (Figure 2). After 12 weeks the necrotic nipple was transformed to eschar and then released from breast tissues spontaneously thus exposing an apparently healthy skin showing a normal sensitivity (Figure 3). During the whole period of treatment the patient was cooperative, adhered to the protocol and did not experience any significant disturbance or complaint.

## DISCUSSION AND CONCLUSIONS

The NAC necrosis is a frequent complication NSM often causing complaints due to its impact on aesthetic and breast functions as well as on psychological/social domains of women (4-6). Often described after cancer therapy in our case the necrosis followed the excision of a lipoma that was surprisingly not detected during the preliminary ultrasound examination before the breast augmentation procedure. Maybe that a preliminary accurate assessment (NMR?) (10) as well as a ultrasound analysis could have revealed the lipoma before surgery thus allowing the surgeon to plan better the intervention and prevent such unwanted side effect. On the other hand the patient was at risk for NAC necrosis being either in overweight and smoker (6).

In order to avoid the undesired pathophysiological, psychological and social burden of NAC necrosis the surgeon must follow firstly the guidelines if available or the good practice in order to warrant a healthy, fast and safe anatomical and/or functional recovery of injured breast tissues. In our case we followed

the conventional protocol of treatment but recognising that the substance leakage did not require a skin graft we applied to the simple cleaning and disinfection of the wound under antibiotic systemic coverage an additional, local and systemic, protocol of oxygen-ozone therapy. Indeed ozone therapy showed great usefulness especially in the management of pain or infections, due to its immune-stimulating, antimicrobial and analgesic effects (19-22). Moreover either in animal models (23-25) or in human diseases, like diabetic foot (26, 27), including skin disorders (28) ozone was able to activate regenerative processes maybe by modulating cell structure and functions (29) through reactive oxidising species (13) thus favouring wound healing processes (30).

In our hand this protocol allowed to reach a satisfying result without any additional surgical intervention or drug treatment thus suggesting the ability of herein used gaseous mixture to work "physiologically". This was evidenced by the absence of any unwanted side effect.

This case report suggests that a locally and systemic combined oxygen-ozone therapy can be of support to the wound healing after NAC necrosis, a condition that complicates 4.8% of all NSM interventions in Italy (31).

However some limitations affected our work and in particular the impossibility to make a comparison of such treatment with other treatments; moreover the lack of both histological examination and oxidative stress biomarkers assessment (32) made almost impossible to establish the eventual mechanism underlying the observed effect; furthermore the patient did not report any eventual changes on lifestyle occurring during the treatment thus impeding

us to evaluate the possible impact of other variables on the results.

Despite such evident limitations this case report can be a possible base for future research aimed to find more physiological safe and fast treatment of NAC necrosis.

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# Rhinoplasty. Serdev mini-invasive techniques. T-Excision for Nasal Tip Rotation.

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## ABSTRACT

"T-excision" for nasal tip rotation is used to reduce long noses as an independent procedure or as a part of primary or secondary rhinoplasties. It consists of "en bloc" excision of the cephalic part of the greater alar cartilages and elongated caudal septum, using: 1. total retrocolumellar incision, prolonged in transcartilaginous incisions, through opposite nostrils, leaving the skin intact, without scars; 2. septal incision, perpendicular to dorsum to form correct dorsum length, prolonged into intercartilaginous incisions, through opposite nostrils, leaving only skin intact. Thus, the cephalic strip resection is done en bloc with the unnecessary excessive and prolonged septum. Two, three mattress transmucosal septo-columellar sutures for 2-3 weeks are enough to support healing. The T-excision technique is mini invasive, and time saving. It is safe, well tolerated by patients, there is no bruising, no pain after surgery, no need of plaster, tampons and bandages. Patients can return next day to social life and work.

**KEYWORDS:** Rhinoplasty; long nose; T-excision en bloc; mini-invasive technique; no downtime.

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## INTRODUCTION

Facial analysis is critical in rhinoplasty. This procedure is not an operation of a separated nose, it is artistic surgery to give aesthetic proportions and angles, as well as properly localized volumes as an aesthetic part of the entire face, which is the goal of beautification. Patient's age, sex, skin quality, ethnicity should be taken into consideration. Nasal tip position has great importance in all rhinoplasty procedures, especially in cases with a long disproportionate nose. Cephalic

strip resection of the lower lateral cartilages is performed to achieve upwards tip rotation. The "en bloc" T-excision technique for adjustment of the nasal tip involves a new understanding of well-known incisions based on anatomical awareness. It minimizes trauma, is nearly bloodless, achieves an acceptable beautifying post-operative result with no downtime for the patient, requiring no plaster, no tampons and nearly immediate return to work and social life. This technique prevents cartilages from iatro-

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genic trauma and devascularization thus permitting faster healing with a stable result. It includes cephalic strip resection of lower lateral cartilages and septal shortening (caudal septum and/or retrocolumellar mucosal elongation) en bloc.

## ANATOMY

The greater alar cartilages (lower lateral cartilages) are situated below the lateral nasal cartilages, forming the columella and the wings of the nostrils. The medial crura are loosely connected to the corresponding portion of the opposing cartilage. Together with the septum they stabilize the columella. In Caucasians the columella is stable, unlike Asians, Afro-Americans and Latino-Americans whose cartilages are thin and do not give adequate support to

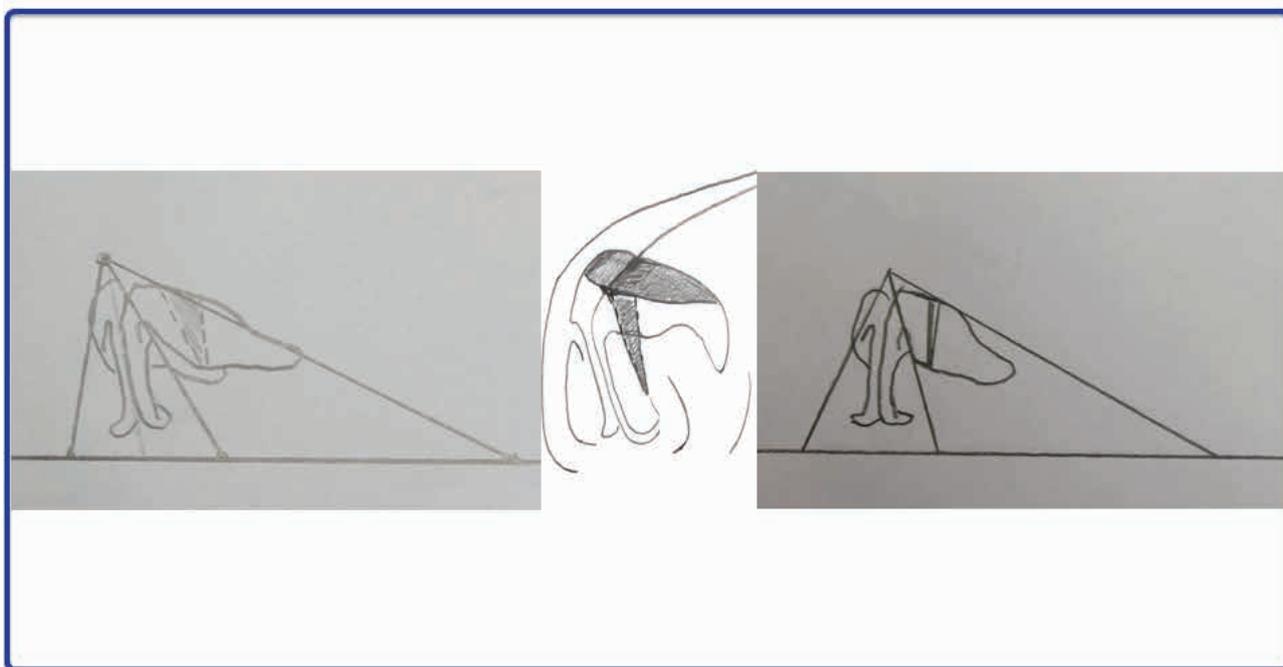
the tip.

The author's observation is that regardless of race, the proper dorso-columellar angle (tip angle) is very close to 90°. Angles different from the right angle change the aesthetic proportions and create an imbalance of the beauty triangle (1-6).

## TIP ROTATION

Using the tripod concept (Figure 1) (7), a long nose has a longer superior leg (septum) including the lateral crura of the greater alar cartilages. Thus the "en bloc" shortening of the elongated distal septum and the lateral crura of the lower lateral cartilages (cephalic strip resection) gives upward rotation to the tip. NB. Projection of the nasal tip is described by the author elsewhere.

**FIGURE 1.** Tripod concept (see reference 7) used for shortening of the dorsal length of the nose via "en bloc" T-excision of elongated distal septum and cephalic part of lower alar cartilages (see also Figures 3, 4 and 7).



## PATIENT CONSENT

If the tip angle is correct, the nostrils in the en-face aspect are slightly visible. Usually, patients with long noses who have never seen their nostrils, have difficulty in accepting that nostrils should be a bit visible in frontal view. It should be clearly explained that in order for the nostrils to be invisible, the tip angle (dorso-columellar angle) should be sharp (about 70°), which is not appropriate and the nose looks long in relation to the face (Figure 2). Patients should be informed, assured and motivated for this change.

## DESIGN OF THE T-EXCISION TECHNIQUE

Excision of the cephalic part of the

greater alar cartilages, including an unnecessary prominent caudal part of the septum, permits rotation of the tip i.e. shortening the length of the nose (Figure 3 and 4).

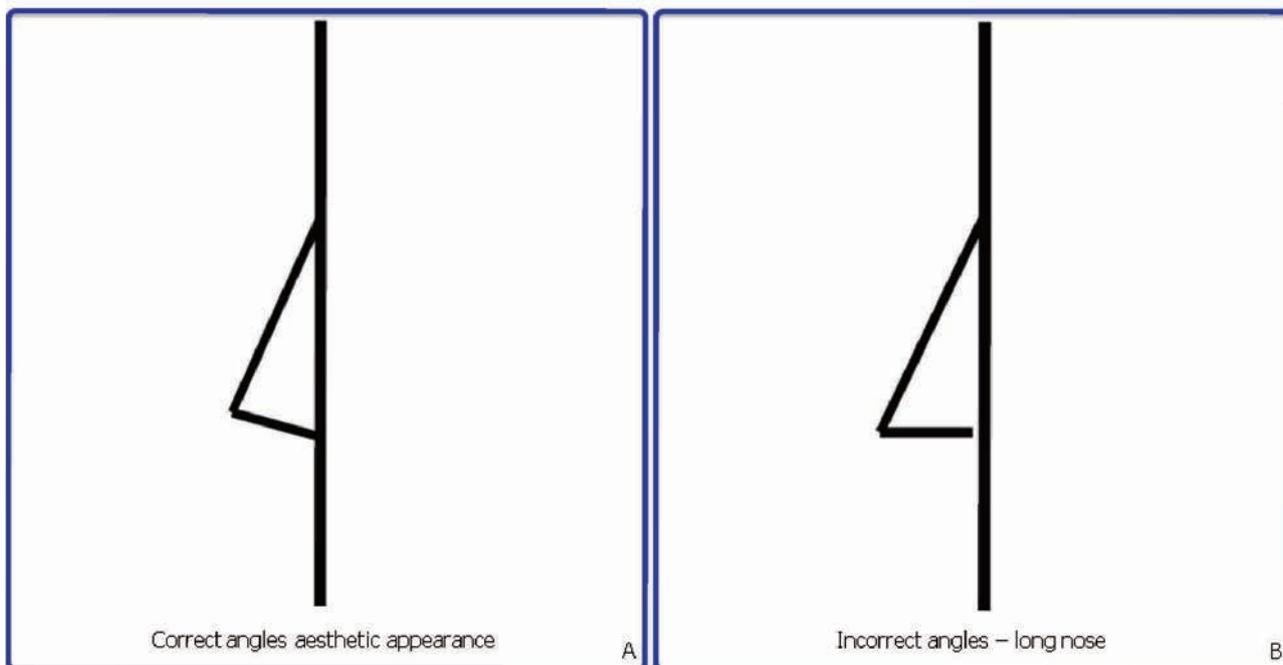
## T-EXCISION: SURGICAL TECHNIQUE

The below described T-excision technique is made en bloc (Figure 5), using a closed rhinoplasty approach. The local anesthesia of the tip, greater alar cartilages and columella, should not deform the nasal tip.

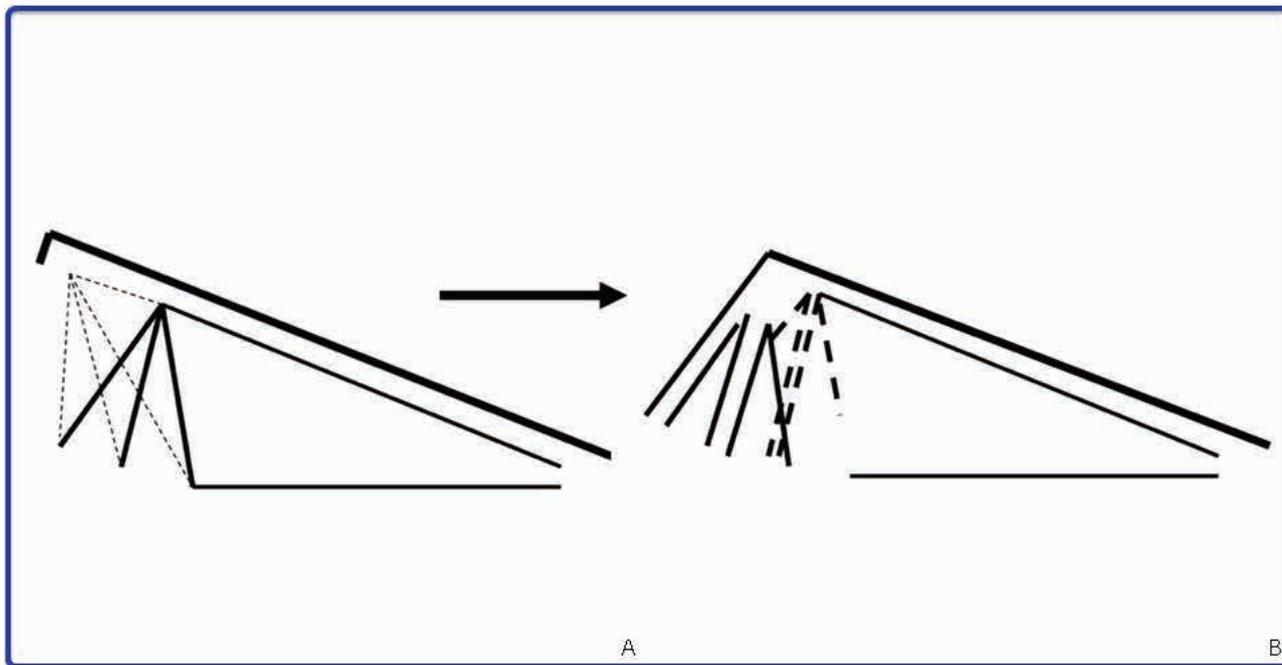
### *First incision*

A total retrocolumellar incision is performed to separate the columella from the septum. In cases of dropping columella this

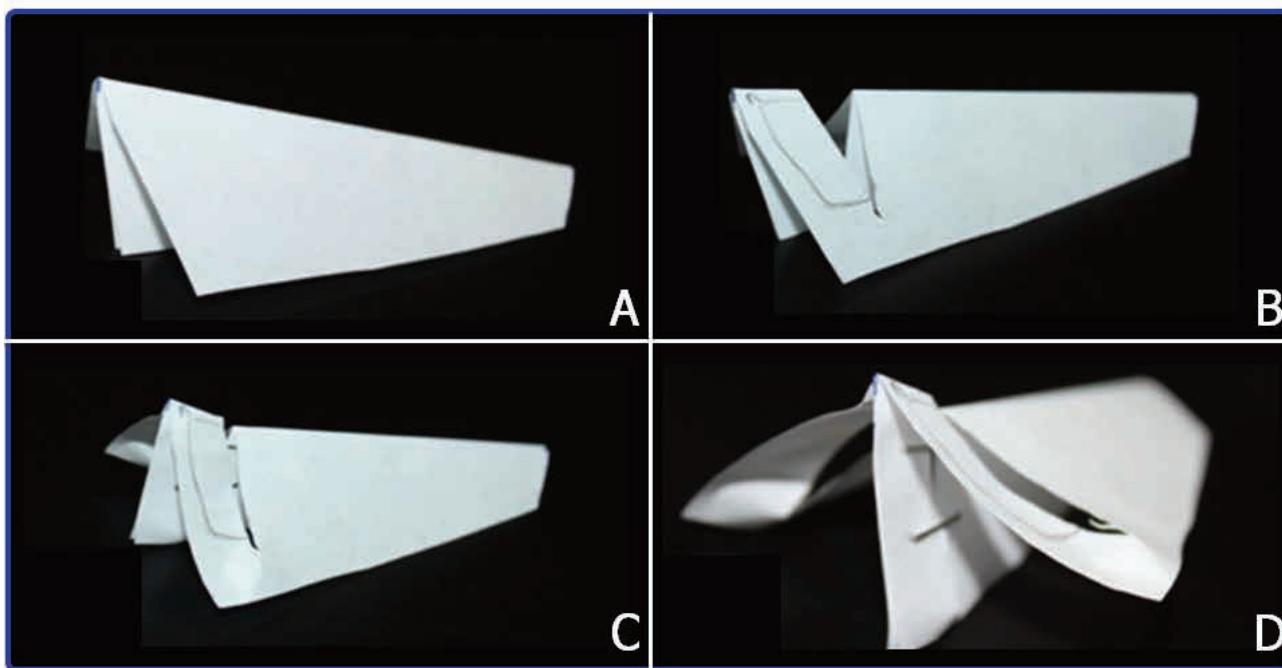
**FIGURE 2.** A. Correct aesthetic proportions and angles. The nose is proportional – 1/3 of the face. Correct 30° dorso-profile angle and nasal tip angles. Nostrils should be a bit visible from a frontal view. B. Visibly incorrect length, angles and lack of aesthetic section of nose and face. The nostrils are not visible from a frontal view (long nose).



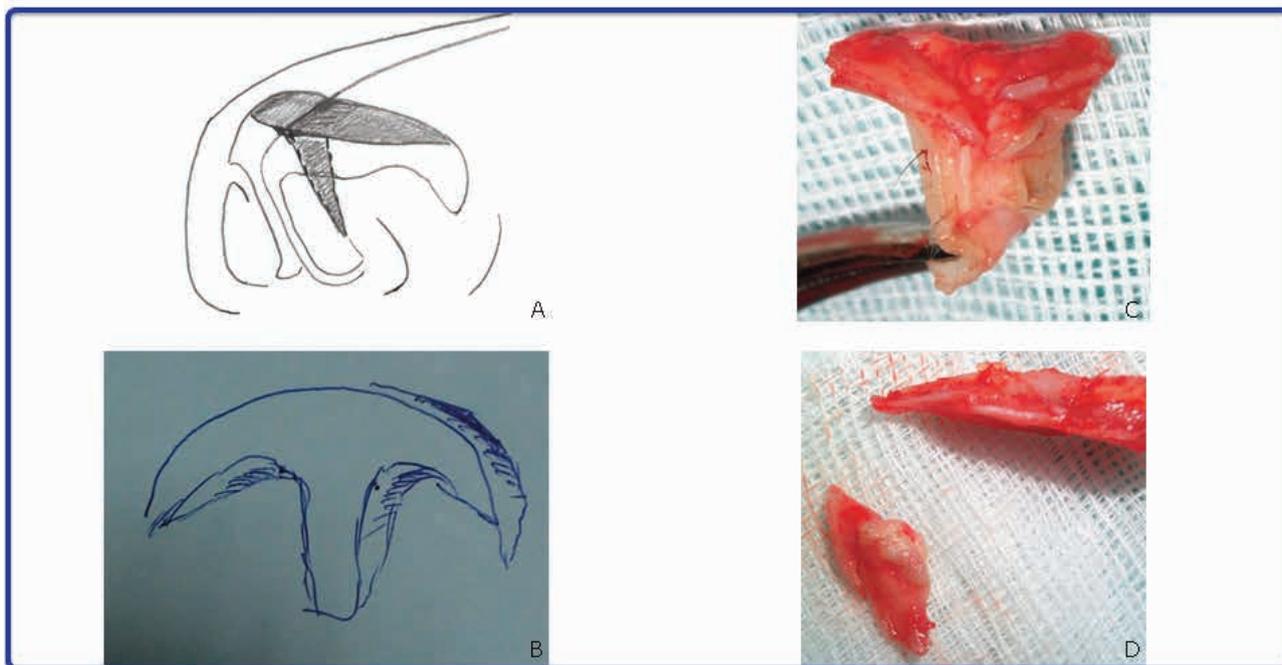
**FIGURE 3.** T-excision drawing. A. Schematic excision of 3 triangles - 2 lateral triangle excisions and one medial triangle excision perpendicular to the nasal dorsum. B. Result after tip rotation gives correct tip position and angles.



**FIGURE 4.** A. Schematic pyramid in a long nose. B. T-excision en bloc, including cephalic part of the greater alar cartilages and elongated caudal septum. C. Tip of the nose rotates easily. D. Two to three transmucosal mattress sutures of columella to caudal septum are enough to hold the tip in position and guarantee good fixation for healing. Stitches are removed after 2-3 weeks.



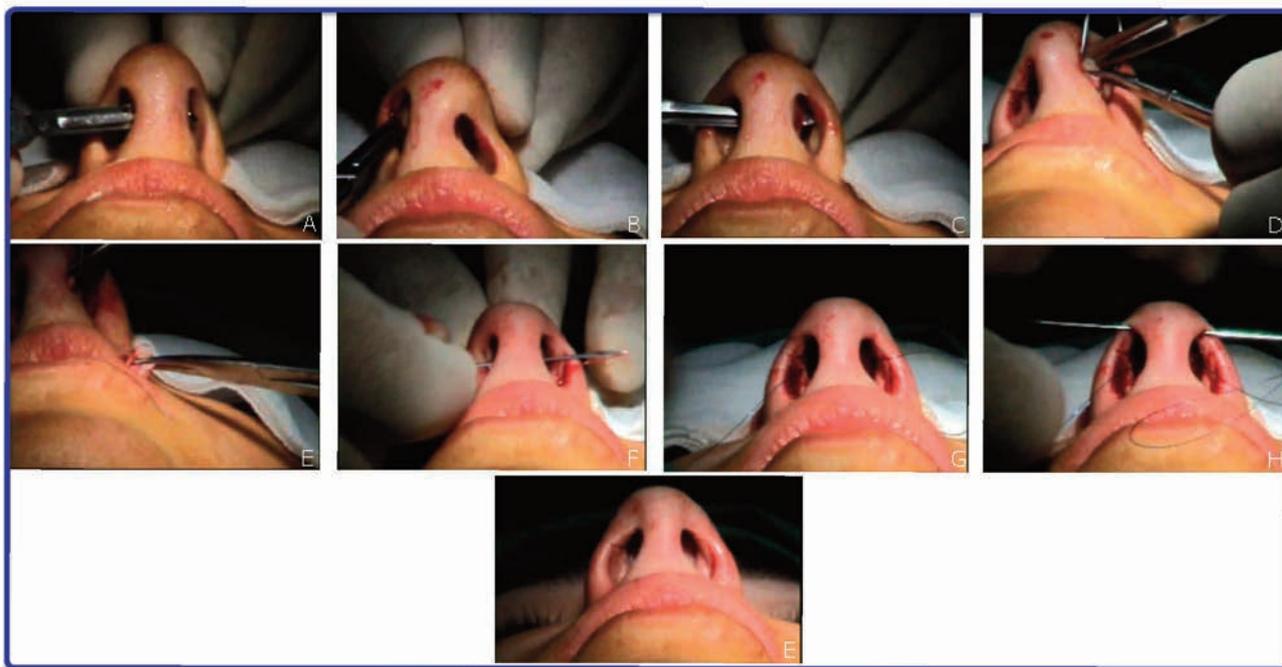
**FIGURE 5.** T-excision en bloc of 3 triangles. A. Transcartilaginous and intercartilaginous incisions form the 2 lateral triangles of the T-excision. The retrocolumnellar and the “90o-to-dorsum” septal incision form the medial triangle of caudal septum excision. The 2 lateral triangles should include the cephalic strip of greater alar cartilages. B. Scheme of excised tissue. C. Excised T-formation en bloc. This particular T-excision en bloc includes caudal septum in the medial triangle and the cephalic part of the greater alar cartilages in the lateral triangles. D. Humpectomy and T-excision will give the new profile to the nose.



incision should follow a desired design. To remove dropping columella, the incision should leave equal thicknesses along the length of the columella. Any other form should be previously designed according to the patient's desire and informed consent. The retro-columellar incision is then extended to the transcartilaginous incision (8), which separates the lateral wing of the greater alar cartilage in cephalic and distal part. In the past, the author used methylene blue dye on the skin to mark the transcartilaginous incision, but it is not always easy to precisely reflect the line that has been drawn on the external skin. This is not entirely necessary, because the transcartilaginous incision is an extension of the retrocolumnellar incision in each nostril, parallel to the nostril border. The tran-

scartilaginous incision is made in each nostril through the opposite nostril, using the opening of the retrocolumnellar incision – this gives better visibility to the surgeon and permits for better orientation. This incision cuts mucosa and cartilages, leaving the skin intact. To be precise, both alae nasi are held with the thumb and index of the opposite hand, feeling the scalpel below the skin with the fingertips (Figures 6 A and B). Transcartilaginous incisions should be located 4-5 mm cephalic to the caudal margin of the lateral crus of the lower cartilages. Finishing both transcartilaginous incisions and leaving only the skin intact, one has separated the lateral wings of the greater alar cartilage into cephalic and distal parts, whereupon the cephalic parts will be removed with the T-excision en bloc.

**FIGURE 6.** A. Total retrocolumellar incision. B. The retrocolumellar incision is extended to the intercartilaginous incisions on both sides through the opposite nostril, using the opening of the retrocolumellar incision. C. Second septal perpendicular to dorsum incision. It will be prolonged into 2 intercartilaginous incisions. D. The T-Excision en bloc is fixed with a mosquito clamp and separated from the dorsal skin with a blunt-tip scissor. E. The T-excision is separated and removed, F. The trans mucosal mattress suture is performed horizontally if the dome is symmetric (or parallel to the asymmetry). G. The trans mucosal septo-columellar suture is ready to be knotted. H. Transmucosal domal suture of medial crura for tip refinement. I. Result after atraumatic, nearly bloodless T-excision procedure for nasal tip rotation.

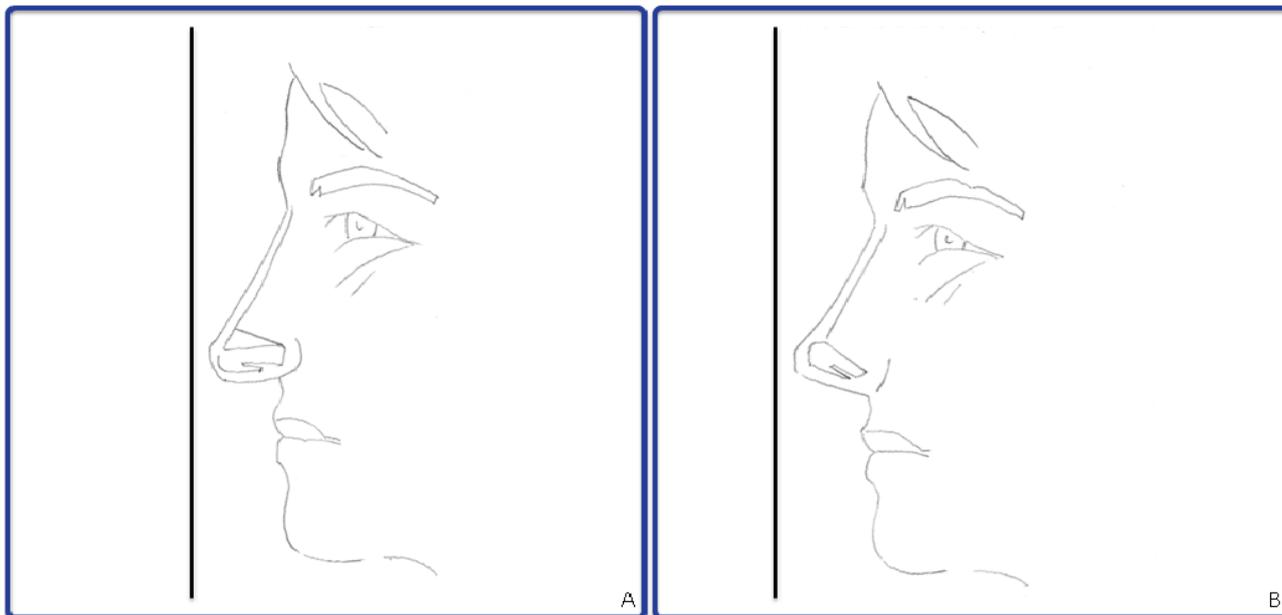


### *Second incision*

The reduction of the length of the nose in the caudal septum region is selective. The second incision line is a “90o-to-dorsum” septal incision, starting from a selected dorsum point in a downward direction, perpendicular to the nasal dorsum to meet the retrocolumellar incision (forming the medial excision triangle), which usually occurs above the nasal spine. This incision is complete, including caudal septum and is then extended into the intercartilaginous incisions in both nostrils, each one through the opposite nostril using the opening of the “90o-to-dorsum” septal incision. The in-

tercartilaginous incision should be placed a minimum of 2 mm caudal to the valve on the lateral crura side in order to prevent nasal valve stenosis. The intercartilaginous incision in this technique leaves only the skin under the fingertips of the guiding hand intact, as described above (Figures 6 A and B). Intercartilaginous incisions meet the transcartilaginous incisions laterally, forming the 2 lateral triangles of the T-excision. Thus, cephalic parts of the greater alar cartilages are separated together with the unnecessary elongated septum (or in some cases only the mucosa), forming 3 triangles of the “T-excision en bloc”: two lateral triangles in the nostrils and one medial triangle in the septal

**FIGURE 7.** A. The superfluous length of septum will be resected. B. The nasal tip is rotated and a correct tip angle is obtained, adapting the nose into 1/3 of the length of the face.



retrocolumellar portion. The tissue of the “T-excision en bloc” is still fixed to the alar skin from which it will be separated and removed by using blunt-tip scissors, guided by the opposite hand to prevent trauma to the alar skin (Figure 6 D). The surrounding skin is slightly undermined with the scissors 2-3 mm to permit rotation of the nasal tip and skin adaptation. For more details see figures 5, 6 and 7 and related legends.

#### *In cases of over-rotation or short upper lip*

If the upper lip is shortened by a septum, which is too long, it is necessary to shorten the entire pyramid of the nose, the prominent posterior septal angle can be excised, together with the prominence of the anterior nasal spine. This maneuver deepens the nasolabial angle. It elongates the upper lip and can also correct an over-rotated nasal tip.

#### *Post-surgery*

Immediately after the operation, local anesthesia and postoperative edema at the tip and nasolabial angle raise the dorsum and make the nasolabial angle obtuse, which gives an impression of over-rotation of the nasal tip, it is a false impression. As the edema subsides during the first 5 to 7 days, the correct angle takes shape and the tip falls into place. There is no bruising post-op.

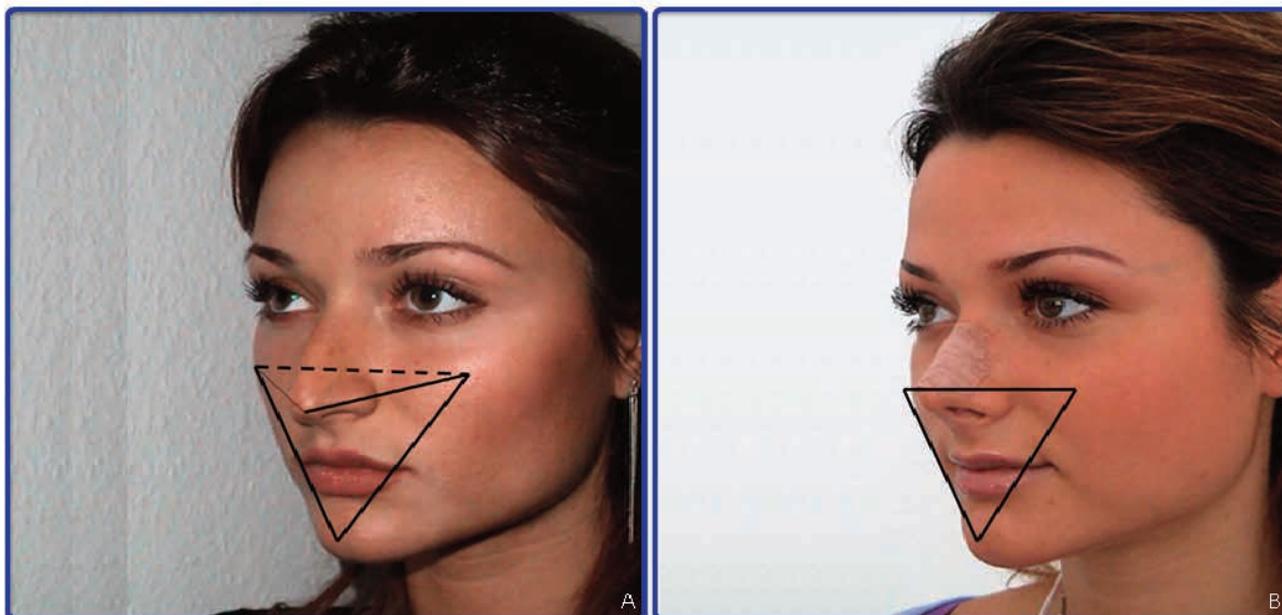
#### **INDICATIONS**

The technique is indicated for long noses.

#### **CONTRAINDICATIONS**

The contraindications are secondary rhinoplasties in cases of malformed greater alar cartilages.

**FIGURE 8.** A. A case of a long nose. Before. The long nose causes incorrect facial proportions. The beauty triangle is disrupted, forming 2 incorrect triangles. The facial features of the patient's face are nice but nearly invisible because of the long and disproportional nose. B. After T-excision for nasal tip rotation and columella sliding for tip projection. The nose is shortened to fit into one third of the face. Correct aesthetic proportions (three equal parts of the face), correct 300 dorso-profile angle and nasal tip volume on the line of the cheekbone prominence. Tape is not necessary – it was requested by the patient (a ballerina) to make the operation visible and thus protect her from trauma at work. The result is beautification of the face by establishing correct proportions, angles, volumes and visible beauty triangle.



T-excision could be used as a stand-alone procedure in long noses, or as a part of a rhinoplasty with hump removal and other additional techniques. The operation is ambulatory, under local anesthesia. The author uses additional IV sedation. The procedure is virtually bloodless and atraumatic. Two to three transmucosal mattress sutures are used to fix the columella to the septum. Stitches, if not absorbed, are removed after 2-3 weeks. There is no need for any bandages or tampons. Patients return to their social life almost immediately.

In aesthetics, there is another important aspect of the T excision – the “beauty triangle”, forming the mid and lower face beauty. It includes the two cheekbones and the chin.

The tip of the nose should not disrupt the upper line of the triangle connecting the projection of the two cheekbones, i.e. its prominence has to be on the line between the two cheekbones. Thus, the nasal tip presents an important aesthetic facial volume, forming a straight line together with the volume of the cheekbones (Figure 8).

## CLINICAL CASES

A series of clinical cases of long nose, long and disproportional nose, small chin (retrognathia), improper ratio in the lower part of the face, aquiline long nose and disproportionally long nose, respectively, is reported and commented in figures 9 to 12.

**FIGURE 9.** T-excision for nasal tip rotation. Immediate result in a case of a long and disproportional nose. A. Before. Long nose with hanging columella, resulting in a disproportional face, containing some nice features, nearly invisible to observers; B. After. Immediate result, a few minutes after T-excision, nasal tip and lower third refinement by transcutaneous Serdev Sutures®. Swelling could be visible to specialists but not to the general public. Aesthetic proportions (three equal parts of the face) are present. The result is beautification of the face – previously invisible beauty is now demonstrated.



**FIGURE 10.** Immediate result (Braunol disinfection is still not totally cleaned) after nasal tip rotation by T-excision and chin enhancement by Serdev Suture® in a case of a long nose, small chin (retrognathia), and improper ratio in the lower part of the face (between upper lip and chin); A. Before. The upper face is nice, but the nose is long, the chin is small and disproportional. Straight line of the noble profile is missing. Due to short mandible and reduced skeleton support, surrounding and submandibular tissue is hanging; B. After. Immediate result after T-excision for nasal tip rotation, columella sliding for tip projection and chin augmentation collecting her own tissue using Serdev Suture® volumising method. Correct volumes are visible – nasal tip on the line of the cheekbone prominence. The result includes: proper aesthetic proportions of the face (three equal parts of the face; chin to upper lip length in correct proportion 2:1); tip is correctly rotated and the nose is proportional. The tip is projected to conform to the proper 300 dorso-profile angle; the chin is augmented using her own collected tissue, without and extraneous materials; jaw line and submandibular line are stretched; straight noble profile is present. The immediate result exalts the beauty of the face.



**FIGURE 11.** A. Aquiline long nose, short upper lip and prognatic jaw. Before. B. One year after T-excision for nasal tip rotation, humpectomy, digital fracture instead of lateral osteotomy, prominent posterior septal angle and nasal spine resection for upper lip elongation, upper lip volumising by Serdev Suture® to bring it to the line of the straight profile. Changes include: proper angles of the nose, it occupies one third of the face, correct aesthetic proportions of the face (three equal parts; chin to upper lip length in correct proportion 2:1 instead of 3:1 ratio before); the upper lip is elongated and brought forward (thus, the prognatic jaw is included in the correct proportions instead of mandibular remodeling resection); a straight noble profile is present. The result is beautification of the face.



**FIGURE 12.** A disproportionally long nose is shortened by tip rotation via T-excision to obtain 3 equal parts of the face with correct angles of the nose. The dropping columella is corrected using a retrocolumelar incision parallel to the columella (see first incision).



## CONCLUSIONS

Beautification is a work of art. Rhinoplasty, including shortening of a long nose aims at obtaining exact aesthetic proportions, volumes and angles of the face. The nose cannot be isolated aesthetically. T-excision en bloc, including cephalic strip and elongated caudal septum resection can rotate the nasal tip to obtain correct proportions of the face. The procedure takes very little time, even less than an injection rhinoplasty.

The procedure is atraumatic, virtually bloodless, does not require plaster fixation, tampons and downtime and the results are permanent. Patients return to work and social life almost immediately. There is no bruising and edema is not visible to untrained personnel. Swelling can minimally change the tip position but this only lasts during the first 5-7 days. After that it falls into a correct and natural position. T-excision is the shortest rhinoplasty procedure for correcting long noses and dropping columella, with the most stable and permanent results, due to minimal or complete lack of trauma to the greater alar cartilages and surrounding tissue.

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