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The skin as the largest organ of the body mediates a continuous flow of information and metabolites where reactive processes like inflammation and oxidative stress play a relevant role. Therefore any disturbance of such adaptive biological mechanisms may accelerate aging and favour the onset of many skin disorders, from common wrinkles to cancer.

Fortunately the development of molecular medicine allows identify very early at cellular level otherwise unperceivable changes of reactive processes thus putting the basis for an integrative approach to skin diseases.

In this context nutraceuticals, that include dietary supplements or fortified foods showing health benefits in addition to their basic nutritional value, are increasingly prescribed alone or as support to conventional skin treatment, sometime by combining the topical application to the oral route.

Taking in account these general concepts, the present issue of EJAMED is completely dedicated to the potential usefulness of nutraceuticals in skin care. The first review-article, made by the Editor in Chief, develops and updates the notions of a previous Editorial [EJAMED. **2016**. 6 (1): 9-47], with the aim to provide to the clinicians a easy-to-use information about the management of supplements in Aesthetics and Dermatology. The remaining articles are focused on specific classes of supplements, including polyphenols (Scapagnini and Coll.), Pychnogenol™ (Passwater and Coll.), omega-3 fatty acids for atopic dermatitis treatment (Selli) and most common micro-nutrients (Romano).

This issue is also a special issue because it hosts in addition to the above articles also the Proceedings of the International Conference SOLGAR MASTERCLASS 2017 – *Beauty and Bendessere* - (Padua, Italy, 2017, 30th April). Most of the Authors of the above reported articles are also speakers of this event and we would like to thank them because they made possible this special issue: *Vittorino Andreoli, MD, PhD, Member of the New York Academy of Sciences, Giovanni Scapagnini,* Department of Medicine and Health Sciences, School of Medicine, University of Molise, Campobasso, Italy, *Immaculata De Vivo, BD*, *PhD*, Harvard Medical School, Harvard T.H. Chan School of Public Health, Boston (MA, USA), *Richard Passwater, PhD*, past-director Solgar Nutritional Research Centre, *Peter Rohdewald, PhD*, Institute of Pharmaceutical Chemistry, University of Münster, Germany, *Arrigo Selli, MD, Maria Concetta Romano, MD, Ivo Bianchi, MD*, *Filippo Ongaro, MD*, *Emanuele Bartoletti, MD, Attilio F. Speciani, MD*, *Lucia Bacciottini*, *PhD in Applied Pathophysiology*, Biologist Nutritionist, *Pierluigi Gargiulo, MD*, *Mario Vignoni, MD, Gianluca Pazzaglia. MD.*

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Skin, oxidative stress, and nutraceuticals.
From the basic research to the clinical practice.

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ABSTRACT

The skin being the largest organ of the integumentary systems shows a very active redox system which main role is to modulate not only energy metabolism (two-electron transfer reactions) but also cell signalling and defence (one-electron transfer reactions). Exogenous and/or endogenous factors can impair such system leading to a condition of oxidative distress. This latter can be considered as an emerging health risk factor which detection is possible only by means of specific test that have been recently included in the novel Redoxomics. Lifestyle plays a relevant role in the prevention and in the treatment of oxidative distress that can take advantage of physiological redox modulators under medical control. This review is an update of a previous paper that has been published one years ago on this Journal [EJAMED. **2016.** 6 (1): 9-47].

KEYWORDS: Oxidative stress, reactive oxidant species (ROS), skin aging, nutraceuticals, redoxomics.

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INTRODUCTION

The skin provides one of the widest interfaces between the body and its surrounding environment (1). As a whole, it works as mechanical barrier against traumas and contribute to the regulation of body temperature as well as water and electrolyte homeostasis (1). At a cellular and molecular level, due to their strategic position as well as features of a perm-selective membrane, its layers – from the epidermis to dermis – mediate an intense, continue or pulsed and bidirectional trafficking of signals and metabolites, where the recently recognised microbiota seems to play a relevant role (2–4). Additional protective/detoxifying/nutritional functions are ascribable to the cutaneous annexes like nails, hairs and exocrine glands, respectively, among which are mammary glands. Finally, the skin is essential in the UV-mediated production of cholecalciferol, i. e. the precursor of the pro-hormone/vitamin D (1,25-dihydrocholecalciferol), from 7,8-dehydrocholesterol, as well as in steroidogenesis (5, 6).

By a structural point of view, the skin is an organ that results from the ordinate organisation of all 4 known tissues i. e. epithelial (epidermis), connective (dermis), muscular (mimic facial and hairy muscles) and nervous (nerve endings), that are responsible of protective/secretive, trophic/mechanical, contractile and sensitive functions, respectively (1). Beyond this the neural cristae-deriving melanocytes protect the body against UV radiation (7). Moreover the extracellular matrix of derma hosts in its highly hydrophilic glucan-proteic three-dimensional network blood and lymphatic vessels, as well as inflammatory and immune cells, that are involved in the nutrition, osmosis and reactive processes (e. g. inflammation), respectively (1, 3, 8).

All above described skin functions are under the control of the so-called psycho-neuro-endocrine-immune system (PNEIS) that warrants the management of any internal as well as external stimuli or stressors in order to maintain the homeostasis of skin as a

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whole and therefore of body (2, 3).

Therefore any physical, chemical or biological, either internal or internal factor able to impair skin structure and/or function may results in a systemic damage (e. g. early aging, invasive cancers) and vice-versa, i. e. any impairment of internal organs can lead theoretically to skin manifestations (e. g. psoriatic arthritis).

On this background, experimental studies and clinical trials made in the last decades – accounting for more than 4,000 academic papers, according to the data base PUBMED – have been identified the skin as one of the most active organs in oxidative-reductive processes. Such knowledge is leading now to identify the molecular basis of most skin cells functioning thus opening new frontiers in skin diseases and skin-related systemic disorders diagnosis, prevention and treatment, where the modern Nutraceutics is candidate to play an increasing role.

Taking in account these concepts, the aim of this review is to provide an update on the oxidative-reactive processes taking place in the skin with a focus on the so-called oxidative stress, its impact on skin diseases, its diagnosis, treatment and prevention.

FROM REDOX REACTIONS TO REACTIVE SPECIES AND ANTIOXIDANTS

Oxidation and reduction are opposite but reciprocal ubiquitous reactions that provide the chemical bases of most biological processes (9). In such reactions – often called redox reactions – one or more reducing equivalent units (i. e. an electron, alone or bound to a proton as an hydrogen atom) are transferred from a "reducing" to an "oxidising" species (9) (*Figure 1*).

The transfer of couples of reducing equivalents (more simply hereinafter referred to as "electrons") is related essentially to the cell energy metabolism (10) (*Figure 2, left side*). Indeed when the ratio $NAD^+/NADPH+H^+$ and/or ADP/ATP increases, specific cell enzymes called dehydrogenase, start to transfer – through specific pathways (mainly glycolysis, b-oxidation and Krebs cycle) – couples of electrons from their substrates (e. g. carbohydrates, fatty acids and amino acids) to the molecular oxygen in order to produce ATP and decrease the ratio $NAD^+/NADPH+H^+$ and/or AD/ATP (oxidation or oxidative phosphorylation) (10). In other words "dehydrogenation" or its synonymous "oxidation" are hallmarks of catabolic processes. When the above ratio exceed a certain critical threshold – e. g. because a hypercaloric diet – the excess of glucose is shunted towards the pentose phosphate cycle where the key enzyme NADPH-dehydrogenase allows to ac-

cumulate the cell "reducing power" as $NADPH+H^+$; the couples of electrons of such coenzyme are then transferred to the exceeding molecules of acetyl-coenzyme A (reduction) thus generating saturated fatty acids and cholesterol (10). Therefore "hydrogenation" or its synonymous "reduction" are hallmarks of anabolic processes (10).

The transfer of an electron alone is related to the metabolism of the so-called reactive oxidant species (ROS) (9, 11) (*Figure 2, right side*). These latter include either radical or non-radical species. A radical specie (suffix "-yl", e. g. hydroxyl) is any alone or grouped atom showing in at least one of its external orbitals one unpaired instead of a coupled electron; in the chemical formula the unpaired electron is indicated by a little dot in exponent position (9, 11). A non-radical specie shows all paired electrons. In both cases the adjectives "reactive" and "oxidant" emphasise a kind of low energetic "inertia" of such species that push them, in opportune conditions of pH and ionic strength, to stabilise themselves only by an oxidation i. e. only by extracting an electron to another chemical specie, being a condition of paired electrons associated to the lowest potential energy (9, 11). Biologically active ROS include a wide range reactive species that can be "centred" not only on the oxygen (reactive oxygen species, properly) but also on other elements like nitrogen, carbon, sulphur and halogens (mainly chlorine) (9, 11). Interestingly on the basis of the above reported definition of redox reaction the species that lose the electron behave as "reducing" species, most of which in biological systems are improperly called "antioxidant" species (*see below*).

BIOLOGY AND PHYSIOLOGY OF THE REDOX SYSTEM

In living organisms the redox system includes the reactive oxidant and reducing species/antioxidants as well as the enzymes that belong to the class of oxidoreductases (9, 10).

Reactive oxidant species can be produced inside or outside the cells with or without a catalyst (9, 11–13).

Paradigmatic examples of non-catalytic generation of ROS are provided by the homolytic breakdown where the administration of energy breaks a covalent bond of the target molecule that is split in two distinct radical species; in particular if such energy derives from a radiant source the breakdown is called photolysis one example of which is done just in the skin by the UV-induced breakdown of a water molecule into an hydroxyl radical and an hydrogen atom (9).

Catalysts able to generate ROS can be either transitional metals or enzymes. Transitional metals like

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.*
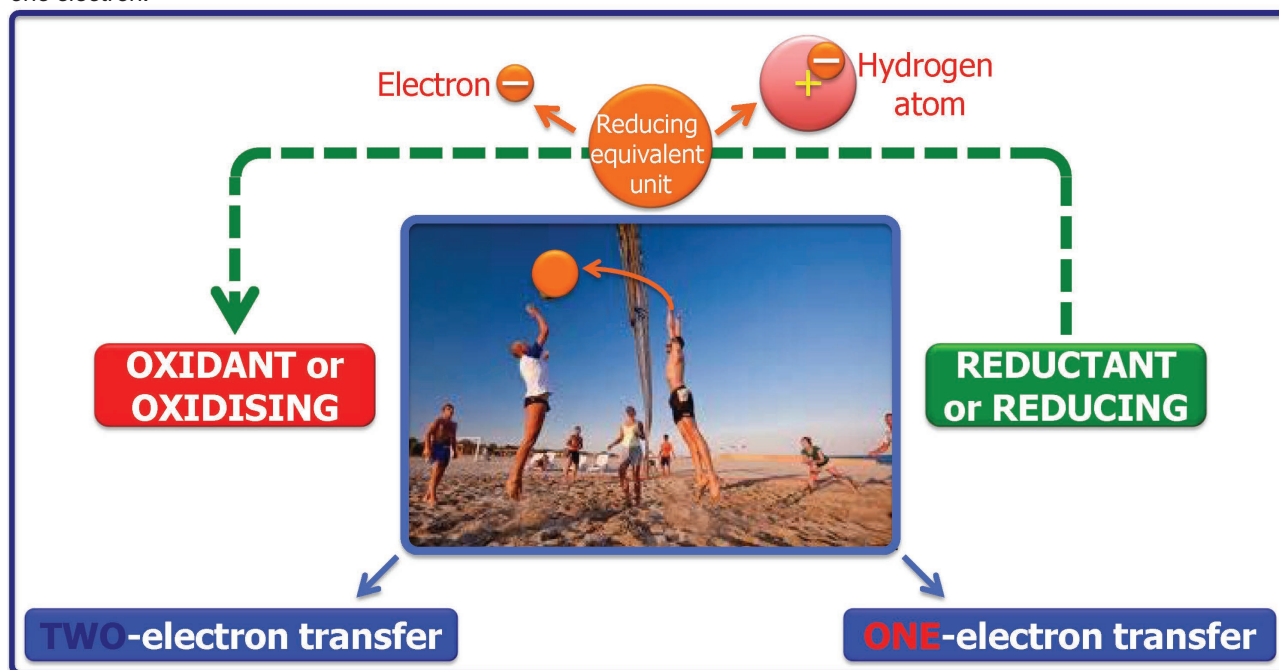
iron (Fe^{2+}/Fe^{3+}) or copper (Cu^+/Cu^{2+}) catalyse the breakdown of peroxides (R–O–O–R) into alkoxyl (RO·) and peroxyl (R–O–O·) radicals, thus providing a well-known example of Fenton's reaction (9, 14).

The enzymes physiologically involved in ROS generation can be located on the plasmamembrane (e. g. NADPH oxidase), into the mitochondria (e. g. respiratory chain), in the endoplasmic reticulum/microsomes (e.g. cytochrome P_{450}), into the peroxisomes (e. g. flavin oxidase) and in the cytosol (e. g. xanthine oxidase) (9, 11). Other reactions which generates free radicals by enzymatic way are described in the biosynthesis of catecholamines (15). By this point of view ROS are almost "forced" intermediates of cell metabolism. And since their production is close related to life, rightly ROS has been defined as "almost irreplaceable journey companions" to living cells.

Once produced ROS can oxidise transiently and reversibly or definitively and irreversibly different targets with diversified biological effects that depend on a lot of variables including i) the nature, the concentration and the oxidant potential of each component od the redox couple; ii) the physical/chemical/biological environment where the redox reactions take place (e. g. temperature, pH, cytosol, membrane, and so on). An example of transient and reversible effect of ROS is done by the oxidation of thiol groups (-SH) belonging to cysteine residues of target proteins to sulfenic derivatives (-SOH); in this case reducing agents like glutathione (GSH, see below) can restore the original reduced thiol group (9, 16, 17). A classical example of definitive and often irreversible effect of ROS is offered by the extraction of one electron from the "π bound" of a double bond –C=C– that generates, in the case of unsatured fatty acids the so called isomer "trans" or the so-called peroxide cascade that can lead to malonyl-dialdehyde or penthane (18, 19).

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 a lot of conditions as above reported, especially the nature and the concentration of the so-called antioxidants, the other side of redox system (9, 13, 16). For instance, many lipid peroxides that are generally believed to be harmful may show beneficial functions in cell signalling (19) while increased levels of exogenous antioxidants can become "pro-oxidants" (20, 21).

Antioxidant "family" includes a number of enzymes (e. g. superoxide dismutase, catalase, peroxidase and thioredoxins) and exogenous compounds (vitamins and vitamin-like antioxidant compounds, such as polyphenols, oligoelements, ecc.) (9, 22)

FIGURE 2. REDOX reactions and cell metabolism. *Left side. Two-electron transfer reactions through oxidation (i. e. dehydrogenation) drives cell metabolism towards ATP production (catabolism). Right side. One-electron transfer reactions are related to the generation of reactive oxidant species that are involved in cell defence, signaling and detoxification; such activities are under the control of antioxidant system.*
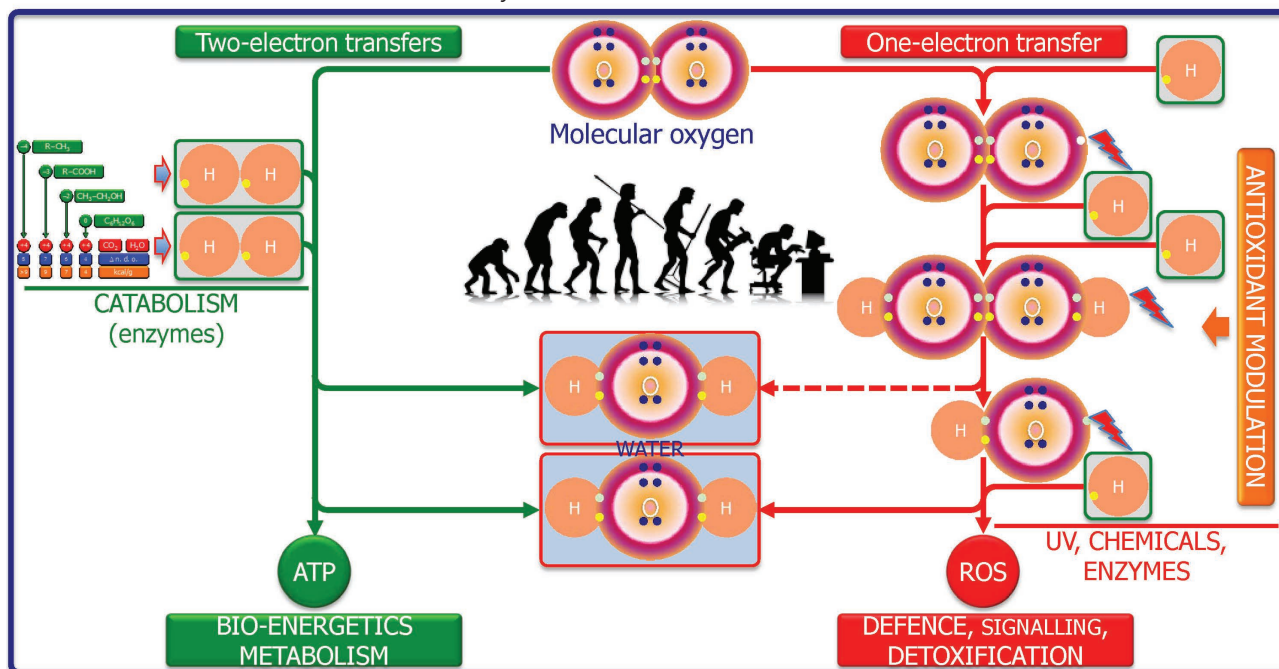
showing preventive, radical scavenging, repair or adaptive functions (23). They are regularly distributed inside a living organism either at extracellular or intracellular level (24). In the extracellular compartment and, particularly, in the blood plasma, all the compounds potentially able to "give" a reducing equivalent "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, and so on) (25–27). In this subject, thiol compounds play a crucial role in ROS modulation (16) (see *also below*). Inside the cell the antioxidant defence 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 defence line against ROS. Later defence line includes the water-soluble vitamin C, several members of the vitamin B group, etc. (28).

Interestingly ROS themselves can stimulate the production of antioxidants as evidenced by the Nrf-2 system that provides an excellent example of signal transduction whit the involvement of DNA (17).

By a physiological point the redox system being highly conserved during the evolution seems to have been planned just to allow the adaptation of living organisms either to external or internal stimuli or stressors (29). According to such concept, exogenous and/or endogenous stimuli should trigger the production of ROS that should activate/inhibit specific biochemical pathways inside the cells thus allowing them to face environmental changes. For instance the ROS hydrogen peroxide was shown able to induce reversible oxidative changes of protein thiols thus modulating key processes involved in cell homeostasis and survival (16, 30). 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 (14) (*see below*). Another relevant example of physiological modulation by reactive oxidising species is provided by nitric oxide pathway in endothelial cells (31).

The generally low energy expenditure required, the fast and easy way of production, the high capacity to diffuse, together with the very short half-life make really these mechanisms essential for Plants as well as Animals survival especially in a context of autacoid modulation. The success of such mechanism is closely related to the efficacy of its restoring machin-

ery. 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, especially if in excess (31–33). For this reason, in the course of millions of years of evolution, the living species have developed, in parallel with the ROS, the so-called "antioxidants" that acts just as "physiological modulators" of ROS (24, 34). Such "physiological modulation 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 unsatured fatty acids or nucleic acid (34). 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) (9). To define these phenomena from the pathophysiological point of view the term "oxidative stress" has been coined (9, 35, 36).

FROM THE OXIDATIVE EU-STRESS TO THE OXIDATIVE DI-STRESS

Oxidative stress (OS) is often but improperly defined as the breakdown of balance between ROS and antioxidants where the firsts should play the role of the bad boys and the seconds that one of "good boys" (9). However OS is not the results of counteracting forces but a necessary mechanism of homeostasis like "emotional stress" (37). Indeed both kind of stress share many features where the first one provides a solid biochemical basis for the second one (12, 13, 37) (*Figure 3*).

Furthermore it has been suggested that the evolution of living organisms and their metabolic, energetic, reproductive and adaptive changes during the last billion of years was driven just by significant redox changes (e. g. increased levels of oxygen in the atmosphere and cysteine in the proteome) according to a specific "redox code", in turn based on the $NAD^+/NADH$ and SH/S ratios (29).

Therefore the so-called "oxidative stress" must be considered by itself as a "positive" adaptive mechanism, of course when it allows through an appropriate oxidation the living organism to successfully respond to environmental challenges (stimuli or stressors) (29). In this case we should use the appropriate word "eustress" that means "good or favourable stress". Of course if 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 (38).

A paradigmatic example of oxidative di-stress can be shown in polymorphonuclear leukocytes after the activation of the plasmamembrane NADPH oxidase by bacteria, that leads to the release of superoxide anion (39) (*Figure 4*).

This latter favours the phagocytosis of the infectious agents but because can be harmful also for the leukocytes themselves and/or for surrounding tissues the cell activates the enzyme superoxide dismutase which role is to convert such ROS to the less harmful hydrogen peroxide, finally responsible for bacteria killing. After that leukocytes enable the second and last 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 same reaction is done by the glutathione peroxidase that converts such compounds to harmless organic alcohols). In other words through such physiological mechanism – oxidative eustress – a stressor (e. g. a bacterium) triggers the production of ROS that after facing the "aggressor" are neutralised by the endogenous antioxidant systems. Unfortunately 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, by the other side, the leukocyte try to dispose of the excess of hydrogen peroxide by activating secondary metabolic pathways like the myeloperoxidase system that leads to the release of power oxidant hypochlorite. Moreover the unprocessed hydrogen peroxide can undergo also to the Fenton reaction thus generating the most harmful ROS, i. e. the hydroxyl radical. Because of this situation a condition of oxidative eustress can switch easily to a condition of oxidative di-stress (38, 39), as it happens in the periodontitis (40, 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 and myocardial infarction), Alzheimer's disease, Parkinson's disease, and cancer (42).

THE EMERGING FIELD OF REDOXOMICS

According to the generally accepted definition of OS 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 i) to a disturbed signalling and/or ii) 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) (42, 43). These latter accumulate either in tissues or extracel-

FIGURE 3. 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.*
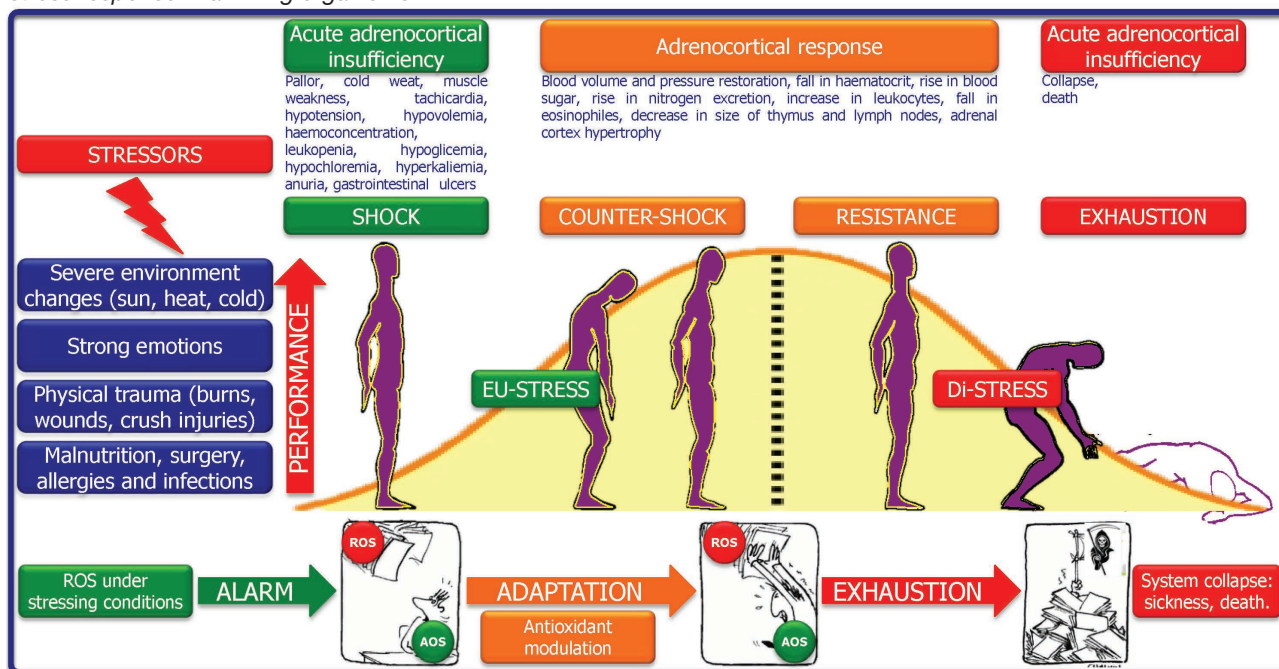
lular fluids, which will represent the optimal specimens in which found specific biomarkers to evaluate the oxidative stress (42, 43).

The first analytical approach therefore involves the direct measurement of the oxidant(s) in a biological specimen (43). This goal can be achieved by using electron spin resonance for radical ROS like hydroxyl or peroxyl radicals, or other photometric/fluorescent methods for non-radical ROS 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 (9, 43). These damaged molecules – or the products resulting from their breakdown – are the "fingerprinting" (43). In other words, oxidative damage is presumed to happen in vivo when it generates identifiable and quantifiable specific by-products in vitro (43). These by-products are assumed 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 (43).

The evaluation of antioxidant defences – 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, 43).

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. 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) (43).

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 (44–49). For this reason very recently we introduced the novel concept of "redoxomics" (49) (a term previously and ambiguously used to identify only some oxidised by-products in the field of proteomics) (50) (*Figure 5*).

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, patophysiology, clinics, pharmacology and so on (50).

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 abnormality of redox status associated to human or animal disease (49, 51).

THE MANAGEMENT OF OXIDATIVE STRESS IN CLINICAL PRACTICE

The clinical approach

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 obvi-

FIGURE 4. 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 alkoxyl radical; NOO·, peroxynitrite; OH⁻, hydroxyl ion; R-NHCl, any organic chloroamine; NO, nitric oxide; O₂⁻, superoxide anion; NOS, nitric oxide synthase; arg, arginine; cit, citrulline; PMN, polymorphonuclear leukocyte; LPS, lipopolysaccharide; Ab, antibody; PL, phospholipid; PLA₂, phospholipase A₂; AA, arachidonic acid; PG, prostaglandins; CAT, catalase; SOD, superoxide dismutase; MPx, myeloperoxidase; HW, Haber-Weiss reaction.*
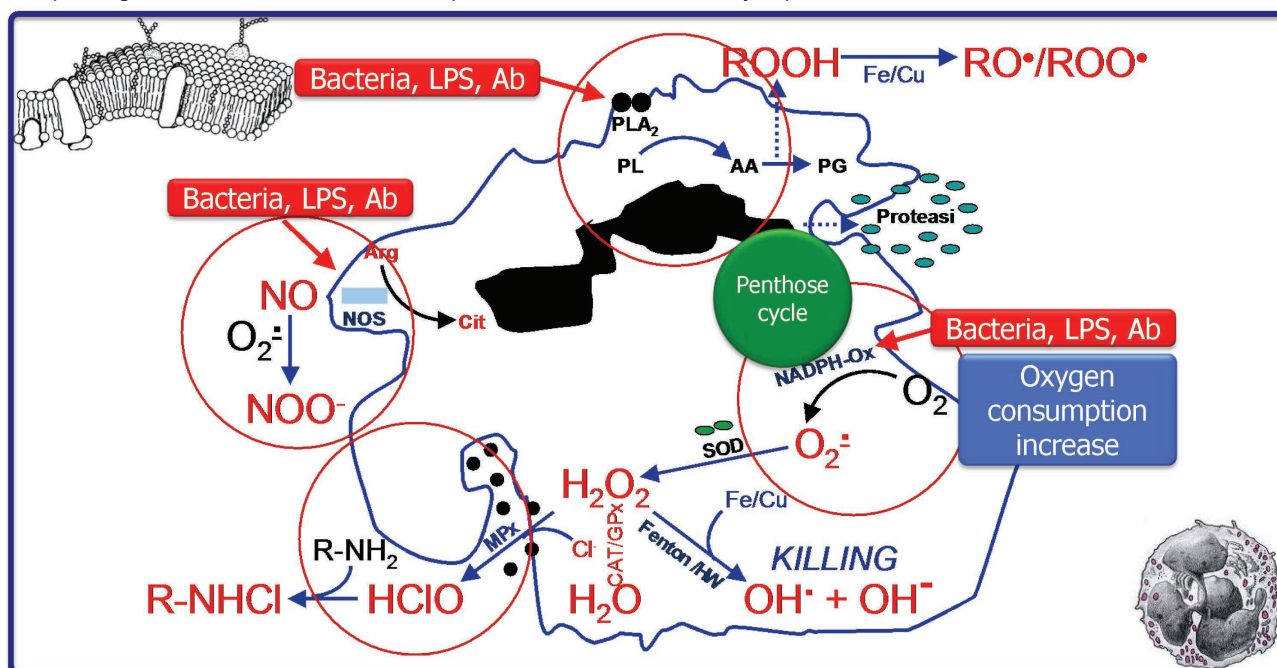
ous the importance of the clinical history that will lead to search the existence of risk factors for oxidative stress, including age, physiological status (pregnancy, lactation, menopause), overweight/obesity, abnormal caloric intake, minerals and vitamins deficiency in the diet, alcohol abuse, cigarette smoke, inadequate exercise, psycho-emotional stress, significant exposure to UV radiations, significant exposure to electromagnetic radiations, significant exposure to environment pollutants, current intake of oestrogen-progesterone combination (especially as contraceptive pill), current chemotherapy, current radiotherapy, current dialysis, current cortisone treatments and so on (9, 36, 43, 51).

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 (9, 51). 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 (43, 51) (*Figure 6*).

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 (51). 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) (14, 52–56). 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 defences (diet/supplementation) (43, 51).

The role of nutrition and supplements: the physiological redox modulation.
Most of oxidative stress-related diseases are closely related to lifestyle. Therefore the first approach to prevent and to control any redox dysfunction, before any supplementation, must consider: a balanced diet, a regular exercise, an effective emotional stress control, and the living in a safe and possibly natural and comfortable environment (42, 57, 58).

Regarding the nutrition, the American Guide Lines for Food Intake, some of which are followed by Oncologists for cancer prevention, clearly suggest take everyday from 5 to 8 portions of fruits and vegetables, preferably fresh and in season (59). However, some Researchers prefer to this "empiric" suggestion more objective criteria, like the one based on the ORAC score (60). 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 (61).

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, and the processes themselves of refinement, transformation, storage and even cooking of vegetables, can variably affect the original content of many micro-nutrients (62).

Therefore, as a precaution, many nutritionists today suggest the indiscriminate use of antioxidants. In our opinion, the use of such supplements should be limited only to the documented cases of oxidative stress, as biochemically detected by specific laboratory tests (43, 49, 57). Indeed nutraceuticals are in some way either drugs or nutrients and since any clinician prescribes a lipid-lowering drugs only after that a laboratory test have been detected abnormally increased circulating fats, it is no matter that the pre-

FIGURE 5. The novel field of redoxomics. *Compared to classical "OMICS", REDOXOMICS appears as a novel multidisciplinary and transversal approach for health and diseases.*
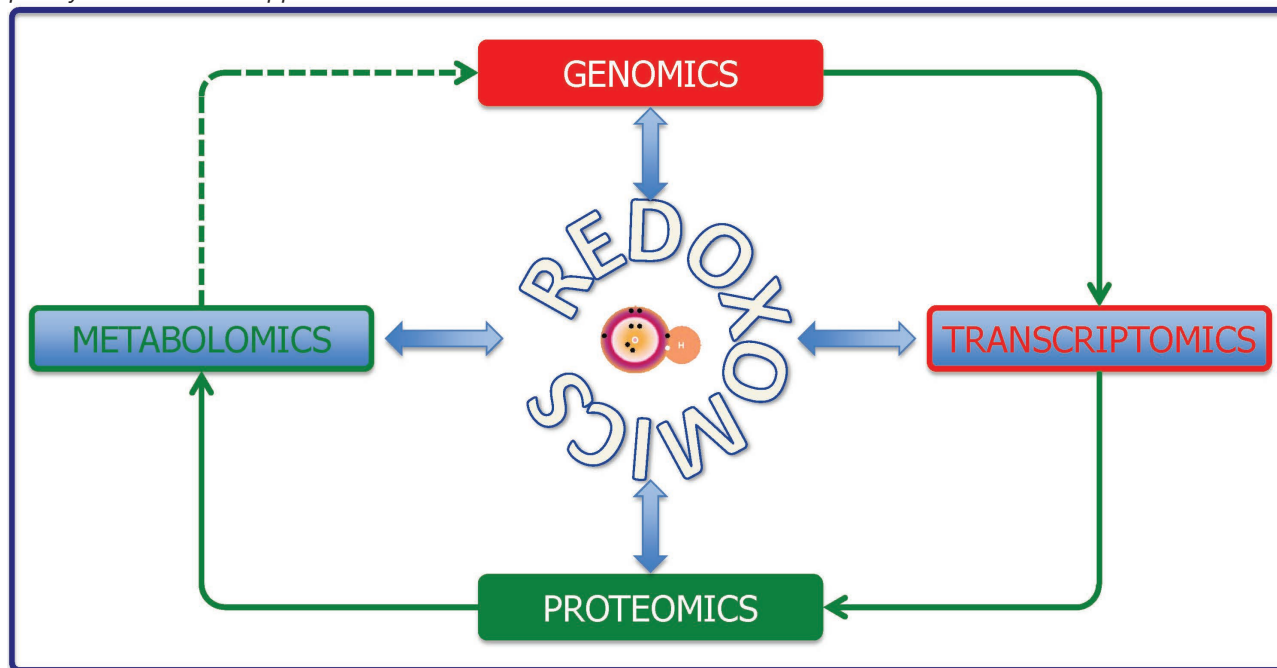
scription of any antioxidant formula should consider a preliminary and specific test for oxidative stress. This "sustainable" approach not only allows to optimize the efficacy but also to reduce the risk of unwanted side effects.

On the basis of oxidative stress biomarkers levels, the clinician should consider any possible way to reduce ROS production and/or to improve antioxidant defences (*see also below 7. 2.*).

A pathological increase of ROS production can be normalised by removing the causes of redox dysfunction, like inflammation, infectious diseases, and so on (51, 57). When this goal is not achievable the administration of nutraceuticals showing both antioxidant and anti-inflammatory features – e. g. curcumin – should be considered (51, 57).

On the other side, reduced levels of antioxidant capacity may suggest the real need of a classical antioxidant supplementation (e. g. vitamins, polyphenols, minerals, and so on) (51, 57).

The prescription of nutraceuticals still remains a delicate as well as open issue, especially regarding the dose. Indeed the opinions of researchers diverge one from another according to two main trends. The first one follows the "American-like" model, according to which we should use a very large amount of antioxidants to prevent and to treat the oxidative stress (57). The second one, prevalent in Europe and in some way conceptually linked to the homeopathy,

suggests the use of low doses of supplements, because high doses can be potentially harmful for our health (57).

Although an accurate biochemical analysis can allow "to tailor" the prescription of a supplement in any patient suffering from or at risk for oxidative stress, some years ago we introduced inside the Nutraceutics, between the above two opposite trends, the concept of "physiological redox modulation" (34, 57, 63, 64).

In agreement with the novel concept of oxidative di-stress (*see paragraph 4*) and the emerging approach of Redoxomics (*see paragraph 5*), we propose herein to "re-baptize" the old terms of "antioxidant supplements" to those of "physiological redox modulators" (PRMs). Physiological redox modulators include a wide class of naturally occurring compounds, mostly deriving from the vegetable world, with proven ability to modulate in vivo and/or in vivo the functions of redox system by acting at the lowest level imaginable (e. g. from mM to μM intracellular concentrations, like for glutathione and vitamin C, respectively, or less, nM, for selenium, for instance) (34, 57, 64). By reproducing that presumably happens naturally in vivo, PRMs are aimed to control mono-electron transfer reactions in order i) to improve or optimize as selectively as possible redox-dependent cell signalling, defence and detoxification mechanisms, and ii) to limit any possible unwanted

FIGURE 6. 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; T_3, triiodothyronine; T_4, 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.*
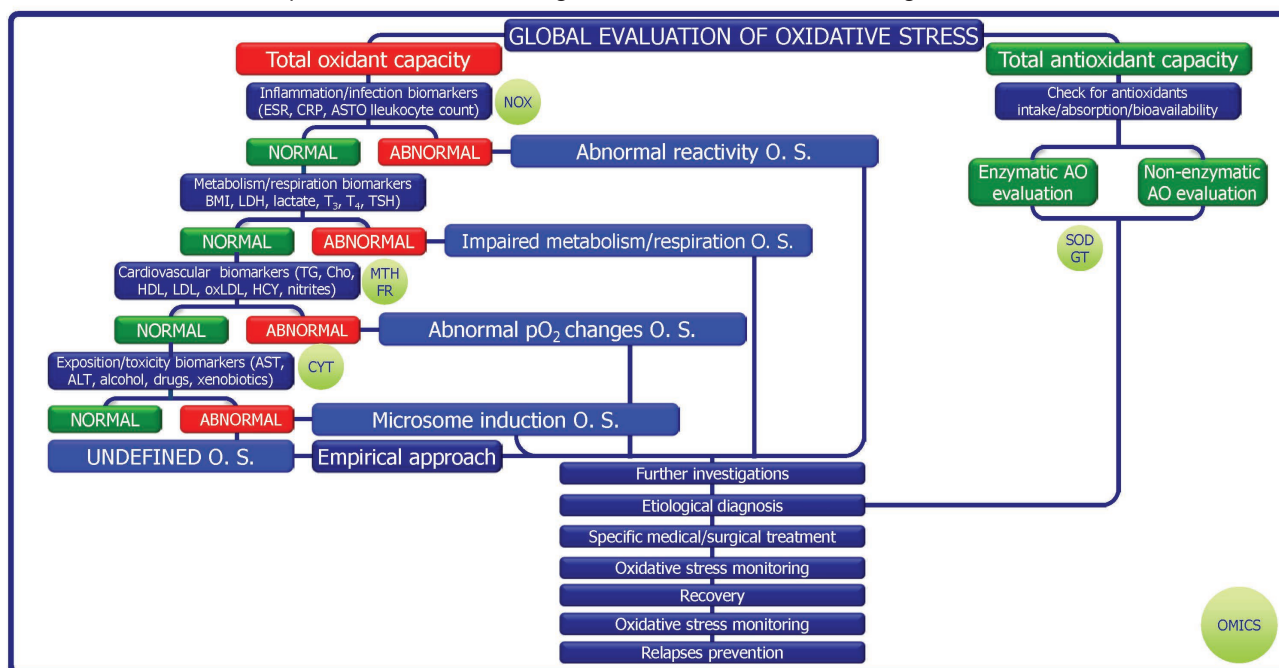
side effect. In other words the aim of PRMs is not to counteract or to face the ROS but, rather, to allow them to exhibit all their positive functions by reaching their specific molecular targets at right time and at the right dose (34, 57, 64).

In the prescription of PRMs the clinician should consider the following issues: i) the physical state of the formula; ii) the redox state and the dose of active principle(s); iii) the route of administration; iv) the bioavailability; v) the interactions among active principles if more than one in the same formula; vi) the required effect; vii) eventual toxicities/unwanted side effects; viii) eventual interactions with drugs; and ix) the scientific evidence (57).

The physical state of the formula (e. g solid or fluid) can have a different impact on specific plasma biomarkers of oxidative stress independently on clinical efficacy itself (65).

The redox state of active principle is crucial. For instance most of commercially available formulas contain "ubiquinone" instead of "ubiquinol", that is the "reduced" and biologically active form of coenzyme Q_{10}. On the other hand, the dose, as below

discussed, should respect the real need of each individuals on the basis of i) the LARN or similar parameters and possibly ii) specific laboratory tests, according to the age, the gender, lifestyle (e. g. cigarette smoke) and so on. Unfortunately "more does not means better" because high doses of antioxidants can show pro-oxidant properties. The risk of overdose is high for supplement containing one active principle alone.

The route of administration plays a specific role. For instance many active principles taken by this way can be neutralized or affected during their transit through the bowel or "sequestered" by the liver, with the final unwanted effect of a reduced "bio-availability" (*see below*) (76). This is the case of the reduced glutathione that can be hydrolysed in the gastro-enteric apparatus like a common peptide (66). On the other hand some clinical conditions, such a celiac disease, by impacting the small intestine can affect the absorption of micronutrients (67). 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 (64, 68). 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, N-acetylcysteine or cysteine from whey proteins were shown to stimulate the endogenous synthesis of glutathione (69, 70).

The bioavailability is a pharmacokinetic parameters that measure the amount (as per cent) of the principle active that is able to reach its molecular target. Unfortunately many common antioxidants like plant polyphenols show very low bioavailability (71). However this parameter can be improved either naturally (e. g. the bioavailability of curcumin is classically increased by piperine) or artificially (e. g. nanotechnologies).

The interaction among active principles is another critical issue because common antioxidants create metabolic chains where any compounds is continuously "recycled" thus reducing their need; for instance vitamin E is recycled by vitamin C and this allows to reduce the intake of vitamin E and therefore its amount in a supplement (57, 71). Moreover the composition of multiple formulas should take in account the stoichiometric ratio between each active principle (57, 71). Furthermore because it is generally accepted that endogenous antioxidant are more powerful than exogenous ones a good supplement should contain at least coenzymes or co-factor able to improve the activity of enzyme like SOD or GPx (57, 71).

Theoretically any supplement should be targeted to induce a specific effect. For instance, among all the plant polyphenols, quercetin is more active on lipid peroxidation while flavones prevent LDL oxidation (57).

Toxicities and unwanted side effects of supplements can be generally avoided firstly by using physiological doses while contra-indication and drug-interaction should be carefully evaluated. For instance omega-3 should be not prescribed in peoples suffering from evident oxidative stress until redox system have been successfully balanced, because such natural compound are very easy to oxidise and then generate potentially toxic by-products. Moreover it is well known that *Ginkgo biloba* extracts can interfere with blood coagulation (72).

Finally convincing scientific evidence should drive the prescription of any antioxidant supplement.

A further improvement of PRMs efficacy is expected by ongoing research on microbioma, gene polymorphisms and epigenetics, that are very closely related to redox metabolism (73).

OXIDATIVE STRESS AND SKIN DISEASES

Biochemical and cellular issues

The skin due to its particular structure/function/position (*see above, Introduction*) hosts one of the most active redox systems of the body. However compared to other organs this system seems differently expressed in the epidermis and in the dermis (1).

The epidermis is mainly composed of epithelial tissue in which layers accumulates keratinocytes; these latter proliferates and differentiate continuously from the basal stratus to the skin surface where they become corneocytes, thus generating a strong and waterproof barrier (1, 74). Because keratinocytes must counteract directly many potential exogenous oxidising agents, like ionizing radiations, chemicals (e. g. ozone, bleach, pollutants, xenobiotics) and parasites (e. g. viruses, bacteria, fungi, toxins), they seems to express a more wide and effective antioxidant network compared to the dermis cells (74). Such system includes not only enzymes (e. g. superoxide dismutase, catalase, and peroxidase) but also small molecules like the endogenous glutathione and melatonin and the exogenous vitamins C, E and A (74). Besides of keratinocytes the epidermis hosts also melanocytes, Langerhans cells, and mast cells, each one with different expression of the redox system (74). Melanocytes produce melanin trough a cascade of reactions that involve redox enzymes (e. g. hydroxylases); abnormal process of melanogenesis can lead to albinism as well as vitiligo (75). Langerhans cells are members of the dendritic cell family and belong to the skin immune system; they acquire antigens in the epidermis, transport them to regional lymph nodes, present to naïve T cells and then initiate the adaptive immune response or mediate the tolerance, for example against commensal bacteria; there are also involved in antimicrobial immunity, skin immune-surveillance, induction phase of the contact hypersensitivity and in the pathogenesis of skin inflammatory diseases; some of these activities seems to be modulated by the redox system; indeed a genetic deficiency of SOD2 leads to an inflamm-aged mouse phenotype (76, 77). Mast cells that are resident in skin and mucosae are key elements of the innate immune response; they are able to rapidly detect pathogens (e. g., viruses, bacteria, protozoa and multicellular parasites) and recruit other cellular effectors of the immune response; in particular the are able to produce ROS after stimulation with *Candida albicans* (78).

The impairment of redox balance in the epidermis is often due to exogenous stressors like UV radiation as widely described (79) However, although DNA is

believed one of the main targets of ionising radiations, nevertheless other factors must be token in account, primarily the relative abundance of water molecules that reside or continuously go across the epidermis layer; such molecules are ideal candidate objective of UV that by the so-called photolysis break them in two radicals including the most powerful and harmful hydroxyl radical; this latter, in turn, can interact with the double bond of unsaturated fatty acids that are abundantly present in the cell membranes and in the lipidic layer of the skin thus generating trans fatty acids and lipid peroxides; the relative deficiency of the antioxidant system can promote such reactions that can lead to the complete oxidation of fatty acids to penthane or malonyldyaldheyde (9, 12, 18, 43).

Reactive oxidising species can have a direct impact also in many pathways, among which are the NF-kB system, which activation is related to the inflammation, and the Nrf-2 system, which activation can stimulate antioxidant, detoxificant and therefore protective responses (17).

In other words the impairment of redox systems and the consequent oxidative di-stress lead leads not only to a damage of crucial cell molecules (e. g. nucleic acids, proteins, and lipids) but also to an impairment of cell signalling with onset or worsening of inflammatory and/or degenerative skin diseases (17, 42) (see below).

The dermis is a classic connective tissue where different kinds of cells (e. g. fibroblasts, mast cells, dendritic cells, lymphocytes, macrophages, and so on) are immerse in the extracellular matrix that, in turns, hosts in its collagen, elastin and glycosaminoglycan three-dimensional network, blood and lymphatic vessels, nerves, sweat glands and hair follicles (1). This layer provides the support for the biosynthesis of many biologically active skin products, including various cytokines, amines, melatonin, hormones, and steroids, which impact on body homeostasis is well known (74). As expected for any connective tissue dermis shows an active redox metabolism that seems most sensitive/reactive to endogenous rather than exogenous stressors, like epidermis. In other words while exogenous stressors (e. g. UV radiations) may impact mainly epidermis (with erythema, burns, photo-allergic reactions, keratosis, cancers) endogenous stressors (e. g. metabolic disorders, systemic inflammation) should have a stronger impact on the dermis (see autoimmune diseases, like psoriatic arthritis). However any external or internal stressor can impact both the layers (79). Into the dermis oxidative stress is often a direct consequence of hypoxia. In this pathophysiological model (Figure 7), a decreased oxygen bioavailability (e. g. due to a local ischemia) leads connective cells to switch their metabolism towards the anaerobic way; the consequent release of acidic cell by-products, mainly lactic acid, in the bloodstream, by lowering the pH in the local microcirculation, can favour the release of transitional metals, like iron or copper, from their carrier proteins, transferrin and ceruloplasmin, respectively, and can activate the so-called Fenton's reaction on plasma circulating unsatured fatty acids, with generation of highly reactive oxidising species (43, 64).

If the antioxidant system is not able to modulate such cascade of reactions, the exceeding amount of ROS may: i) oxidise circulating lipoproteins to oxidised-LDL, i. e. the major actor of atherosclerosis drama; ii) oxidise nitric oxide to peroxynitrite, a powerful vasoconstrictor, pro-inflammatory and pro-aggregative compound, thus leading to the endothelial dysfunction (43, 64). Moreover the activation of leukocytes and macrophage in the extracellular matrix can contribute to the tissue damage by the release of additional ROS and cytokines. Unfortunately an uncontrolled reperfusion can suddenly worsen the tissue damage (ischemia-reperfusion damage). Finally, the excess of ROS may impair the antiprotease system with consequent and permanent activation of proteases and digestion of collagen and elastin. This although herein oversimplified mechanism can explain the impact of oxidative stress in the pathogenesis not only of aesthetic disorders like wrinkles or cellulite but also of systemic disease like diabetes (see diabetic foot), inflammatory/autoimmune diseases (see erythematous system lupus), cancer, and so on (79).

Most importantly the skin is one of the most sensitive organs to the emotional stress: vitiligo provide a clear example of this close relationship that builds a bridge between the two kinds of stresses. In this object can be enlightening to remember that one of the major hormones of the stress, i. e. epinephrine /adrenalin, after bound to their skin receptors is metabolised by the enzyme mono-amine oxidase (MAO) which by product is hydrogen peroxide, a powerful ROS (80). On the other hand the exogenous antioxidant ascorbate (vitamin C) is required to activate the endothelial enzyme nitric oxide synthase (eNOS) that leads to the release this powerful endogenous vasodilator (81).

A recently published study shows that ROS play a relevant role not only in many cell signalling pathways but also in supporting body circadian clocks (74).

The list of skin diseases – besides of wrinkles and aging processes - that were shown to be related to oxidative stress is wide and includes: erythema, oedema, heat, pain, photo-allergic reactions, autoimmune diseases, porphyrias, psoriasis, neutrophilic disorders (e. g. acne/rosacea), atopic dermatitis, urticarial, ischemia-reperfusion injuries, cancers and so

on; in such diseases evidence shows the potential usefulness of redoxomic approach and antioxidant supplementation (79, 82–94) (*see next paragraphs*).

Diagnostic issues

Oxidative di-stress is not a common disorder but, rather, a health risk factor which specific biochemical changes are not related to any particular clinical picture (43, 49). Therefore as it happens for other organs it can be identified and quantified only by means of specific tests (43, 49) (*Figure 8*). At the moment, unfortunately, the ideal biomarker has not yet been discovered but a combination of more than two tests can be useful to identify a condition of oxidative stress (43, 95).

In this object a double approach is available: i) to evaluate the level of specific biomarkers directly in the skin; ii) to evaluate the level of specific biomarker indirectly, i. e. in the blood or in the urine.

The evaluation of oxidative stress in the skin is normally done for research purposes, because it requires a biopsy specimen. This makes the approach too much invasive and therefore not suitable for clinical routine. Keeping in the mind these limitations suitable methods to measure oxidative stress have been successfully applied in several skin diseases by evaluating: i) some by-products of lipid (per)oxidation, like the so-called thiobarbituric reactive substances (TBARs), because the skin relatively rich in unsatured fatty acids) or protein oxidation (96–98); ii) some antioxidant enzymatic activities (mainly catalase and peroxidases) (99-100). Other invasive methods have been applied also to measure the level of hypoxia because a reduced oxygen bioavailability is the leading causes of oxidative stress (64, 101). Luckily a new device has been recently developed for clinical practice with the aim to measure the so-called advanced-glycation end-products (AGEs) that are suitable biomarkers of oxidative stress-related skin diseases (102).

The evaluation of oxidative stress in biological samples like blood or urine has been described below (42, 43). Increased evidence supports this approach because the skin due to its extent and its level of vascularisation can influence the systemic oxidative balance thus making positive many common biomarkers if sick.

The measurement of oxidative stress in the skin may allow the Clinician to identify a not otherwise detectable health risk factor that in turns can be useful in the prevention and in the follow-up of patients suffering from skin-related redox imbalances; moreover the skin oxidative stress evaluation is crucial to establish the real need of supplements (to much often token without any laboratory test) and to monitor their efficacy over the time. This latter is a critical issue because

some antioxidants become pro-oxidant if improperly taken (e. g. to much high dosage) (43, 57, 64).

Potentially usefulness of antioxidant nutraceuticals in skin diseases

The detection of an oxidative di-stress condition in skin disorders raises the question how to "re-balance" the redox system functions without substitute or interfere with any eventual ongoing medical/surgical treatment. The question is still open because oxidative stress may affect many biochemical pathways and implies a lot of variables that cannot be controlled by a unique "antioxidant" approach (43, 57, 64). However it can be argued that a health lifestyle (see above) can positively influence the redox functioning. Indeed a healthy diet can make available many antioxidants while a regular exercise may stimulate the endogenous production of antioxidant via Nrf-2 (103).

However on the basis of significantly abnormal values of oxidative stress biomarkers and/or in the occurrence of a severe skin disease the Clinician can decide to integrate the medical/surgical approach with additional physiological modulators. These latter can be done either locally or systemically: the ideal approach should consider a combination of both treatments (104).

Local nutraceuticals can be dispensed by different way like creams, gels, and so on. Evidence supports in some case their ability to improve oxidative stress and some time the clinical signs of the underlying skin disease (105).

The list of systemic nutraceuticals (by oral route or rarely by parenteral way) that show the potential ability to modulate physiologically the redox system is long (105). Such supplements can help to modulate oxidative stress by three ways: i) by reducing the production of ROS; ii) by enhancing the level of exogenous, non-enzymatic antioxidants by dietary and/or pharmacological interventions; iii) by increasing the endogenous antioxidant enzyme level/activity (105).

The production of ROS can be theoretically reduced by: i) scavenging transition metals that are responsible of the so-called Fenton's reaction by means of plant-derived polyphenols (e. g. the flavonoid quercetin that proven useful in irone chelation) or chemicals (e. g. EDTA or penicillamine); ii) by inhibiting the key enzyme NADP-oxidase (e. g. with some triazolopyrimidine derivatives) (106).

The level of antioxidants can be enhances by taking natural or synthetic compounds including β-carotene, vitamin C and E, coenzyme Q10 (as reduced form, ubiquinol, polyphenols, edaravone, and so on. In particular, the potential benefits of vitamin C are related to the ability of this lactone: i) to scavenge free radicals; ii) to regenerate vitamin E; iii) to inhibit the

FIGURE 7. 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·, peroxynitrite; OH⁻, hydroxyl ion; R-NHCl, any organic chloroamine; NO, nitric oxide; O₂⁻, superoxide anion; NOS, nitric oxide synthase; arg, arginine; cit, citrulline; PMN, polymorphonuclear leukocyte; LPS, lipopolysaccharide; Ab, antibody; PL, phospholipid; PLA₂, phospholipase A₂; AA, arachidonic acid; PG, prostaglandins; CAT, catalase; SOD, superoxide dismutase; MPx, myeloperoxidase; NOX, NADPH oxidase.*
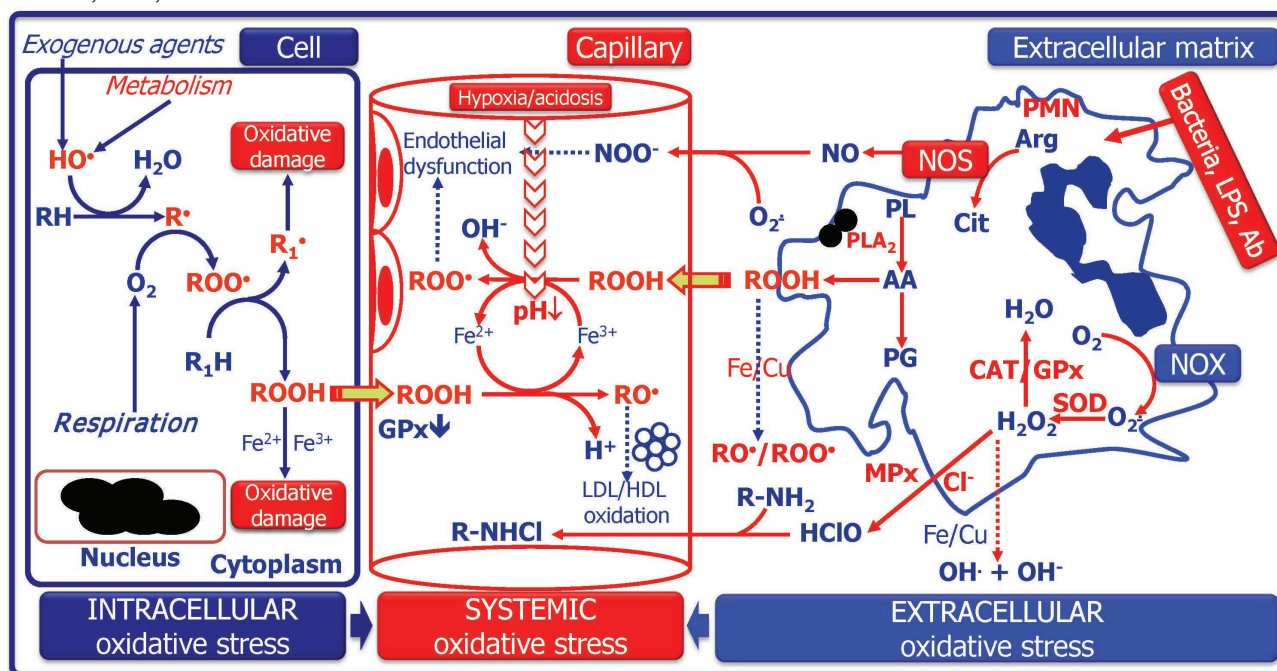
hypoxia-inducible factor HIF1a (that play a crucial role in cancer); iv) to activate eNOS; v) to favour the biosynthesis of endogenous key compound like carnitine and catecholamines; vi) to improve the collagen biosynthesis (due to the hydroxylation of L-proline and L-lysine moieties) (81). The beneficial effects of such antioxidants improve when combined each other (e. g. vitamin C plus vitamin E) possible at physiological dosages (106).

Finally the level/activity of antioxidant enzyme that can be preliminarily evaluated on biological samples (e. g. on red blood cells for SOD or GPx) or predicted on the basis of some specific SNPs can be increased by supplementation with co-factors or coenzymes (e. g. selenium or L-cysteine for GPx, Mn for SOD) or inducing HO-1 system (e. g. polyphenols) (106).

CONCLUSIONS

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 (79). 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 (34, 57). Indeed oxidative distress – 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 (42, 58). Oxidative stress is also involved in the pathophysiology of aesthetic as well as dermatological diseases like photo-aging, wrinkles, and cellulite (107). Unfortunately oxidative stress does not show any specific clinical picture but can be diagnosed only by means of specific biochemical tests on biological fluids (43). This approach led to the development of new branch of applied biochemistry and molecular diagnostics called Redoxomics (49). 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 (58), physiological redox modulators

(57), biocompatible biomaterials (e. g. threads) (108, 109), gases (e. g. oxygen infusion/propulsion, carboxy therapy, ozone therapy) (110) 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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FIGURE 8. Oxidative stress and skin diseases: from cell damage to diagnostics. *Physical, chemical and biological factors, including sun UV radiations, trigger the production of reactive oxidizing species (ROS), including the powerful hydroxyl radical (HO·), either in epidermis or in dermis layers, with different effects; the impairment of antioxidant modulation machinery can lead to oxidative intracellular and extracellular di-stress; this latter is responsible of DNA, lipid and protein damage, clinical outcomes (e. g. wrinkles) and ultimately to the release of oxidative stress biomarkers that can be analytically detected. ECM, extracellular matrix; PX, peroxisomes; XO, xanthine oxidase; Nrf-2, nuclear factor (erythroid-derived 2)-like 2; 8-OH-dG, 8-hydroxy-2'-deoxyguanosine; 2-NH$_2$-3-KBA, 2-amino-2-ketobutyrrate; MMPs, matrix metalloproteinases; GSH, glutathione; ßCA, ßcarotene; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; UCA, urocainic acid; BB, bilirubin; HE, heme.*
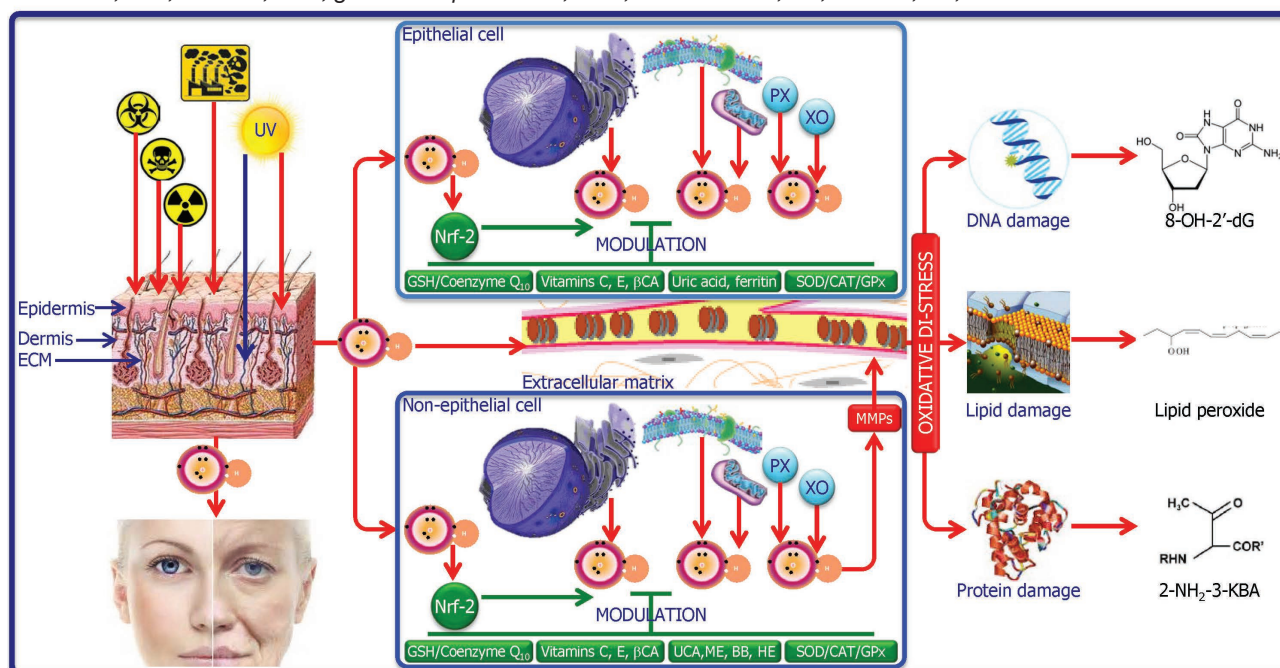
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Nutraceuticals for skin health: an update on polyphenols.

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ABSTRACT

Skin aging depends by both intrinsic and extrinsic factors. Among the latter, solar UV exposure represent the main cause of photoaging. The quality of skin aging has a great social relevance because its aesthetic impact, but also in terms of global health system, being skin cancer the most diffused type of tumor. Nutrition, providing a consistent amount of active compounds, is a direct factor affecting well-being, health and proper skin condition. Natural derived polyphenols have recently attracted considerable attention because of their skin photoprotection effects. They have been shown to modulate different molecular targets, impinging on several signalling pathways, and showing pleiotropic activity on cells and tissues. In the skin context they display anti-oxidant, anti-inflammatory and immunomodulatory activities and also control dermal extracellular matrix remodeling. In the present review, we will give an update on the recent scientific advances in the field of dermatology about the efficacy and mechanisms of action of some of the most used polyphenols.

KEYWORDS: Skin, photoaging, polyphenols, flavanols, curcumin, resveratrol.

SKIN AGING

Cutaneous aging consists of distinct processes due to either intrinsic or extrinsic factors. Intrinsic aging depends on time, reflects genetic background, epigenetic traits, and it is highly influenced by hormonal and metabolic changes. The extrinsic aging, also known as photoaging, is clinically, biologically, and molecularly distinct from intrinsic aging (1). Photoaging is typically characterized by prominent alterations of the skin cellular components and the extracellular matrix of the connective tissue and depends on the adverse acute and long-term effects of solar exposure (2). Solar UV radiations hurt epidermal and connective tissues, activating complex molecular cascades able to accelerate physiological aging. Photoaging is characterized by specific and peculiar clinical and histopathologic features. The former includes deep wrinkles, roughness and dryness, laxity, atrophy, yellowish complexion, hyperchromic areas (solar lentigo, flat seborrheic keratoses, freckles) and hypochromic areas, telangectasie, purpura, cutaneous fragility and pseudostellate scars, finally resulting in preneoplastic and neoplastic lesion development on chronically photoexposed areas. It is also well established that exposure of the skin to UV radiation contributes to the development of skin cancers. Epidemiological, clinical and pre-clinical studies have implicated that solar UV radiation is the major etiological factor in the development of cutaneous malignancies including the nonmelanoma skin cancers that represent the most common malignant neoplasms in humans (3). Photon energy carried in UV (particularly UVB at 280–315 nm, and UVA at 315–400 nm) induces alterations that accumulate and promote the majority of the typical manifestations of both skin aging and cancer. Because the atmospheric ozone filter, UVB makes up only 5% of the UV radiation that reaches the surface of earth and has little penetrance, but it displays great biological activity. UVA makes up the remaining 95% of incident light and is more penetrating, promoting photo aging. However, UVA carries less energy and therefore promote carcinogenesis to a lower extent than UVB. UV damages can be linked mostly to the photochemical overproduction of

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DISCLOSURES

ROS and reactive nitrogen species (RNS). ROS and RNS UV-generated in skin layers can directly alter cellular components (DNA, proteins, lipids), and also affect regulation of gene expression of signaling molecules/cascades (4) (*Figure 1*).

The predominant pathways regulated by photooxidative stress include the mitogen-activated protein kinase (MAPK), the nuclear factor-kappa beta (NF-kβ), and the transcription factor activator protein-1 (AP-1) (5). The activation of NF-kβ and AP-1 transcription factors, result in the secretion of pro-inflammatory cytokines, tumor necrosis factor alpha (TNF-α), interleukin (IL-1, IL-6, IL-8), thereby inducing skin inflammation. Furthermore, photo-exposure induces the activation of the enzymatic systems, e.g. lipoxygenase (LOX) and cyclooxygenase (COX), which are responsible for the production of additional inflammatory mediators. Of particular interest is gene regulation and oxidative activation of matrix metalloproteinases (MPPs), a family of Zn-dependent endopeptidases that are produced by different cell types and taken together are capable of degrading all the components of the intercellular matrix of the connective tissue. MPP takes part in the development of the alterations, typical of photoaging. The pro- and active forms of MMPs are inhibited by the tissue inhibitors of MMPs or tissue inhibitor of matrix metalloproteinases (TIMPs) (6). The remodeling of collagen and elastin,

for angiogenesis, metastasis, and tissue destruction, is largely from the increased expression or activation of MMPs and reduced expression of TIMPs. Even limited exposure to solar light may induce MMPs synthesis beyond the control of specific inhibitors. The role played by ROS in controlling MPPs activity has been largely documented, and a critical role is due to the activation of the transcription factor AP-1 (7). Further, AP-1 inhibits the transcription of type I collagen gene. Hence, the damage to the ECM and tissue integrity is from the enhanced degradation of ECM by MMPs as well as the reduced expression of the structural ECM proteins. However, both intrinsic and extrinsic mechanisms, in a vicious circle, through ROS production and telomere shortening, are responsible for a pro inflammatory status of skin, hence worsening skin aging (8).

NUTRITION AND PHOTOPROTECTION

Photoaging depends primarily on the degree of sun exposure and skin pigment. It is widely assumed that sensitivity to UV is directly related to pigmentation or tanning ability, and this assumption is primarily based on epidemiological evidence that shows that skin cancer and photoaging are much less common in people who tan well or who have high levels of constitutive pigmentation (9). The most obvious strategy

FIGURE 1. The Molecular pathways involved in UV radiation-induced photoaging. UV radiation leads to increase in oxidative stress, increase in inflammatory mediators which cause activation of matrix metalloproteases (MMPs)]
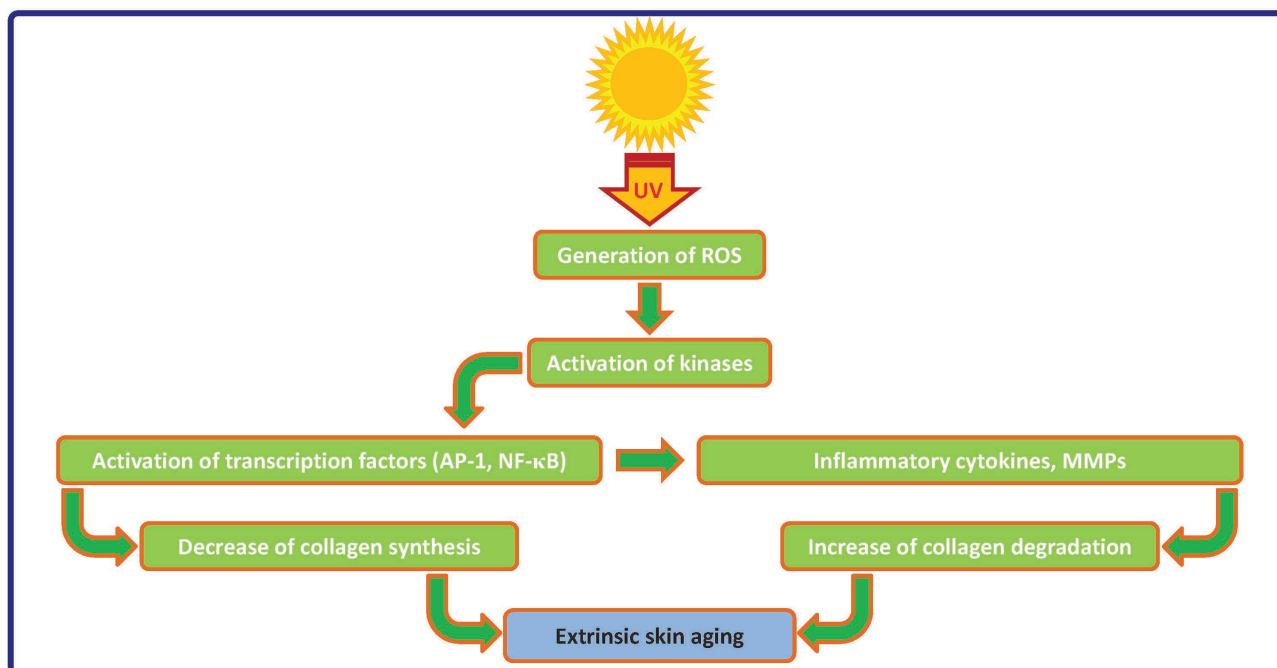
to prevent the deleterious effects of UV radiation is to avoid its incidence on the skin; thus, physical blockers and screens are the most widely accepted and used countermeasures (10). Topical sunscreens are the most important preventive measures against photoaging and photocarcinogenesis and can be divided into reflective and absorbing substances. Nevertheless, their use requires application of the correct amount, and frequent replenishment upon changing environment conditions (e.g. increased perspiration, water immersion, and so on). Additional difficulties to their use include displeasing sensitivity, (e. g.. stickiness, aesthetic issues due to whitening, and so on). Finally, complete blockade in cases of extreme photoprotection may lead to vitamin D deficiency, which can promote carcinogenesis. Apart the phototype and the sunscreens, individual ability to counteract noxious molecular events induced by UV, depends by the activation of a complex defense system against oxidative stress. As we have highlighted before, oxidative stress and consequent inflammation, are key process underlying both, photo aging and photo carcinogenesis. Endogenous systems to prevent their deleterious effects include antioxidant enzymes, (e. g., superoxide dismutase, catalase, transferrin, and so on), and other substances obtained from the diet, e. g., vitamin E (β-tocopherol), vitamin C (ascorbic acid) and carotenoids (β-carotene). Thus, proper nutrition, providing a consistent amount of these antioxidant compounds, is a direct factor affecting well-being, health and proper skin condition (11). Diets rich in fresh fruits, vegetables, and their antioxidants may help reduce photoaging and the risk for developing skin inflammatory disorders, such as psoriasis. A case–control study linked increased consumption of carrots, tomatoes, and fresh fruit, as assessed by patient questionnaires, with a significantly decreased risk for skin diseases. Antioxidant supplementation is an integral part of a multi-faceted approach in photoprotection, and includes myriad of extracts or isolated/purified substances from different parts of plants, including roots, leaves, flowers, seeds, and so on. Although some of these substances have been used topically, its route of administration is mainly oral, as food supplements, concentrates and purified extracts. Topical delivery of antioxidant compounds, such as Vitamin E, is an attractive alternative, so that they can be used as cosmetic ingredients against skin aging, especially as curative/therapeutic in addition to their prophylactic action. The active phytochemicals or the extracts of their sources have become major photoprotective compounds characterized by peculiar mechanisms of action. Although they mainly function as antioxidants, they also display anti-inflammatory and immunomodulatory activity and also control dermal extracellular matrix remodel-

ing. In this context, natural derived polyphenols have recently attracted considerable attention because of their skin photoprotection effects.

POLYPHENOLS AND SKIN PHOTOPROTECTION

Polyphenols are a group of chemical compounds diverse in terms of structure and properties, which are classified as plant's secondary metabolites (12). They can be found in different parts of plants, including flowers, fruits, seeds, leaves, roots and bark layers, and their main biological activity is to protect them against biological stressors, harmful effects of UV radiation, viruses, bacteria and fungi and also to assist in the process of adaptation to changing environmental conditions, cellular signal transduction or gene expression. Polyphenols are chemicals characterized by the presence of more than one phenolic group (a hydroxyl group bound to an aromatic ring) per molecule. Their intrinsic antioxidant function resides in the hydroxyl (–OH) group that, bound to the aromatic ring, act as a hydrogen or an electron donor, giving it to a free radical or other reactive species. The typical classification of these molecules takes into account the number and type of phenolics, which determine their biological properties. According to this, polyphenols are either flavonoids (the most numerous) or nonflavonoids, appearing in numerous plants. In addition to their antioxidant capability, some of them display metal (Cu and Fe) chelating properties, thereby preventing the Fenton reaction, which involves formation of free radicals from hydrogen peroxide (H_2O_2). Nonflavonoids comprise mono phenolic acids and alcohols, benzoic and cinnamic acid and stilbenes. The flavonoids include catechins, isoflavones, proanthocyanidins, and anthocyanins.

In recent years, there has been a growing interest, supported by a large number of experimental and epidemiological studies, for the beneficial effects of some phenolic substances, contained in commonly used spices and herbs, in preventing various age-related pathologic conditions, ranging from cancer to neurodegenerative diseases. Although the exact mechanisms by which polyphenols promote these effects remain to be elucidated, several reports have shown their ability to stimulate a general xenobiotic response in the target cells, activating multiple defence genes, having a number of different molecular targets, impinging on several signalling pathways, and showing pleiotropic activity on cells and tissues (12). A major mechanism relates to the ability of polyphenols to upregulate antioxidant defences by overexpressing highly protective inducible genes involved in the cellular stress response, such as the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) sig-

nalling pathway (13). Nrf2 is a conserved master regulator of cellular antioxidant responses. Nrf2 belongs to the Cap'n'Collar family leucine zipper transcription factors and regulates the expression of genes encoding anti-oxidant and detoxifying proteins such as glutathione S-transferase (GST), glutathione synthetase (GSS), heme oxygenase-1 (HO-1) and NAD(P)H:quinone oxidoreductase. In addition, polyphenols can also suppress oxidative stress by reducing inflammatory responses via interfering with NF-kβ and MAPK controlled inflammatory signalling cascades.

Current evidences strongly support that dietary phenolics can act as regulatory molecules that have the ability to restore the redox homeostasis (activating Nrf2) and prevent systemic or localized inflammation (inhibiting NF-kβ) (Figure). Despite the low absorption rate of the dietary phenolics, studies have shown that low concentrations of these compounds with physiological relevance can still modulate the expression of various inflammatory bio-markers via different signalling pathways as discussed above. These properties can be exploited for the prevention of variety of skin disorders caused by excessive exposure to solar UV light (14). *In vitro* and *in vivo* systems have both shown the protective effects of polyphenols on the biochemical processes that are induced or mediated by UV radiation, suggesting that routine use of natural polyphenols both topically and orally may provide effective protection against UV radiation, photoaging (15).

Some phenolic compounds, such as epigallocatechin-3-gallate (EGCG) from green tea, have been shown to protect against UV-induced DNA damage and immune suppression, allowing this component to be incorporated in conventional sunscreen formulations to boost the photoprotection provided by the UV filters.

In vitro and in vivo studies have recently demonstrated that plant extracts rich in polyphenols, particularly EGCG, curcumin, resveratrol and flavanols present in cocoa powder can significantly reduce photoaging and skin inflammation caused by exposure to UV (*Figure 2*).

CURCUMIN

Curcumin(1,7-bis[4 hydroxy 3 methoxyphenyl]1,6 heptadiene 3,5 dione), a coloring agent and food additive commonly used in Indian culinary and traditional medical preparations from time immemorial, is extracted from the rhizome of *Curcuma longa*. Its anti inflammatory properties and cancer preventive activities have been consistently reported using in vitro and in vivo models of tumor initiation and promotion. Although the exact mechanism by which curcumin promotes these effects remains to be elucidated, the electrophilic properties of this yellow pigment appear to be an essential component under-

FIGURE 2. Principal food derived polyphenols endowed with skin photoprotection effect
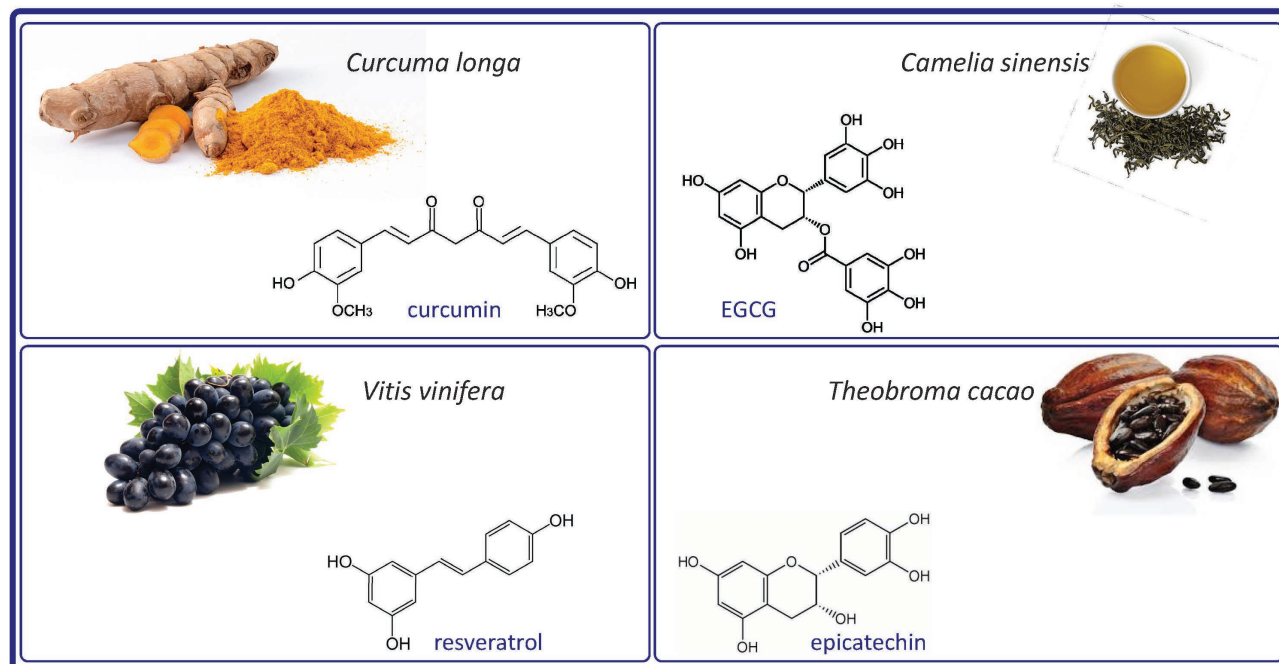
lying its pleiotropic biological activities. Recently, several researches have established that these effects are mediated through the regulation of various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other enzymes. Of particular interest is the ability of curcumin to inhibit COX 1 and COX 2 enzymes and to reduce the activation of nuclear transcription factor NF-kβ, AP-1, and MAPK pathways. Furthermore, data from our and other groups revealed that in different cellular types, low concentrations of curcumin, potently activate Nrf2 translocation to the nuclei and the consequent induction of protective and antioxidant genes (16, 17). The involvement of curcumin in restoring cellular homeostasis and rebalancing redox equilibrium by the activation of defensive genes, suggests that it might be a useful adjunct also for skin photoaging and for some skin inflammatory diseases treatment. Cho et al. also showed that curcumin inhibited NF-kβ and MAPK pathway to decrease cell proliferation as well as reduce the expression of IL-6 and IL-8 in TNF-α-treated keratinocytes (18), while Lima et al. reported that curcumin enhances antioxidant defenses, via Nrf2 activation, in human skin fibroblasts (19). These effects have been also proposed as beneficial treatments for different skin inflammatory conditions, ranging from photoaging to psoriasis. Various investigators have recently shown that curcumin treatment reduces wound-healing time, improved collagen deposition, and increased fibroblast and vascular density in wounds thereby enhancing impaired wound healing. It has also been shown that curcumin acts as a proangiogenic agent in wound healing by inducing TGF- , in both normal and impaired healing wounds. However, to date, the amount of human clinical trial using curcumin supplementation in skin disorders are extremely limited. Skin gets most heavily damaged upon exposure to sulfur mustard (SM) and causes pruritus, the most common chronic skin complication of SM. A clinical trial on 96 male Iranian veterans showed Curcumin supplementation effectively mitigates inflammation in these patients suffering from chronic SM-induced cutaneous complications (20). This anti-inflammatory effect might account for the observed pruritus alleviation and QoL improvement by this phytochemical (21). A randomized, double-blind, placebo-controlled clinical trial to assess the ability of curcumin to reduce radiation dermatitis severity in 30 breast cancer patients, has been recently performed. Oral curcumin at 6.0 g daily during radiotherapy, significantly reduced the severity of radiation dermatitis, supporting a strong protective effect of this compound on skin oxidative stress caused by ionizing radiation (22).
Recently, a systematic review was conducted to ex-amine the clinical evidence for the use of both topical and ingested turmeric/curcumin to improve certain skin diseases and overall skin health (23). A total of 18 clinical studies met inclusion criteria and 10 studies noted statistically significant improvement in skin disease severity in the turmeric treatment group.

EGCG AND GREEN TEA CATECHINS

Green tea, from *Camelia sinensis*, one of the most widely consumed beverages, has recently attracted scientific attention as a potential nutritional strategy to prevent a broad range of age-related chronic disorders. Moreover, a number of epidemiological studies have suggested that consuming green tea on a daily basis, as part of a healthy lifestyle, may reduce the onset of all causes of mortality and improve longevity (24). The health-promoting effects of green tea consumption are mainly attributed to its polyphenol content, which represents 35% of its dry weight (25). Green tea is particularly rich in catechins, which include EGCG, (-)-epicatechin-3-gallate, (-)-epigallocatechin, and epicatechin. EGCG is the most active and abundant compound in green tea, representing approximately 43% of the total phenols. EGCG possesses antioxidant and anti-inflammatory properties, which include the capacity to inhibit overexpression of cyclooxygenase-2 and nitric oxide synthase (26). Several reports have also shown that EGCG is able to induce a general xenobiotic response in the target cells, activating multiple defense genes (27). These evidences suggested its potential use in photoprotection and photo carcinogenesis. Pretreatment of human epidermal keratinocytes with EGCG suppressed UVR-induced activation of NF-kβ in a dose- and time-dependent manner (28). EGCG can also directly inhibit the expression of metalloproteinases such as MMP-2, MMP-9 and MMP-12 (29).
Several laboratories have reported that topical treatment or oral consumption of green tea polyphenols inhibits UVR-induced skin tumorigenesis and improves numerous skin diseases in different animal models (30, 31). Although green tea catechins reduce UV radiation (UVR)-induced inflammation in experimental models and have been successfully introduced in cosmetology as an anti-aging active compounds for topical use, there are much less information about efficacy of oral treatment for photoprotection in human studies. Heinrich e al. reported in 60 female volunteers that a regular consumption for 12 weeks of a green tea rich in polyphenols contributed to protect skin against harmful UVR and helped to maintain skin structure and function (32). In an open oral intervention study, sixteen healthy human subjects (phototype I/II) were given low-dose green tea

catechins (GTC) (540 mg) with vitamin C (50 mg) daily for 12 weeks. Pre- and post-supplementation, the buttock skin was exposed to UVR and the resultant erythema quantified. GTC intake results in the incorporation of catechin metabolites into human skin associated with abrogated UVR-induced 12-HETE; this may contribute to protection against sunburn inflammation and potentially longer-term UVR-mediated damage (33).

RESVERATROL

Resveratrol is a polyphenolic phytoalexin present in grape, peanuts and some plants, mainly the Chinese herb *Polygonum cuspidatum*. During the last decade, resveratrol has been shown to possess a fascinating spectrum of anti-aging and pharmacologic properties (34). In human skin resveratrol has been shown to delay, or even arrest, the course of skin aging by blocking apoptotic events and mitochondrial dysfunctions in keratinocytes (35, 36). Studies with human keratinocytes have shown resveratrol to be able to modulate cytokines such as IL-6, IL-8, and TNF-α, and also stimulate the expression of Hsp70, which is important for cell repair and cytoprotection (37, 38). A recent study has shown that resveratrol suppresses senescence, and preserve cell proliferation ability, in human keratinocytes, by activation of key longevity pathways, such as AMPK-SIRT1 and FOXO3 (39). Involvement of AMPK-SIRT1 and FOXO3 in a number of fundamental processes including stress resistance, insulin signaling and longevity suggests that nutritional modulation of these targets, by supplementation with resveratrol, might offer protection against two phenotypes observed in aged sun-protected skin: senescence and impairment of cell renewal.

COCOA FLAVANOLS

Cocoa powder, obtained by the beans of *Theobroma cacao*, is recognized as a rich source of polyphenols (40). Total polyphenol content of the cocoa beans is about 6–8% by dry weight and its product black chocolate is considered one of the major sources of antioxidants. Flavanols that include Catechins, and procynanidins are the major polyphenolic compounds in cocoa. Cocoa flavanols possess a direct ability to induce endothelial NO synthase (eNOS), and accordingly, to induce a relaxation of vascular smooth muscle cells, leading to vasodilation (41). Besides their direct effects on eNOS activity, cocoa flavanols exert strong antioxidant effects (42). Cellular studies, and results from oral treatment and topical application studies in both animals and humans, provide evi-

dence that cocoa polyphenols, especially those belonging to the flavanol family, can offer effective photoprotection (43). Furthermore, the antioxidant and anti-inflammatory properties of cocoa polyphenols may constitute the basis of possible antitumor promoting effects of these phytochemicals. A study conducted on rodents has demonstrated that an high phenolic extraction from cocoa powder (containing 468 mg/g of gallic acid-equivalent phenolics and 413 mg/g epicatechin-equivalent flavonoids) strongly inhibits the induction of COX-2 expression, the activation of MAPKs, and NF-kβ signaling in 12-O-tetradecanoylphorbol-13-acetate (TPA) treated mouse skin (44). In particular, oral administering of cocoa polyphenols (4, 20, 40, and 200 mg/kg body weight) to mice 1 h prior to TPA exposure (10 nmol) inhibited ear oedema at 5 h in a dose-dependent manner. A more recent study has demonstrated the ability of cocoa polyphenols to block TNF-α-induced activation of the nuclear transcription factors AP-1 and NF-kβ, and VEGF expression, in mouse epidermal cells (45). Other studies have explored the protective action of cocoa directly applied on the skin. Topical application of cocoa polyphenols has been shown to positively affect several parameters of skin elasticity and skin tonus, and prevent UV-induced wrinkle formation in hairless mice (46).

In vivo human skin studies have also shown strong anti-inflammatory, antioxidant, photoprotective, and chemopreventative effects of cocoa, after oral consumption. In a double blind clinical trial, 2 groups of women, with healthy and normal skin of type II consumed either a high flavanol (326 mg/d) or low flavanol (27 mg/d) cocoa powder dissolved in 100 mL water for 12 weeks. Dietary intervention with a cocoa beverage rich in flavanols decreased the sensitivity of human skin toward UV light, which was determined by the degree of erythema (reddening) following irradiation with a solar light simulator (47). The same study has found an increase in cutaneous and subcutaneous blood flow in women supplemented for 12 weeks with a cocoa beverage rich in flavanols. Microcirculation is important factor for thermoregulation, nutrient and oxygen supply, and it affects skin condition and appearance. The relationship between dark chocolate consumption rich in flavanols and skin photoprotection has been investigated in a double-blind *in vivo* study in 30 healthy subjects. In the high flavanol chocolate group the mean minimal erythema dose (MED) more than doubled after 12 weeks of chocolate consumption, while in the low flavanol chocolate group, the MED remained without significant change. These results demonstrated that regular consumption of a chocolate rich in flavanols confers significant photoprotection and can thus be effective

at protecting human skin from harmful UV effects (48).

CONCLUSIONS

Although more clinical evidences are probably needed to better evaluate the clinical relevance of polyphenols in dermatology, the data presented above clearly showed that most of the compounds described have important antioxidant, anti-inflammatory, and photoprotective functions on the skin (*Figure 3*). The ability of these phytochemicals to modulate critical biochemical functions, through oral and topical formulations makes such polyphenols promising candidates for further dermatological applications, ranging from cosmetic wellness to prevention of carcinogenesis.

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FIGURE 3. Main molecular targets and signalling pathways modulated by polyphenols.
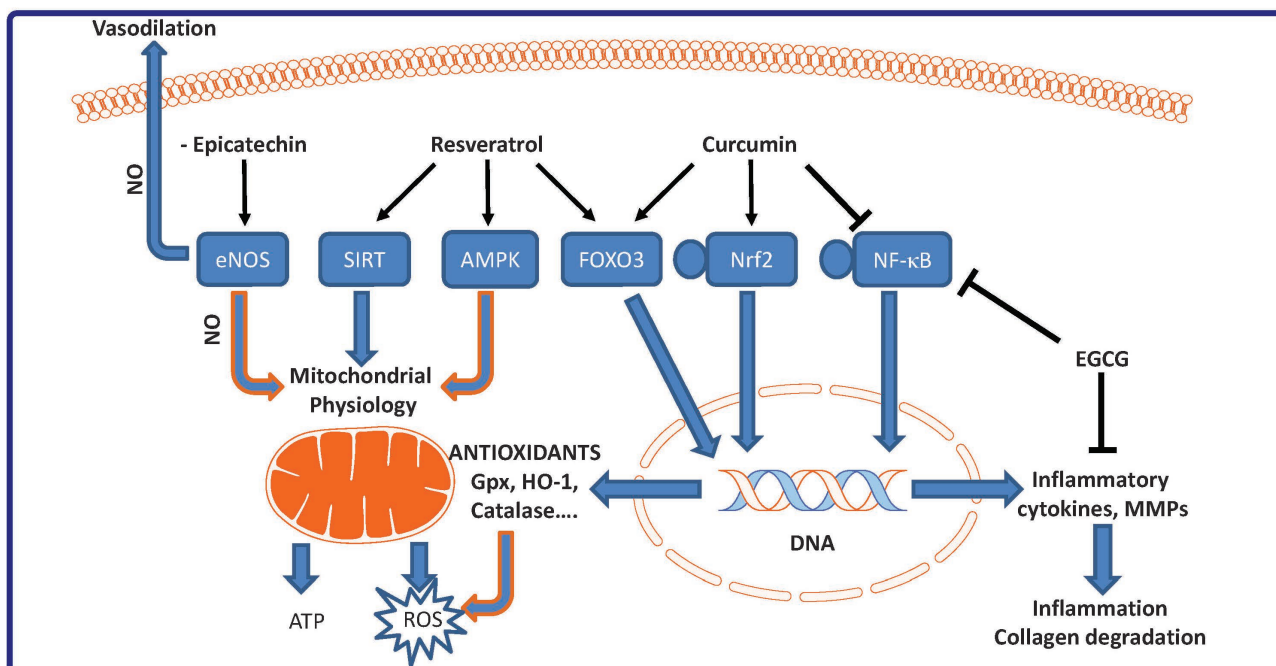
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Pycnogenol™ in oral skin care: a mini-review.

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ABSTRACT

Nutrients can play a significant role in optimizing the levels of main proteins (collagen, elastin and keratin) and hyaluronic acid (HA) that are natural compounds of the skin. This review examines the skin benefits of the lesser known nutrients of Pycnogenol™ an Unique Nutrient. The skin proteins and hyaluronic acid are especially sensitive to the needs of micronutrients such as those contained in Pycnogenol™, plus vitamin C and silicon. Less than optimal nourishment with these and other nutrients are manifested visibly as rough, reddened, wrinkled, scaling or even itchy skin. The demonstrated synergies of key vitamins and minerals acting in concert with Pycnogenol show the potential of Pycnogenol™ for radiant and healthy skin. Pycnogenol™ supports in-creased presence of collagen and elastin, improves skin micro-circulation, elevates skin hydration and elasticity by upping dermal hyaluronic acid generation and, furthermore, balances pigmentation for brighter skin complexion and quenches inflammatory processes, such as during UV exposure. Finally, Pycnogenol™ contributes to a variety of physiological functions for improvement of both health and aesthetic appearance of human skin.

KEYWORDS: Pycnogenol, skin elasticity, photo-ageing, key micro-nutrients, hyaluronic acid

Both health and skin appearance are largely affected by its main proteins (collagen, elastin and keratin) and hyaluronic acid (HA). Nutrients can play a significant role in optimizing these levels. As well-known examples, the vitamin ascorbic acid is involved in collagen formation and the mineral silicon is required for silicon in collagen and glycosaminoglycan formation (1–3).

This mini-review examines the skin benefits of the lesser known nutrients of Pycnogenol™. Pycnogenol™ contributes to a variety of physiological functions for improvement of both health and aesthetic appearance of human skin.

There are at least 20 published clinical investigations on Pycnogenol™ in dermatology. Pycnogenol™ supports increased presence of collagen and elastin, improves skin micro-circulation, elevates skin hydration and elasticity by upping dermal hyaluronic acid generation and, furthermore, balances pigmentation for brighter skin complexion and quenches inflammatory processes, such as it happens during UV expo-sure.

Pycnogenol™ is an unique nutrient. Its has been the subject of more than 140 published clinical studies and 470 scientific publications. It is a precise extract from the bark of the French Maritime Pine tree (*Pinus pinaster atlantica*) consisting of a mixture of nutrients, primarily bioflavonoids. The monograph "Maritime Pine Extract" of the US Pharmacopoea describes the composition of Pycnogenol in detail (4)

Pycnogenol™ helps skin rebuild elasticity which is essential for smooth and youthful looking skin. It has a high and specific affinity for the main skin proteins, collagen and elastin, protecting them against free radicals and destructive enzymes (5, 6). The most dangerous enzymes destroying the essential structure elements of the skin are the collagenases and elastases. Both enzymes are inhibited after intake of Pycnogenol™ (6). Besides protecting collagen, a study has also shown that Pycnogenol™ helps produce new fibers which make skin smoother and more elastic, with fewer wrinkles (5). Pycnogenol™ also stimulates

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the body's production of hyaluronic acid which is important for water retention, wound healing and filling in (volumizing) wrinkled skin to smooth it (5). This action counteracts the thinning of skin that develops with aging. Pycnogenol™ helps the skin rebuild its thickness and elasticity. Skin fullness and elasticity are essential for skin smoothness. The *Figure 1* reports the health benefits of Pycnogenol™ on the skin.

Pycnogenol™ stimulates collagen synthesis in women and correspondingly significantly elevates their skin elasticity. A study with 20 healthy women, presenting with Caucasian skin types II and III, found that daily supplementation with Pycnogenol™ over a period of twelve weeks, significantly increased new collagen (type 1) synthesis in their skin by increasing gene expression by 41% (5). Correspondingly, women's skin elasticity, as measured by means of a cutometer, was in average elevated by 25% after six weeks supplementation with Pycnogenol™ and remained at this value until trial completion (*Figure 2*). In parallel, skin fatigue decreased by 30% during the same time.

The elevated collagen synthesis identified in the dermis of 20 women supplementing with Pycnoge-

nol™ coincided with significant increase of their skin elasticity parameters, as judged from cutometer measurements, after both 6 and 12 weeks of supplementation with Pycnogenol™, respectively.

Pycnogenol™ metabolites represent potent inhibitors of destructive enzymes matrix metallo-proteinases (MMPs) 1, 2 and 9, which break down dermal tissue proteins collagen, elastin and gelatin, respectively (6, 7). The reduced activity of lytic enzymes extends connective tissue half-life in the dermis, representing the basis for maintaining an elastic, smooth and youthful looking skin.

Pycnogenol™ has been demonstrated to stimulate hyaluronic acid generation in women's skin, to naturally improve skin moisture. Pycnogenol™ supplementation significantly elevates generation of hyaluronic acid synthase by increasing gene expression in women's skin, the enzyme representing the natural source of water-binding hyaluronic acid in the dermis (5).

The gene expression of the hyaluronic acid generating enzyme was increased significantly by average 44% in women taking Pycnogenol™ for twelve weeks, as compared to baseline values (*Figure 3*). Furthermore, Pycnogenol™ stabilizes dermal barrier functions which further contributes to counter skin dryness.

In parallel to increased hyaluronic acid synthesis taking place in women's skin, in response to supple-

FIGURE 1. The nutrients in Pycnogenol™ provide numerous health benefits to the dermis. Indeed such nutrients: 1. decreased pigmentation; 2. increased hyaluronic acid synthesis that keeps epidermis taut and smooth; 3. elevated micro-circulation (perfusion) that improves vitality (hydration nutrient and oxygen supply and detoxification); 4. increased collagen and elastin presence that yelds greater skin elasticity while firmness prserves youthful skin.
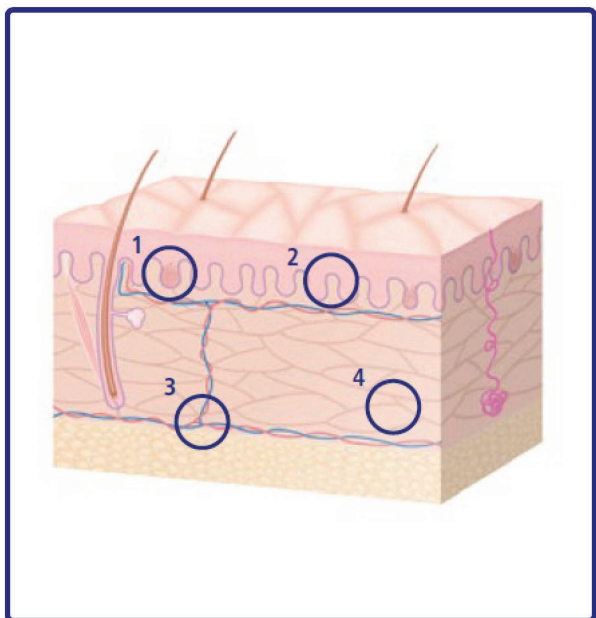

FIGURE 2. Pycnogenol™ increased skin elasticity by 25% and skin collagen production by 40% in the 12-week clinical study by Marini et al. (5)
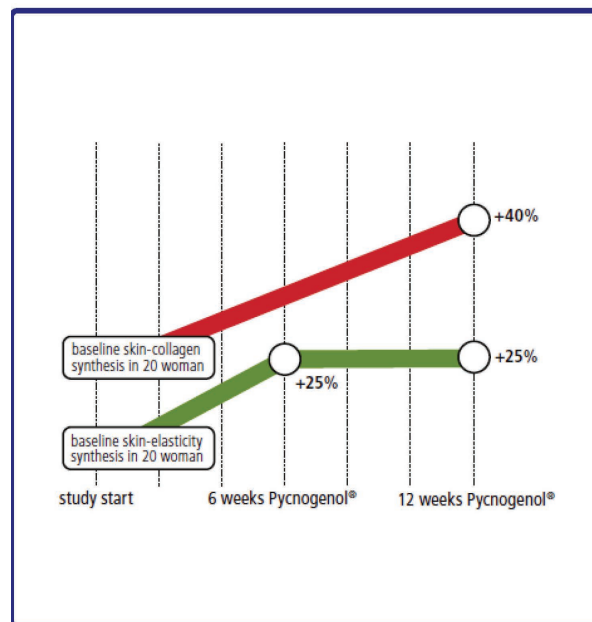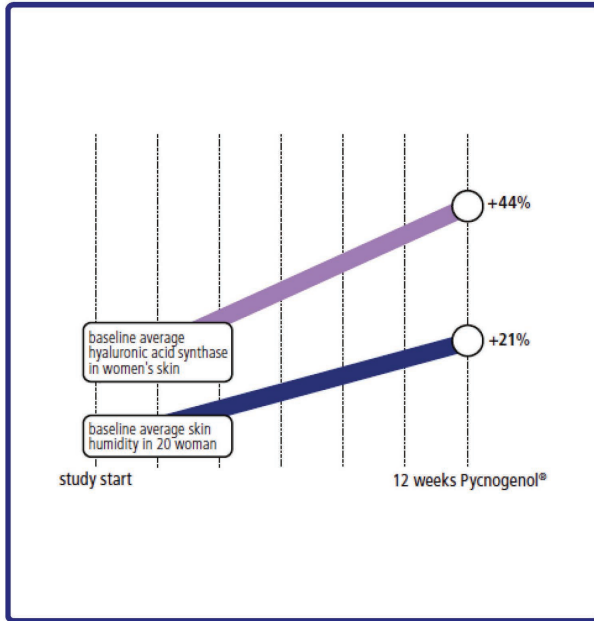
FIGURE 3. The nutrients in Pycnogenol™ increased hyaluronic acid synthase by 44% and skin hydration by 21% in the 12-week clinical study by Marini et al. (5)

FIGURE 4. The nutrients in Pycnogenol™ effectively reduced skin over-pigmentation intensity by 37% and over-pigmentation area by 22% in a 330-day clinical study by Ni et al. (10)

mentation with Pycnogenol™, the more abundant hyaluronic acid binds larger water quantities in the dermis, thus significantly increasing skin hydration particularly in women presenting with dry skin. In women presenting with normal skin moisture, as measured by corneometry, supplementation with Pycnogenol™ was demonstrated to still further improve skin humidity significantly by 8% (5).

Pycnogenol™ also has been shown to lighten over-pigmented skin, lowering pigmentation intensity resulting in brighter skin complexion (*Figure 4*). Pycnogenol™ dose-dependently inhibits α–MSH (melanocyte stimulating hormone) stimulated pigment formation (melanogenesis) in melanocytes (8).

In a clinical trial with 20 women oral supplementation with Pycnogenol™ was shown to significantly lower UV-induced expression of pigment synthesizing enzymes, tyrosinase-related protein 1 (TRP1) and tyrosinase, related to melanogenesis (9). This further supports Pycnogenol's ability to reduce hyperpigmentation.

A clinical study by Ni et al also demonstrated that supplementation with Pycnogenol™ effectively lightens-up over-pigmented skin areas (10).

Pycnogenol™ enhances blood-microcirculation in dermal capillaries for improved perfusion, hydration, nutrient and oxygen supply, as well as waste removal. Pycnogenol™ improves endothelial function, resulting from expanded endothelial nitric oxide synthesi.

(11). Supplementation with Pycnogenol™ was demonstrated to increase blood perfusion of the dermis, resulting in greater oxygen and nutrient abundance as well as better waste removal (12).

Dermal capillaries are fragile, yet they carry the burden of supplying required nutrients, meet the required oxygen and hydration demand, as well as waste removal. Pycnogenol™ was demonstrated in clinical trials to significantly elevate dermal oxygen prevalence and, correspondingly, reduce carbon dioxide presence (13).

Pycnogenol™ contributes to save the skin from photo-ageing. Exposure of skin to energetic light, especially UV radiation, exacerbates skin ageing processes. Pycnogenol™ has been shown in clinical trials to significantly contribute to photo-protection, while it is not suggested to replace adequate skin-protective measures in situations of intense sun exposure. Taken as daily dietary supplement Pycnogenol™ provides potent photo-protective effects from inside the skin, which is very beneficial in addition to topical skin protection and shadowing.

Oral supplementation of 21 healthy volunteers, presenting with fair skin (predominantly skin types I and II) with Pycnogenol™, at different dosages in repeat experiments, demonstrated significant photo-protective effects, reducing the minimal erythema dosage (14). In the study by Saliou et al., it was shown

that the minimum UV dosage leading to first visible signs of skin reddening (erythema) was demonstrated to increase in response to Pycnogenol supplementation in a dose-dependent fashion (14) (*Figure 5*).

Pycnogenol™ acts in concert with key vitamins and minerals to significantly elevate skin elasticity and smoothness in double-blind, placebo-controlled trial. A double-blind, placebo-controlled clinical study with 62 women supplementing with a complex dietary formulation with Pycnogenol as lead active ingredient, demonstrated significantly increased skin elasticity after 6 weeks by 9% as compared to placebo (15). In addition to Pycnogenol™, this complex formulation contained various natural antioxidants, minerals and vitamins. Continuous intake of the complex formulation for 12 weeks demonstrated significantly improved skin smoothness by 6% as compared to placebo.
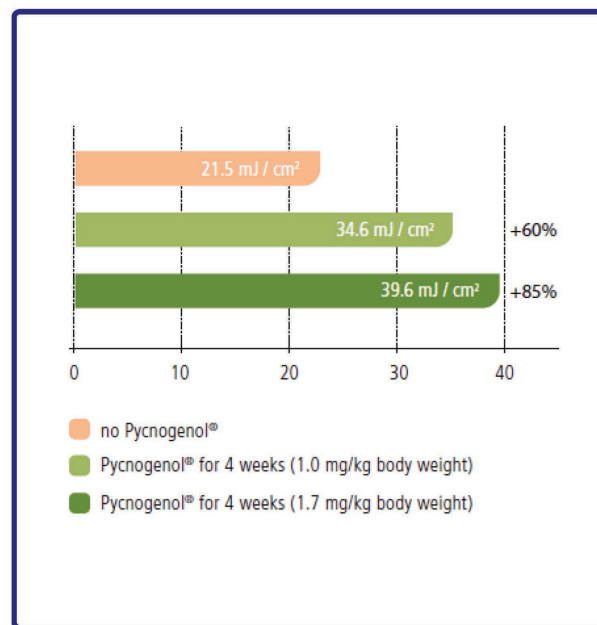
This study demonstrates the theme of this review in that the visible attractiveness and healthy skin physiology are inseparable from another. The dermis largely requires the same key micro-nutrients, vitamins and minerals as most organs, the quantities however may vary considerably.

In conclusion, the skin proteins and hyaluronic acid are especially sensitive to the needs of micronutrients such as those contained in Pycnogenol™, plus vitamin C and silicon. Less than optimal nourishment with these and other nutrients are manifested visibly as rough, reddened, wrinkled, scaling or even itchy skin. The demonstrated synergies of key vitamins and minerals acting in concert with Pycnogenol™ demonstrates the potential of Pycnogenol™ for luminous and healthy skin. In review the above studies demonstrate that Pycnogenol™ stimulates synthesis of new collagen, increases skin elasticity and reduces skin fatigue, elevates hyaluronic acid generation, moisturizes the skin, arrests activity of enzymes breaking-down collagen and elastin contributes to limit photo-ageing, and reduces pigmentation for a more even, brighter-looking skin.

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FIGURE 5. Pycnogenol™ dose-dependently increases resistance to solar UV exposure (solar light dose needed to trigger skin redness) (14)

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Atopic dermatitis and medicinal plants: a review.

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ABSTRACT

"Atopic eczema/dermatitis syndrome" (AEDS) can have a genetic basis or an intrinsic predisposition to produce antibodies or a clinical manifestation through phenotypic aspects. The use of plant drugs for topic application and oral supplementation has been studied for many years: in particular, gamma-linolenic acid (GLA) from *Oenotera biennis L.* and *Borago officinalis L.* have influence on the skin barrier balance. The review includes other herbal drugs that must be approved by clinical trials using the double-blind vs. placebo approach.

KEYWORDS: Oenothera, borage, astaxanthin, resveratrol, essential fatty acids.

ATOPIC DERMATITIS. DEFINITION

In this paper I will use the definition "atopic eczema/dermatitis syndrome" (AEDS) for all forms of eczema formerly indicated with the term 'prurigo Besnier' or 'atopic dermatitis' (1).

In addition to that there is also an additional "clinical" definition of atopic dermatitis. 'Intrinsic atopic eczema' is the term used by Wüthrich (2), for patients who have the phenotype of atopic dermatitis, but who have no allergen-specific IgE, which in other words means patients having a genetic, and hence intrinsic predisposition to produce IgE. In the extrinsic form external allergens penetrating through a compromised epidermal barrier sensitise the patient.

Finally, I have to point out that some Authors, basing on the pathogenesis of the injury introduced a new definition as a variant of atopic dermatitis, i.e. "atopiform dermatitis" (3), that is a disease with the phenotype of atopic dermatitis but without the constitutional stigmata of atopy or allergen-specific IgEs.

ATOPIC DERMATITIS. DIAGNOSTIC CRITERIA

The diagnosis of atopic dermatitis (AD) is clinical. In 1980, (4) a study was published with a list of all the signs and symptoms of eczema, dividing them into major and minor signs. These include itching, a chronic course with a tendency to relapses, lesions with a recognisable appearance and position, personal or family history including other atopic disorders (allergic oculorhinitis, asthma and so on).

According to Hanifin criteria, an AD diagnosis is accurate when at least three major and three minor criteria are met:

A. MAJOR CRITERIA
- itching
- chronic or recurrent dermatitis
- symmetric distribution in typical sites (facial and extensor involvement in children; flexural lichenification in adults)

B. MINOR CRITERIA
- xerosis
- palmar hyperlinearity
- follicular ichthyosis
- pityriasis alba
- dennie-morgan fold
- subauricular fissures
- cheilitis
- anterior neck folds
- orbital darkening
- course influenced by environmental or emotional factors
- blepharitis
- conjunctivitis
- cataract
- keratoconus
- tendency toward nonspecific dermatitis
- tendency toward cutaneous infections

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- white dermographism
- reactivity to skin tests
- raised serum ige

Sampson identified "three main criteria for atopic dermatitis: i) family history of atopic diseases; ii) typical (eczematous or lichenified) dermatitis on the face or the extensor surfaces; iii) presence of itching" (5).

The most recent diagnostic criteria were provided by the Williams team (6, 7, 8, 9, 10, 11) and are based on a number of specific symptoms or signs. According to the researchers the definition of atopic dermatitis for patients older than 2 years can only be applied to a situation characterized by a MAJOR CRITERION, i. e. either an itchy dermatosis in the last 12 months or the parents' relating that the child and experienced itching or rubbing, accompanied by at least three of the following signs:

- disease manifestation before the age of 2 (not useful if the child is younger than 4);
- personal history featuring skin folds (antecubital, popliteal) and so on (including the cheeks in children younger than 10);
- positive personal history of skin dryness for at least one year;
- positive personal history of other atopic diseases (asthma or hay fever) or history of atopic diseases in first degree relatives (for children younger than four years);
- Visible flexural eczema (or eczema on the cheeks/forehead and the outer parts of the legs, in children younger than 4 years).

ATOPIC DERMATITIS. PATHOGENETIC HYPOTHESES

Atopic dermatitis is a heterogeneous multifactorial disease that arises as a result of the interaction between environmental and genetic factors. The fact that the pathogenesis of this disease is multifactorial makes it difficult to act on a specific determinant agent and therefore any attempt at treating is undermined by this indeterminacy (difficulty in locating a single underlying cause was seen already in the definition of the disease: intrinsic atopic dermatitis v. extrinsic and atopiform dermatitis).

Below we will analyse various pathogenic hypotheses that refer to the appearance of the clinical picture.

A) Genetic elements
Immune dysregulation
Genetic factors determine the expression of one atopic dermatitis or skin pathology accompanied by intestinal and respiratory allergic reactions (12). This theory is called immunocentric because it places particular emphasis on the immune element as the prime

mover of the whole clinical picture.

We have seen that the immune system is involved as lymphocyte activation causes eczema in the skin and increased incidence of type I and type IV allergies induced by environmental allergens. The presence of altered concentrations of certain interleukins in atopic patients explains the increased activation of T lymphocytes and the increased amount of IgE polyclonal antibodies in many patients.

It has been speculated that the cause of atopic dermatitis was associated with an inborn error of the maturation of the epithelial tissue (13).

The accumulation of T lymphocytes activated at skin level, which induces a chronic relapsing eczema not accompanied by specific allergies in two thirds of patients has led researchers to think that atopic dermatitis is a cytokine-dependent but antigen-independent disease. This indicates the existence of T cells located in the skin whose growth is induced by different stimuli than those who act for mature T lymphocytes circulating in the blood. For this reason, some authors have suggested that atopic dermatitis is a genetically determined change of ectodermal tissue; the thymic epithelium is formed from the ectoderm, and therefore researchers have speculated that the maturation of T cells in people who develop atopic dermatitis is altered because of an abnormal selection of T lymphocites in the thymus. According to this theory, the T lymphocytes that have undergone an abnormal maturation and have been selected in an anomalous way leave the thymus and continue their growth into the skin; the increased proliferation capacity of aberrant T cells causes an alteration in the production of cytokines. In some patients, the overproduction of cytokines leads to the development of allergic reactions of type I due to the stimulation of the humoral immune system towards IgE production (14).

The model proposed by some authors (15) is a biphasic pattern in which initially the Th2 lymphocyte involves macrophages and eosinophils which produce interleukin 12. This interleukin, in turn, activates the Th1 response. In other words, cytokines produced by induction by the Th2 are involved in the early stages of the acute phase, while the combined action of TH1 and TH2 is responsible of chronic wounds.

The pathogenesis of atopic dermatitis would, basically be mainly due to a dysregulation of the adaptive immune system in which an important role is played by the T-helper 1 (Th1)/Th2, the production of IgE, the humoral messages of dendritic cells and mast cell hyperactivity (16).

In recent times we have been identified two elements, which have been assigned a decisive role in the pathogenesis of dermatitis. The first one is the genetic mutation of a skin barrier protein called filaggrin,

whose alteration is present in 25% of affected patients. The second one is the identification of IL-31 as a molecule involved in itching.

Another pathogenetically interesting element is the role of thymic chemokines (TARC) that bind to the surface of TH2 lymphocytes, are assigned to the activation and proliferation of these cells and induce the acute phase of dermatitis. In atopic dermatitis the skin is invaded by TH2 lymphocytes in the acute phase, TARC chemokines are produced by keratinocytes in the lesions, and the chemokine receptors are excessively xpressed in skin lymphocytes exhibiting the CLA+ (cutaneous lymphocyte-associated antigen) receptors and therefore, basing on an in vivo response researchers have speculated that this anomaly is the root of the inflammation mediated by TH2 on the skin (17) (3).

Alteration of the skin barrier

Some authors have listed impaired skin barrier functioning as one of the most important pathogenetic factors.

Some authors (18), basing on the faster regeneration reported have speculated that the pathogenesis was due to a slight disturbance of the barrier function. They hypothesized that repair mechanisms are activated permanently, which provide a faster recovery although complete restoration of the epidermal barrier function is never achieved, perhaps due to the decrease in ceramides (19).

Others (20) think that the structural lipids (ceramides) inserted between the cells of the stratum corneum play an important role in the stratum corneum's liquids retention capabilities.

More recently (21) the identification of alteration of genes that encode structural proteins, epidermal proteases and protease inhibitors that predispose to a defective skin barrier has led to the hypothesis that these abnormalities are the basis of atopic dermatitis. In particular, mutations in the FLG gene inducing the loss-of-function of the structural protein filaggrin currently are the predisposing genetic factor most significant.

An increase in dermal pH increases the activity of degrading protease and decreases the activity of enzymes responsible for the synthesis of lipids, thus the strong association between skin barrier and environmental insults led to believe that the malfunctioning of the skin barrier is the primary event in the development of the disease.

Another hypothesis has been developed basing on the above. This hypothesis is called "immunocentric" and is based on an immune alteration localized at the level of the skin barrier; there is also a "corneocentric" hypothesis which speculates that the prime mover is based is the alteration of the skin barrier.

Deficiency in essential fatty acids (EFA)

Another opinion is that the lack of essential fatty acids may play a crucial role in the pathogenesis of the disease (22, 23). Eczema can be caused by a defect or deficiency of the delta-6-desaturase enzyme and possibly also of the delta-5-desaturase enzyme (24). These enzymes are responsible for the conversion of linoleic acid into gamma-linolenic acid, a substance with anti-inflammatory properties. A reduced conversion may result in a relative deficiency at skin level of gamma-linolenic acid, alpha-linolenic acid and stearidonic acid.

B) Environmental factors: the hygiene hypothesis

According to this hypothesis the disease is more frequent in the more affluent social classes and in industrialized societies. The term "hygiene hypothesis" was first proposed by Strachan to explain the inverse relationship between the prevalence of allergic rhinitis and the number of siblings (25). The best environmental hygienic conditions lead to the reduction of infectious diseases which in turn supposedly leads the immune system to produce less interferon-gamma (IFN-y), which in turn risks leading T lymphocytes to move towards phenotype Th 1.

Let me now consider some key elements (26).
- *Number of siblings*. Nine studies out of 11 report higher disease incidence in the first-borns; the risk of developing specific immunoglobulins E (IgE) is reduced by the number of siblings (27).
- *Environmental conditions and presence of mites or dust*. Cross survey in the UK showed a statistically significant association between atopic eczema and humidity in the house, the use of the radiator for heating the child's bedroom and the use of synthetic fabric pillows. The frequent use of the vacuum cleaner has been associated with a decreased prevalence of eczema (28). The perception that eczema is caused by mites of dust and food allergies often dominates the lives of people with eczema (29)
- *Effects of Infections.* Certain infections do appear to have a major protective effect: among them there are tuberculosis (30) and fecal-oral transmission diseases, such as hepatitis A (31 – 32 – 33) (34), toxoplasmosis, Helicobacter pylori infections and enteritis caused by *Salmonella* (34). The frequency of intestinal infections is shown to be in an inverse proportion with frequency of atopic dermatitis. Infection with EBV and cytomegalovirus reduce IgE synthesis (35). According to the study there is an inverse correlation between viral infections of the upper respiratory tract (especially if accompanied by fever) and AD (Atopic dermatitis) while inflammation of the lower airways is considered a factor favoring the onset of atopy (36). A prospective

study showed that children with at least one episode of fever in the first year of life have a reduced incidence of atopy at the age of 6-7 years (37). The influenza virus infection enhances allergic response in patients with atopy by having an effect on dendritic cells located in the lower respiratory tract (38); infections reduce proliferation and activation of memory T cells (the independently of the presence of the allergen (39)) through the production of interleukins (IL-2 and IL-I5). When the infectious stimuli are triggered at the moment the allergic reaction is taking place they act as adjuvants (Th2 can also be activated by the infection, even in absence of the allergen) but if the infection occurs before the atopic response or in its early stages they have an inhibitory effect (40, 41). However, there is a positive association between infections in early life and atopic dermatitis, and the risk is increased by about 30% in children with frequent infections (42).

- *Exposure to bacterial endotoxins*. Children who grow up in a close contact with cattle farms have a lower incidence of allergic diseases compared to their peers, the protective effect persists into adulthood, but the child should be exposed early in life because after the fifth year the protective effect no longer occurs (43 - 48). The effect is stronger if exposure occurs during intrauterine life (49). The protective mechanism is due to exposure to endotoxins of gram-negative germs and muramic acid, a component of peptidoglycan which is part of the cell wall of the bacteria commonly found present in the faeces of cattle that can induce the proliferation of CD4 T lymphocytes with consequent release of IFN-γ, IL-10, IL-l2 and tumour necrosis factor-alpha (TNF-alfa) (44) (46) (50 – 57).

- *The role of Staphylococcus aureus (SA)*. The stratum corneum is a barrier because it contains lipids with antimicrobial activity, such as free fatty acids, and free free glycosilceramides and sphingosines (which are reduced in atopic skin) (23). Atopic skin also features a reduced number of secretory immunoglobulin A (20). Enterotoxins (ET) of the SA are also becoming increasingly more important; some authors (17) have shown that the severity of the DA is directly related to the production of ET-C by isolated SA in the skin and nostrils of people suffering from AD. Studies have shown that in subjects under AD ET-B stimulates the production of interleukin IL-5 (which is directly related to eosinophilia) (58). The ET would cause the release of histamine and leukotrienes from basophils in AD patients and this suggests that the ET act as antigens and stimulate the production of IgE (59). By binding directly to the T receptor and to second class molecules of the major histocompatibility complex it short-circuits

the classical mechanisms of stimulation linked to a specific antigen and can induce a generalized reaction (60).

- *Maternal stress during the gestational period*. A study carried out in Denmark (61) determined the association between psychosocial work stress during pregnancy and atopic dermatitis in children born of said pregnancy. An association between high job strain and atopic dermatitis was reported in children aged 7; these children were 15% more likely to develop atopic dermatitis compared to other children born to women exposed to lower stress during pregnancy.

ATOPIC DERMATITIS. EPIDEMIOLOGY

Atopic dermatitis is a problem "that affects 10-20% of children of school age in developed countries", and 1% to 3% of the general adult population". The prevalence of the disease has increased by two to three times in the developed world over the past three decades. "The prevalence in the US alone has almost tripled in the last thirty or forty years " "However, the disease's prevalence is much lower in rural regions, such as China, Eastern Europe, and rural areas of Africa " (62). The reasons for the increasing prevalence are not clear; However, the prevalence seems to increase along with the populations' degree of urbanization.

PLANT DRUGS IS USE IN CLINICAL SETTINGS

Gamma-linolenic acid(GLA)-rich plants drugs
Gamma-linolenic acid (GLA). Overview.
Gamma-linolenic acid (GLA) or gamoleic acid or 18:3n-6; 6,9,12- octadecatrienoic acid is a fatty acid that cannot be synthetized by the human body (essential fatty acid, EFA). it is a poly-unsaturated fatty acid of the omega-6 class (PUFA); the molecule is made up of 18 atoms of carbon and three double bonds (*Figure 1*).

Gamma-linolenic acid is usually found in seed oil: its concentration in oenothera oil is 7-14%, in borage oil the concentration is 20-27%, in blackcurrant oil it is 15-20% and GLA is also found hemp seed oil.

In the human body it can derive from linoleic acid through the enzyme delta-6-desaturase, though when enzymatic activity reduces GLA may become conditionally essential.

Linoleic acid is first converted into GLA by the enzyme delta-6-desaturase and then into DGLA (dihomogammalinolenic acid) by the elongation of the chain through an elongase and, finally, into arachidonic acid by the enzyme delta-5-desaturase.

The supplementation with a GLA-based diet by-

passes delta-6-desaturation and therefore in case of shortage of GLA (enzymatic activity is hindered by several factors, including ageing, lack of nutrients, trans fatty acids, hydrogenated oil, smoking and ethanol), it still allows the secretion of metabolites downstream.

Excessive supplementation of omega 6 may cause an increase in arachidonic acid, hence the risk of a pro-inflammatory effect ensuing the secretion of PGE2, while the combination of alpha-linoleic acid, eicosapentaenoic acid (EPA) and docosaesaenoic (DHA) acid with GLA antagonizes the conversion into arachidonic acid (63 – 64).

The overall share of GLA in oil is not the only factor explaining the biologic efficacy; indeed, the bioavailability in affected by several factors, including the stereo-specific composition of triglycerides and the cellular dynamics of phospholipase and acetyltransferase (65); In fact, although the concentration of GLA in borage oil is twice as much as in oenothera oil, the secretion of PGE1 it is the same (66).

GLA is converted into the polyunsaturated fatty acid DGLA, which in turn is the oxygen cycle in prostaglandin E1 (PGE1); PGE1 carries out its biological activities by binding to surface receptors of the smooth muscle cells.

The actions attributed to PGE1 include:
- anti-inflammatory activity,
- vasodilation and hypotension,
- inhibition of cholesterol biosynthesis,
- inhibition of platelet aggregation.
- increase of ampc through inhibition of phospholipase a$_2$.

The action of PGE1 at the immune system level has a particular inhibitory action both at the level of lymphocytes and polymorphonuclear granulocytes as it induces an increase of AMPc inside of the inhibition of phospholipase A$_2$ cells. At polymorphonuclear leukocyte level, this increase of intracellular AMPc causes:
- reduction in the release of lysosomal enzymes,
- reduced chemotaxis
- reduction of margining and adherence to blood vessels.

The intake of fat and protein in diet can affect the intake of essential fatty acid: a high intake of saturated fatty acids can increase the requirement of EFA due to decreased synthesis and decreased bioavailability.

A randomized, double-blind trial on pharmacokinetics showed the presence of steady plasma levels after seven days after administration, regardless of the dosage: therefore therapeutic levels can be achieved within one week (67).

The influence of different combinations of gamma-linolenic, stearidonic and eicosapentaenoic acid induces important changes in the composition of blood lipids and in mononuclear cells.

More specifically, the intake of GLA (2g/day), in the absence of stearidonic acid (STA) or eicosapentaenoic acid (EPA), increases plasma levels of DGLA,

FIGURE 1. The chemical structure of some polyunsatured essential fatty acids.
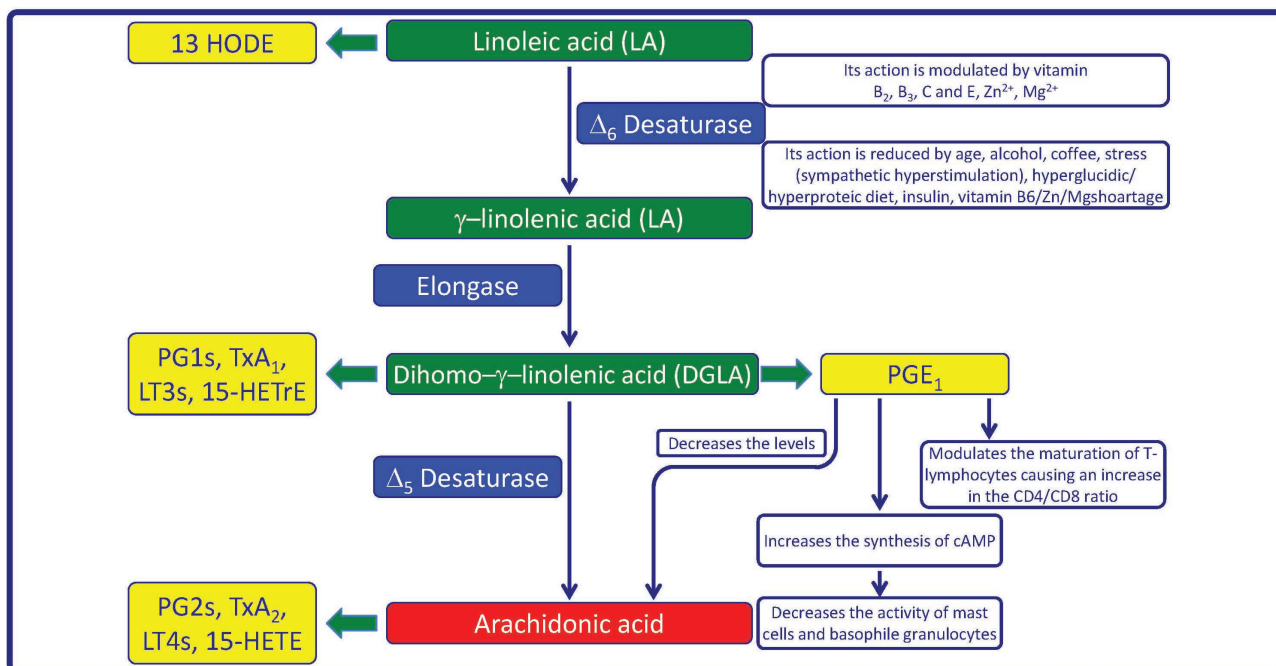
but also raises the content of arachidonic acid. This effect can be avoided by combining the administration of GLA and EPA.

Gamma-linolenic acid, after being converted into PGE1, has an anti-inflammatory, antithrombotic, antiproliferative action and improves smooth muscle cell relaxation and vasodilation.

All EFA (including GLA) are constituents of the membrane phospholipids (GLA and DGLA are not found in the free state, though only as components of cell membranes) and contribute to the integrity and fluidity thereof.

The action of delta-6-desaturase in newborn babies is low; breast milk is rich in GLA and DGLA.

The GLA-deficient exhibit dryness, flaking, and thickening of the skin, as well as growth stunting (68 – 69).

The composition of fatty acids of membrane structural lipids can affect the function of the membrane by changing the degree of fluidity, which in turn determines the thickness of the membrane, or influences the interaction of fatty acids with membrane proteins (44).

GLA has bactericidal activity against *S. aureus* colonizing the skin (70).

Some pathogenetic hypotheses singled out as the primary defect in atopic dermatitis (AD) the maturation and differentiation of T cells that infiltrate the skin or that are not capable of controlling the colonization of T-lymphocytes at the level of the dermis.

A dysregulation element could consist of a deficiency in omega-6 essential fatty acids and E-type prostaglandins that are important for the maturation of T cells of thymic derivation and to the hormonal action of the thymus (71).

Shortage of omega-6 unsaturated fatty acids were observed:
- in plasma, epidermal and erythrocyte phospholipids of patients with DA:
- in lecithin in the plasma of the umbilical cord of newborns with increased levels of IgE in umbilical cord blood;
- in T cells in umbilical cord blood of newborns "at risk of atopy":
- in monocytes of the atopic;
- in lipids in the adipose tissue of patients with AD;
- in lipids in breast milk of mothers with a history of AD;
- in lipids in breast milk mothers of children with AD.

The decreased synthesis of prostaglandin E2 (PGE2) was observed in monocytes of atopic patients and in the epidermis of atopic patients.

The response in plasma phospholipids in a group of 50 young adults atopic patients (72) in connection with the increase of cis-linoleic acid matched with gamma-linolenic acid deficiency, of dihomo-γ-

linolenic acid and arachidonic acid has led some authors to formulate the hypothesis whereby atopic patients have an altered function of the enzyme delta-6-desaturase.

The lack of precursors of prostaglandins as the outcome of the altered function of the enzyme delta-6-desaturase is supported by the following elements:
- patients with cystic fibrosis tend to be phenotypically atopic (thus corroborating the hypothesis whereby the defect at the basis of cystic fibrosis would lie in the enzyme delta-6-desaturase);
- atopic patients are particularly sensitive to side effects of non-steroidal anti-inflammatory drugs (reaction hindered by prostaglandins);
- the application of niacin-based compounds on the skin does not induce redness (a reaction mediated by prostaglandins)

A study (22) found out that the shortage of n-6 essential fatty acids (EFAs) leads to an inflammatory skin condition in animals and humans. However:
- in atopic dermatitis have it has never shown low blood concentrations of EFAs,
- it lacks a therapeutic response at exceptionally high doses of linoleic acid;
- there is no deficiency of linoleic acid, while linoleic acid concentrations instead tend to be elevated in blood, milk, and in the adipose tissue of patients with atopic eczema.
- it has been observed that the concentration of metabolites of linoleic acid is reduced; this finding has therefore led to believe that there is a reduced conversion of linoleic acid into gamma-linolenic acid (GLA) (*Figure 2*).

Oenothera biennis L.

Oenothera biennis L. is a biannual herbaceous plant of the Onagraceae family. The drug is derived from the seeds from which the oil is obtained by cold pressing (like all oils rich in unsaturated fatty acids, it preserves badly).

The main ingredients of the oil are (9):
- cis-linoleic acid (LA) 72% (65-80%);
- cis-γ-linolenic acid (gamoleic acid, GLA) 2-16%;
- oleic acid 9%(6-11%);
- palmitic acid 7% ;
- stearic acid (3%).

Pharmacokinetics

The serum concentration over time of eight fatty acids was measured after oral administration of oil to six healthy volunteers. Six oil capsules (500 mg. each) were administered between meals in the morning upon awakening and in the late afternoon. The administration of GLA oil is followed by a precise pattern of absorption-elimination for which blood concentration up to 24 hours from administration is significantly
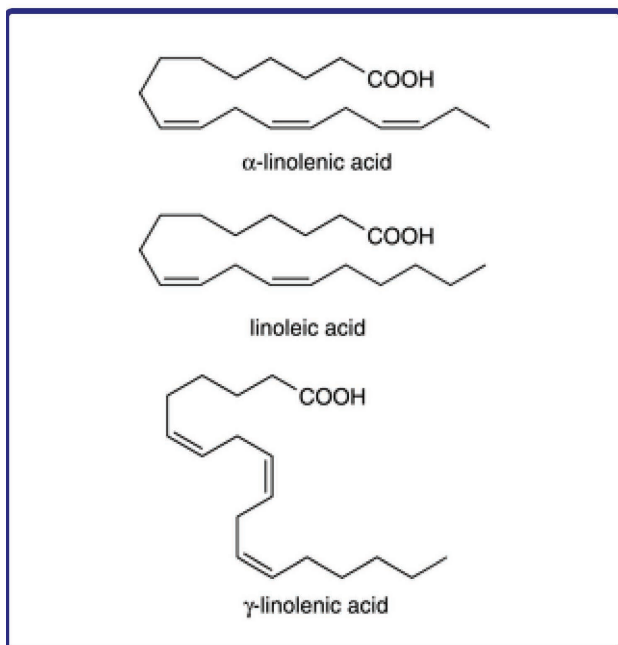
increased as compared to baseline values. Bioavailability is high, with the achievement of the highest peak of plasma concentrations after approximately 4 hours after administration and the presence of significant plasma levels for about 8 hours. The half-life of the cis-γ-linolenic acid is significantly lower in the evening dose (2.7 h) compared to the morning one (4 h).Serum levels of dihomo-γ-linolenic and arachidonic acid on the other hand did not increase (73).

Pharmacology

The therapeutic effects in the pathogenesis of immune and inflammatory diseases are attributed to the direct action on immune cells (74), which also contribute indirectly by interacting with the synthesis of eicosanoids.

Gamolenic acid and its metabolite dihomo-γ-linolenic acid (DGLA) are both precursors of prostaglandin E2 (PGE2), which has an inflammatory action (in an indirect way since prostaglandin is derived from Arachidonic acid, which in turn derives from GLA) and prostaglandin E1 (PGE1), which has properties that range from anti-inflammatory to immune-modulating, vasodilating, inhibiting platelet aggregation and the biosynthesis of cholesterol, hypotensive and inhibiting the elevation of cyclic AMP (inhibits phospholipase A_2).

Dietary supplementation with gamolenic acid induces a marked increase of DGLA, while the increase of arachidonic acid concentrations is much smaller; the lower transformation into arachidonic acid is related to PGE1 and 15-hydroxy-DGLA. The latter metabolite inhibits the conversion of arachidonic acid to inflammatory metabolites by blocking lipoxygenase, while PGE1 inhibits the enzyme phospholipase A_2 which is involved in arachidonic acid mobilization from phospholipid membrane deposits. In addition, the desaturation of DGLA into arachidonic acid is a limiting factor in humans and stands out for a very slow process (40).

In vivo studies

The topical application on the skin (of a pig) for six weeks is known to increase cell proliferation (75). In rats fed a diet rich in Oenothera b. oil and subsequently exposed to radiation he protective effect on skin damage was evaluated; plasmatic levels of free fatty acids in erythrocytes and plasma were also measured. It was observed that this oil reduces skin sensitivity to phlogogenic effects and increases blood flow. In animals treated with Oenothera b. oil, plasma levels of linoleic, gamma-linolenic, arachidonic and dihomo-γ-linolenic acid increased significantly and in their erythrocytes there was a significant increase in gamma-linolenic and linoleic acid (76).

Clinical trials

In clinical trials, the administration of Oenothera oil in atopic patients has produced conflicting results. Positive reviews come from:

- A meta-analysis (77) carried out on nine studies with a total of 311 patients, reporting the clinical efficacy of Oenothera Biennis oil on symptoms compared to placebo.
- The study by S. Wright of 1985 (78) and the study of Stewart C of 1990 (79) reported very positive effects in cases of atopic eczema of moderate and severe grade; a significant improvement was reported in itching after administration, and a reduction of steroids, antibiotics and antihistamines use was also reported in terms of both oral and topical use.
- A randomized, double blind, placebo-controlled trial of 99 patients evaluating the efficacy of oral administration of oil in the symptomatic treatment of atopic eczema. Patients treated with 2-4 g per day of oil for 12 weeks showed an improvement of 30-45% in the overall severity of the eczema, including a significant reduction of itching and scratching (p <0.002), compared to those who received placebo (80).
- A clinical double-blind study carried out on 51 chil-

FIGURE 2. In atopic subjects lacking the enzyme Δ6 desaturase, normal levels of linoleic acid may be observed as well as low levels of α-linolenic acid; moreover, since GLA is a precursor of both PGE1 and arachidonic acid, its shortage causes the inhibition of phospholipase A_2 and the balance shifts towards the secretion of flogogen metabolites (e. g., PGE_2 and leukotrienes) derived from arachidonic acid.

α-linolenic acid

linoleic acid

γ-linolenic acid

dren with atopic dermatitis (81). The trial was made up of three groups identified and treated as follows for 8 weeks:

1. One group with Oenothera b. oil (0.5 g/kg/day)
2. One placebo group,
3. A third group with a combination of 50% placebo and Oenothera b. oil

The daily dose was 0.5 g/kg/day. At the end of the trial the group treated only with Oenothera b. oil showed a significant improvement in clinical symptoms with an increase in the percentage content of n-6 fatty acids in erythrocyte membrane and dihomo-γ-linolenic acid in plasma. No changes were observed in microviscosity the erythrocyte cell membrane, despite the increase of long-chain polyunsaturated fatty acids in it.

- A meta-analysis of nine clinical double-blind trials on the effectiveness of Oenothera b. oil in atopic eczema. From the analysis it appeared that the use of this oil allowed to obtain statistically significant clinical improvements (p <0.001), thus restoring of cis-linoleic acid and its metabolites plasma levels almost to normalcy (82).

In contrast, no significant benefits were detected in the following:

- Bamford JTM in 1985 (83): In a double-blind study vs. placebo carried on 123 patients (by administering 2-4 g for children, 6-8 g for adults, daily for 4 weeks);
- Berth-Jones J in 1993 (84): In a double-blind study versus placebo carried out on 102 patients;
- Whitaker in 1996 (27): In a double-blind study versus placebo, evaluating the efficacy of Oenothera oil in the treatment of 39 patients with chronic dermatitis in the hands; the patients were administered 6 g of oil or placebo for 16 weeks.

Dosage

The oil daily dose (WHO) is 320-480 mg/die (calculated as γ-linolenic acid) divided into two administrations, preferably in between meals.

The doses recommended by Barnes for the treatment of atopic eczema are:

- 160-320 mg of gamoleic acid daily divided into two doses, preferably between meals for children aged 1 to 12 years;
- 320-480 mg of gamoleic acid daily divided into two doses and preferably in between meals for adults; such therapy is indicated for a period of at least three months.

Other authors propose the following dosage: 6-8 g per day (adults); 2-4 g per day (children). It may be necessary to extend the treatment for three months before any clinical response may be observed (48).

The doses are based on a standardized content of 8 % gamolenic acid.

Toxicity

The following symptoms were reported: headache, nausea, diarrhea.

Toxicity studies indicate the lack of toxicity.

The World Health Organization at the Uppsala Monitoring Centre (WHO-UMC; Collaborating Center for International Drug Monitoring) has received reports of suspected adverse reactions by national pharmacovigilance centers in more than 70 countries around the world. In late 2005, the WHO-UMC Vigisearch database contained a total of 291 reports, reporting a total of 489 adverse reactions to products containing Oenothera biennis.

The reports are not homogeneous, at least as regards the origin or the likelihood that the pharmaceutical product caused the adverse reaction.

Interactions with drugs

Oenothera b. oil should not be used together with anticonvulsant drugs (especially phenothiazines) because it can lower the threshold at which the seizures occur. The WHO recommends the use of this oil during pregnancy, breastfeeding and in childhood only upon medical indication.

Precautions and contraindications

Patients taking anticoagulants should be monitored in that Oenothera b. oil inhibits platelet aggregation (44) while it also inhibits the platelet aggregation factor (85). Oenothera b. oil can cause the onset of seizures in undetected temporal lobe epilepsy; moreover it can also worsen the symptoms of patients with schizophrenia or those taking epileptogenic drugs such as phenothiazines (WHO).

Pregnancy and breast-feeding

Animal studies have shown that Oenothera oil is not teratogenic; however, data on the safety in human pregnancy are not available and therefore patients should be advised not to take Oenothera oil during pregnancy, unless the potential benefits should exceed the potential damage.

Both LA and GLA are normally present in breast milk and it has been calculated that an infant receives a greater proportion (mg/kg) of LA and gamolenic acid from human milk than from Oenothera oil ingested by the mother during breastfeeding. Therefore it is reasonable to assume that Oenothera oil may be taken during breast-feeding because it is very difficult that it could end up in the mother's milk at toxic doses (86).

Borago officinalis L.

It is an annual herb belonging to the family Boraginaceae, originally from Syria but nowadays wide-

spread in Europe and America. The term "Borago" comes from the Latin "borra", referring to the hair that covers the leaves. The drug is derived from the seeds from which the oil is obtained by cold pressing (like all oils rich in unsaturated fatty acids, it preserves badly).

Its constituents are:

- alkaloids: they are pyrollizidine alkaloids i. e. lycopsamine, intermedine, acetyllycopsamine, acetylintermedine, amabiline, supinine and thesinine (unsaturared). The reported concentration is 0.01% (that is 2-10 ppm for dry samples). The concentration is the same for fresh and dry samples; alkaloids are present in the fresh samples of roots as free base and are present in leaves as N-oxides (39 – 61);
- mucilage: it represents 1%; glucose, galactose and arabinose are the main ones;
- oil: it is rich in EFA i. e. 35-40% linoleic acid and 22-24% GLA;
- other constituents: acids (acetic acid, lactic acid, malic acid, silicic acid), cyanogenic compounds and tannins (up to 3%).

Characteristics of borage oil and its role in the pathogenesis of AD

In atopic dermatitis, there are skin barrier defects which cause transepidermal water loss (TEWL) and a higher permeability to irritating substances and allergens.

It is assumed that the altered activity of the delta-6 desaturase enzyme leads to a reduced transformation of linoleic acid in GLA, resulting in an altered incorporation of EFA in membrane phospholipids, which maintain the barrier structural integrity and control the permeability and alterations of the structure of associated proteins (receptors and enzymes) (22)(29)(72)(84).

EFA anomalies could contribute to the development of the disease through a direct effect on the skin structure, influencing the maturation and the sensitisation of the immune system (active eicosanoids, such as prostaglandins and leukotrienes, are EFA derivatives, and control the inflammatory, immunological and proliferative response).

Until now the most common source used to check this hypothesis has been Oenothera oil, but its use has led to conflicting results.

More recently borage oil has been taken into consideration due to its high GLA content, that is two to three times higher than in oenothera oil (87 - 88). However, it should be pointed out that the studies carried out using only borage oil or mixtures of borage oil with other oils could not be valid for the oil considered individually, because the activity of the different oils that contain GLA is influenced not only by the GLA content but also by the the position of GLA on triglycerides and by the percentage of omega-6, -3, -9 and other fatty acids present in oil (89).

Borage oil contains high levels of omega-6 and no omega-3 (which are less important for the skin) (Connor WE. 2000); it should be pointed out that the desaturase bond is competitive, therefore an increase in the levels of omega-6 can influence the metabolism of omega-3 and vice versa.

The GLA content in borage oil is higher than in most of similar oils, with blackcurrant oil (GLA 12-20%) as the only possible exception, and is consistently different from oenothera oil, which normally contains 70-80% of linoleic acid and 8-12% of GLA (it is not clear whether the higher content of GLA in borage oil can directly translate into in vivo efficacy and an increased biological activity).

The linoleic acid contained in borage oil plays a direct role in maintaining the integrity of the skin barrier (90) (19) and significantly improves the barrier function with an average decrease of 11% of TEWL (91).

The main metabolite of linoleic acid in the skin is the 13-hydroxyoctadecadienoic acid (13-HODE), which has an antiproliferative action, borage oil produces similar or lower levels of 13-HODE in the skin, compared to safflower oil (in animal models) (1).

However, the therapeutic effects of borage oil mainly depend on the derivatives that are more "downstream" in the metabolic chain of omega 6, namely GLA and DGLA.

Many studies have shown that atopic dermatitis is often linked to an increase in the levels of linoleic acid and decreased levels of GLA, DGLA and / or arachidonic acid, supporting the hypothesis of a dysfunction of the delta 6 desaturase enzyme.

Such EFA imbalances were found:

- in the blood of the umbilical cord of newborn babies who later developed atopic dermatitis (92);
- in the breast milk of mothers of children who later became atopic dermatitis patients (37) (93);
- in the adipose tissue, in the serum and plasma phospholipids, in the erythrocytes and monocytes of children and adults with atopic dermatitis (65) (72) (94) (96) (97).

These data seem to show the potential therapeutic importance of borage oil which apparently provides directly a high level of GLA; the GLA content is also important because linoleic acid is not converted into GLA in the skin to a significant extent (only 5-10%) because the desaturase enzyme is not present in a sufficient quantity.

Borage oil does not contain DGLA or arachidonic acid, however GLA is rapidly converted into DGLA in the body by the elongase enzyme, so the GLA deriving from borage oil administered orally increases the DGLA levels in phospholipids.

GLA supplementation increases DGLA levels in

the skin, increasing the production of PGE1 and 15-hydroxyeicosatetraenoic acid (15-OH-DGLA) (98).

The increase in the production of PGE1 regulates the immune response and inhibits phospholipase A1, which contributes to the release of arachidonic acid during the inflammation of cell membranes, producing eicosanoids, that have a proinflammatory action (see LTB4).

The relation between DGLA, arachidonic acid and their metabolites seems to be one of the key factors that results into a pro- or anti-inflammatory effect.

Since the activity of the delta-5-desaturase enzyme that converts DGLA into arachidonic acid in the skin is absent, borage oil supplementation through oral administration causes a slight increase of arachidonic acid in phospholipids and a much higher increase in DGLA levels, the presence of high levels of DGLA hinders the conversion of arachidonic acid into pro-inflammatory metabolites; the ability of borage oil to suppress the generation of LTB4 is mainly mediated by 15-HETRE.

Clinical trials on borage oil

A review (99) was carried out, analysing clinical trials that were performed exclusively with borage oil administered orally (100 – 109) for the treatment of atopic dermatitis. In another study supplementation was performed on newborn babies at risk of developing atopic dermatitis (110). The total number of patients was 812. All tests were controlled and in most cases they were double-blind randomised trials, however a minimum number of 120 patients, necessary to achieve a statistically reliable result, was reached only in three trials (85) (110) (118). Many studies were based on heterogenous patients without precise exclusion criteria and a wide variety of measures was used for the results obtained (this shows the current lack of consensus on how to measure the severity of the disease). All trials were carried out in Europe.

A significant effect was highlighted in five trials (70) (101) (106) (108) (119), five trials proved the inefficacy of the treatment (85) (102) (104) (105) (107) and in the remaining trials the therapeutic response was noticed only in some patients. Most of the trials showed a slight improvement of the symptoms, and unfortunately the data from studies that showed significant improvements are not reliable due to the low sample size and methodological limitations.

An important trial was carried out by Takwale et al. in 2003; in this trial, that was well designed and adequately supported by an adequate number of cases, a high dose of borage oil was administered orally (4,000 (GLA 920) mg / day for adults; 2,000 (460) mg / day for children) for 12 weeks. The trial showed that borage oil has no efficacy, since the results obtained from the placebo differed only little from the verum group; however the authors concluded that their data cannot exclude the possibility that borage oil can have a moderate beneficial effect.

In the trial by Henz et al. about one hundred patients were treated with borage oil administered orally at the dose of 1,500 mg twice a day(GLA z 690 mg / day) or placebo for 24 weeks. There were no significant differences between the verum group and the placebo group after 24 weeks of treatment at the primary efficacy endpoint, which consisted in the total quantity of corticosteroid used until that moment (defined as a 50% reduction in the Costa Score); some improvements were reported in regard to the rash, blisters, scabs, lichenification and insomnia, but not in regard to itchiness, which is the most disturbing symptom.

It is important to highlight that this trial identified a subgroup of patients with an adequate systemic absorption and GLA metabolism, for which borage oil produced a significant effect. The results were difficult to interpret because they varied significantly in the four treatment centres that were involved, nevertheless this trial is highly important for the large sample size; besides it was one of the few trials that defined a homogeneous group of patients (including only patients with a moderate atopic dermatitis, up to 14 years of age).

Only a small trial compared borage oil with evening primrose oil in similar dosages of GLA (10 patients for each group) (106); the results are controversial due to the lack of placebo, however they seemed to show a response rate of 90-100%.

Dose

The following doses were reported for adults: 3.7 – 5 grams of oil per day, containing:
- 23-37% of linoleic acid;
- 18-27% of gamma-linolenic acid;
- 0.2-10% of alpha-linolenic acid.

Side effects and toxic effects

None documented, however it contains low concentrations of unsaturated pyrrolizidine alkaloids, which are known to be hepatotoxic both in animals and in man.

Interactions with drugs

No interactions were documented, however the therapeutic effects should be carefully considered to avoid possibile interactions with co-administered drugs, in particular with those that have similar or opposite effects, so the different synergies will be evaluated below.

Borage oil:
- I can increase the risk of bleeding if taken with drugs that increase the risk of bleeding, see Warfarin. An increase in the risk of bleeding was reported in

combination with Ginkgo biloba, Allium sativum and Serenoa repens.
- can have anti-inflammatory properties, so it should be used with caution in combination with NSAIDs due to possible additive effects;
- can reduce the levels of plasma triglycerids and can increase the concentration of HDL cholesterol, so it should be used with caution in patients that take red yeast rice, due to possible addictive effects;
- can alter heart function, so it should be used with caution in patients with heart diseases or who take plant drugs or drugs with effects on the heart;
- can alter the immune responses, so it should be used with caution in combination with other immunomodulators;
- seems to have antibacterial effects against H. pilori, so it must be used with caution in combination with plant drugs and drugs that can have an antibacterial or an antiulcer activity (111).

Contraindications

Like oenothera, the oil must be used with caution in patients with epilepsy, especially in patients with schizophrenia and / or patients who use phenothiazines, because it contains high concentrations of gamolenic acid, so it could cause seizures. Due to the presence of pyrrolizidine alkaloids, excessive or prolonged ingestion of borage should be avoided.

Pregnancy and breastfeeding

Considering the presence of pyrrolizidine alkaloids and the lack of toxicity data, borage should not be used during pregnancy and breastfeeding. In a reported case a pregnant woman who had been treated with borage seed oil turned to a doctor due to moderate abdominal meteorism. GLA could alter the production of breast milk.

Discussion on the use of oenothera oil and borage oil
- Until now EFA supplementation trials in atopic dermatitis have had conflicting outcomes and the licence for the use of oenothera oil that was initially granted as drug for atopic dermatitis was withdrawn in the United Kingdom in 2002, after an inspection by the United Kingdom Medicines Control Agency and by the drug safety committee, due to the lack of efficacy tests (75);
- borage oil could be more effective because of its much higher GLA content, but it also does not comply with the strict efficacy standards, even when administered at high doses; however it should be pointed out that most of trials carried out with borage oil in atopic dermatitis have shown at least a moderate efficacy and a subgroup of patients has had a statistically important improvement;
- the effect of the treatment with GLA has been de-

scribed as moderate and not clinically important, that means a 1.5 reduction in Costa score values or a 5% reduction in severity.
- The current guidelines for atopic dermatitis do not support the use of borage oil or any other EFA supplementation, because the efficacy has not been clearly proven.

In the review carried out in 2013 (*Cochrane Database Syst Rev.*) 27 trials were examined with a total of 1,596 participants; 19 trials of which had been carried out on oenothera oil and 8 of which on borage oil.

There is no clinical evidence of efficacy for the oral administration of borage oil and oenothera oil in eczema; the improvement has been similar to that obtained with the placebo, besides they have the same light and temporary side effects, mainly gastrointestinal side effects.

According to a case report, if oenothera is taken for a prolonged period (more than a year), there is a potential risk of inflammation, thrombosis and immunosuppression; another study showed that it can increase bleeding in patients receiving warfarin therapy.

Later two trials highlighted that the dose of oenothera oil can affect the results, in particular the trial by Bo Young Chung of 2013 (112) aimed at finding out the useful dose and the duration of the treatment. To this end, forty patients were enrolled and divided casually in 2 groups: a group received 160 mg of oenothera oil per day for 8 weeks, while the other group received 320 mg of oenothera oil twice a day for 8 weeks. Later, all patients were given scores according to the EASI score (Eczema Area Severity Index) at week 0, 2, 4 and 8. Then the levels of fatty acids in serum were evaluated, divided into palmitic acid, linoleic acid, linolenic acid and arachidonic acid, with the gas chromatography method. The authors observed higher levels of linolenic and arachidonic acid in serum in the group treated with 320 mg, compared to the group treated with 160 mg. After the treatment the EASI scores of the two groups decreased, however the improvement was higher in the 320 mg group than in the 160 mg group. No side effects were observed in both groups during the trial.

The trial by Simon D. of 2014 (120) evaluated whether oenothera oil supplementation caused a GLA and DGLA increase in plasma, the clinical improvement was assessed with the SCORAD index (SCORing Atopic Dermatitis). The trial (open-label-trial) included 21 patients. EPO was administered (4-6 g per day) for 12 weeks. Before the treatment, 4 and 12 weeks from the start of the EPO supplementation, the SCORAD index and the GLA and DGLA concentrations in plasma were evaluated. At 4 and 12 weeks from the start of the treatment there was a significant GLA and DGLA increase in plasma and a SCORAD

decrease. A significant inverse correlation was found beween changes in GLA plasma levels and SCORAD (P = 0.008). According to the conclusions of the authors, the activity of the treated clinical disease is linked to the increase of GLA plasma levels. Therefore the outcomes of this pilot study highlight that a GLA increase in plasma could be used as a predictive parameter for the efficacy of the EPO therapy.

Supplements and herbal drugs, whose use may improve DA symptoms

Astaxanthin

Astaxanthin, a xanthophyll derived from the microalgae Haematococcus pluvialis has been investigated for its properties on the skin in two clinical trials in 2012 (113).

In the first trial (an open, uncontrolled trial), 30 healthy female subjects were tested for 8 weeks; significant improvements were observed by administering an oral supplement of astaxanthin (6 mg/day) and using 2 mL per day topical application of astaxanthin, significant improvements were noticed as regards skin wrinkling, size of age spots, skin elasticity, skin texture, moisture content of the stratum corneum (corneocyte layer) and corneocyte condition. The authors concluded that astaxanthin could improve skin condition in all the layers by combining oral supplementation and topical treatment.

In the second trial (a randomized double-blind trial vs. placebo), 36 healthy male subjects were tested for 6 weeks; after oral administration of 6 mg/day of astaxanthin, skin elasticity and transepidermal water loss (TEWL) were evaluated. An increase in the aqueous and oily percentage of sebum was observed; therefore also the oral administration alone induces an improvement of skin hydration and the hydrolipidic film.

A recent trial (121) assessed whether astaxanthin (AST) can improve the inflammation and itching in a murine model of dermatitis similar to atopic dermatitis, using NC / Nga mice. The trial was conducted using olive oil as a placebo. The effects were assessed through:
- behavioural observation;
- clinical situation of the skin (defined in degrees according to a severity scale);
- serum levels of IgE;
- skin histological analyses;
- reverse transcription polymerase chain reaction (RT-PCR) and Western blotting analysis for inflammation-related factors.
- Astaxanthin (100 mg/kg) was orally administered once a day and three times a week for 26 days.
- The administration of astaxanthin significantly reduced:
- the clinical severity score of the skin;

- spontaneous scratching;
- the level of IgE in serum;
- the number of total eosinophils and degranulated mast cells in the skin.

A significant reduction in mRNA levels and protein levels of eotaxin, MIF, IL-4, IL-5 and L-histidine decarboxylase was observed in the skin of treated mice.

These results suggest that astaxanthin can improve dermatitis and itching through the regulation of inflammatory agents and the expression of inflammatory cytokines.

Astragalus membranaceus L.

The *Astragalus membranaceus* is a plant belonging to the *Fabaceae* family and is known for its ability to modulate the immune response. A trial (114) investigated whether astragalus can suppress the skin lesions induced in mice by DNFB. 2,4-dinitrofluorobenzene (DNFB) is a chemical antigen that, when administered subcutaneously evokes a clinical situation similar to atopic dermatitis (AD) in NC/Nga germ-free mice.

Oral administration inhibits the increase in ear thickness and skin lesions induced by DNFB; moreover, the production of IFN-gamma by CD4 (+) lymphocytes coming from the lymph nodes was significantly inhibited by treatment.

A more recent trial (115) analyzed Dangguibohyultang (DBT), an herbal formula consisting of *Astragalus membranaceus* and *Angelica sinensis* in a ratio of 5:1 traditionally used for allergic diseases. The effect of DBT on the skin reaction in an atopic dermatitis model was investigated; the treatment with the combination of 2 plants induced the remission of clinical symptoms, namely a decrease in skin thickness, scratching, total level of serum IgE and the number of mast cells. The levels of cytokines (IL-4, IL-6, IFN-y, TNF-alpha and IL-1β) and inflammatory mediators (NF-kβ, phospho-IKBA and phospho-MAPK) were significantly decreased by the use of both individual plants and the combination of the 2 plants; however the combination induces a significantly greater improvement.

Korean red ginseng

The Korean red ginseng was recently studied (116) in a murine model of atopic dermatitis to assess its ability to suppress itching and inflammation. Thirty NC/Nga mice were randomly divided into 5 groups and the typical skin lesions of atopic dermatitis were induced by percutaneous administration of TNCB on the ears and backs of the NC/Nga mice. Red ginseng extract, oenothera oil, cyclosporine and phosphate-buffered saline were orally administered through a gastric tube. Each group was further divided into subgroups, in which scratching was allowed or inhibited to evaluate the impact of the habit to scratch. The ef-

fects were evaluated by measuring the clinical severity score, ear thickness, the magnitude of the transepidermal water loss (TEWL), the number of scratching movements, systemic values of immunoglobulin E (IgE) and interleukin (IL-31), histological changes of skin lesions, the levels of mRNA expression of (TNF-α), interferon (IFN-γ), thymic stromal lymphopoietin (TSLP) and IL-31. Korean red ginseng was observed to exerts their effects by inhibiting any Th2-mediated inflammation (T helper 2), as well as decreasing the itching feeling (the reduction of the scratching behaviour suppresses the vicious cycle that, starting from itching, leads to the habit of scratching even when itching has disappeared thus, paradoxically, originating itching again).

Resveratrol

A trial (104) investigated the effect of resveratrol (a polyphenol with immumodulatory action) in a murine model with lesions similar to the atopic dermatitis lesions induced by the application of the dust mite extract on the dorsal skin of NC / Nga mice. Resveratrol was subsequently administered (20 mg/kg) daily for 2 weeks. Later, the severity of the dermatitis was evaluated, as well as histopathological alterations, serum levels of T helper (Th) interferon (IFN-γ), interleukin-4 and the changes in protein expression by Western blotting for HMGB1, receptors for the final products of advanced glycation (RAGE), toll-like receptor (TLR4), nuclear factor NF-Kβ, PI3K, ERK, COX 2, tumor necrosis factor (TNF-α), IL-1β, IL-2RA and other inflammatory markers in the skin. The treatment with resveratrol inhibited the growth of DA-like skin lesions. The histological analysis has shown that resveratrol inhibits the hypertrophy, intracellular oedema, migration of mast cells and infiltration of inflammatory cells. Furthermore, the treatment blocks the production of HMGB1, RAGE, P- NF-kβ, p-PI3K, p-ERK1 / 2, COX2, TNF-α, IL-1β, IL-2Rα, IFN-y and IL-4.

A trial carried out in 2016 (117) evaluated the effects of resveratrol on epithelial-derived cytokines and apoptosis of epithelia in a murine model of atopic dermatitis induced by repeated application of 2.4-dinitrofluorobenzene on the shaved dorsal skin. Twenty-one BALB / c mice were divided into three groups and one group was systemically administered resveratrol (30 mg/kg/day) more times a day for six weeks. Later, the mice were sacrificed and their skin tissues were histologically examined; the epithelial apoptosis (caspase-3) and epithelium-derived cytokines [interleukin IL-25, IL-33 and thymic stromal lymphopoietin (TSLP)] were evaluated using an immunohistochemical method. An improvement of the epithelial thickness was observed in the verum group ($p < 0.05$); the concentrations of IL-25, IL-33 and TSLP-positive cells in the epithelium were lower in the verum group than in

the control group ($p < 0.05$). In the epithelium, the number of caspase-3-positive cells, used as an indicator of apoptosis, were significantly lower in the verum group than in the control group ($p < 0.05$). The treatment with resveratrol is therefore effective in the improvement of histological alterations and inflammation, since it acts on epithelium-derived cytokines.

Oolong Tea (Camellia sinensis)

A trial (85) tested the effectiveness of the Oolong Tea in 121 patients suffering from unresponsive atopic dermatitis and 118 patients completed the open trial. Patients were advised to maintain their traditional drug treatment; however they were instructed to drink oolong tea using a 10-gram tea bag soaked in 1,000 ml of boiling water for 5 minutes. This dose was then divided into 3 equal portions and each portion was administered after the 3 regular meals. Photos of 2 or 3 lesion sites were taken at the start of treatment and after 1 and 6 months, and the itching severity was assessed on a Likert-type 6-point scale. After 1 month of treatment, 74 (63%) out of 118 patients showed a marked to moderate improvement. The beneficial effect was still observed after 1 or 2 weeks of treatment. The good response to treatment was still observed in 64 patients (54%) after 6 months.

CONCLUSIONS

Atopic dermatitis is a disease of multifactorial aetiology, caused by a combination of genetic and environmental factors. The data about borage oil in its treatment lead to the following conclusions:

- borage oil has not been compared to the pharmacological treatments of atopic dermatitis and borage oil's role would be to aid in the treatment, along with continued use of topical products with softening effect, to control the inflammation and prevent mild-to-moderate disease outbreaks; it does not play therefore the role of medication in the treatment of acute exacerbations or severe diseases, where the use of topical corticosteroids or other immunodulators is recommended;
- use of borage oil may be decided when a patient expresses interest in a more natural treatment approach rather than the use of traditional treatments. In this case, nutritional supplementation with borage oil may be recommended along with the continued use of softening lotions for a trial period of 8 to 12 weeks if the disease is not serious (99); treatment may need to be extended for up to three months before observing a clinical response (48);
- there must be a realistic expectation of the (small) benefit that can be obtained and, in case of worsening of the disease, treatment should be discontinued;

- dosages should not exceed the dosages tested in clinical trials (1-2 g/day for children and 2-4 g/day for adults) for borage oil (99), 6-8 g per day (adults); 2-4 g per day (children) for oenothera oil (65) and, more generally (5), 320 mg/day for children and 480 mg/day for adults (calculated as GLA) divided into two administrations preferably between meals;
- the big advantage is a good tolerance, which may attract people worried by the negative effects of corticosteroids.

However the true fact that the definition of the disease is controversial (see atopic dermatitis and atopiform dermatitis) leads me to think that, sometimes, the results obtained in the various experiments with vegetal supplements that do not have the action potential (but not even the negative, systemic and local, effects) of corticosteroids or immune response mediators may be due to a lack of clarity in defining the groups of patients for which they are used.

Therefore, the supplementation with GLA-rich oils could exert a stronger action if the disease was substantially derived from an enzyme deficiency and an element of confusion may arise from the age of patients, because it is plausible that supplementation is more effective in the ages at which the enzyme is less functionally active.

To clarify these issues, please refer, therefore, to numerically useful trials, selected by age and geographical location (in order to have a uniform environmental element) and carried out using the double-blind vs. placebo method.

Finally, *in vivo* and *in vitro* trials of various drugs or substances of vegetable origin (e. g., astaxanthin, resveratrol, *Astragalus,* Red ginseng, Oolong tea) should be considered, since they open up new therapeutic possibilities, but which must be validated by appropriate clinical trials before being combined with conventional therapy.

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The role of nutrition and Nutraceutical for the well-being of skin.

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ABSTRACT

While the nutritional approaches linked to cardiovascular system, autoimmune system and gastrointestinal tract's functionality is enough investigated instead the skin organ is gaining scientific interest in the last years. The established belief that using food supplements combined with a correct nutritional diet can improve the beauty of skin as an organ is gaining ground in the minds of many of dermatologists.
That's why nutraceuticals is becoming an important tool to control oxidative stress and promote a healthy skin. The key concept that vitamin C, which can only taken through diet, is one of the most important micronutrients, since it is involved in the regulation and control of collagenases could be taken as a starting point, along with the fact that foods or supplements containing polyunsaturated fatty acids (PUFA) such as omega-3 have been prescribed in the last few years in different ways for their clinical effectiveness not just on the signs of aging but also in the prevention of aging.

KEYWORDS: Hyaluronic acid, collagen, vitamin C, vitamin E, healthy skin barrier skin

History, traditions, empiricism, but also epidemiological studies and more recent clinical studies confirm the close link between nutrition and health, nutrition and beauty, power and longevity. From a strictly scientific point of view, it is now very clear, if you consult a qualified search engine (PUBMED), that the link between diet and skin is definitely the least investigated, compared to other similar initiatives that have focused nutritional importance to protect and control the disease of the cardiovascular system, cancer, autoimmune diseases. But as any other tissue, skin requires an optimal supply with nutritive compounds including macronutrients such as lipids, amino acids or carbohydrates and micronutrients including vitamins and essential minerals (1)

And therefore attention recently guidance of many pathways and cofactors that are involved in biological mechanisms of the skin, regulating its cellular metabolism, and that today allow us to state that various skin conditions are also regulated by dietary habits.

Food is our first "nutraceutical", since we find all or most of the elements and molecules needed to our whole body functioning. In case of increased metabolic demands, decreased intake, and nutritional deficiencies people could rely on the assumption of Dietary Supplements. Picking out the "right combinations" it can be managed a series of deficit or altered situations that often are the basis of many skin disorders. For example, it has been shown that a supplement mixture consisting of carotenoids, vitamin E and selenium increases skin density and thickness when ingested over a period of 12 weeks. Also skin surface parameters including scaling and roughness were improved upon supplementation (2).

All components of this supplement are either direct or indirect antioxidants. It has been suggested that under stress conditions topical and/or systemic application of antioxidants contribute to the maintenance of healthy skin barrier.

Oxidative stress has been shown in many dermatological diseases, including vitiligo, atopic dermatitis, alopecia areata, photoaging,

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carcinogenesis, and chemotoxicity. Most free radicals in the body exist in the form of reactive oxygen species (ROS). Excessive free radicals impair not only DNA, but also cellular proteins and lipids. In living cells, ROS are continuously generated as a byproduct of oxidative energy metabolism. In addition, external stimuli, such as ionizing and ultraviolet (UV) radiation, environmental pollutants, contact allergens, and drugs, are potent inducers of ROS production. Inflammatory cytokines are also responsible for ROS generation. Since the skin is the outermost organ of the body, it is inevitably exposed to UV rays and environmental pollutants. These external stimulants induce oxidative stress, adversely affect the proper differentiation and barrier function of the skin and accelerate skin aging, leading to skin inflammation and carcinogenesis (3).

It has been suggested that under stress conditions topical and/or systemic application of antioxidants contribute to the maintenance of healthy skin barrier (4).

Recent evidence has confirmed that adherence to a healthy diet over time reduces the risk of long-term inflammation (5, 6). In particular, Barbaresko et al. (7) reported that fruit and vegetable-based healthy dietary patterns were associated with lower biomarkers of inflammation, such as CRP levels.

The traditional Mediterranean diet (MD) is a healthy diet characterised with the abundance of vegetable foods and cereals, such as green and yellow vegetables, salads, legumes, bread, pasta, fruits and nuts (8). MD is a highly palatable and favourable diet and may lead to a higher adherence among dieters in the long term. EVOO is the main source of fat and the intake of fish, poultry, dairy products, and eggs is moderate. In addition, different amounts of wine are usually consumed in moderation with meals. Animal fats used in butter, cream, and lard are not included in this diet. The MD is considered a healthy eating pattern, associated with reduced risk for metabolic (9), cardiovascular (10), neoplastic (11), and chronic inflammatory diseases (12). One of the most accredited hypothesis of this association is that the high content of different beneficial compounds, such as antioxidants and polyphenols, largely present in Mediterranean foods, such as plant foods, fruits and red wine, have anti-inflammatory and antioxidant properties. The association between MD and the lowered incidence of chronic inflammatory diseases is well-supported by intervention studies with the MD (13). Considerable amounts of micronutrients such as antioxidant vitamins and carotenoids are present in the skin and are suggested to contribute to the maintenance of skin health (14).

Vitamin A is a group of unsaturated nutritional organic compounds. Vitamin A and its derivatives, *e.g.*, retinoids and carotenoids, play an important role in regulating proliferation, differentiation, and apoptosis of different cell types, including skin cells (15-17). While the beneficial effects of carotenoids are thought to be due to their role as antioxidants, carotenoids first need to be converted to retinoid forms to provide physiological functionalities in skin (18). Since epidermal keratinocytes and dermal fibroblasts express both retinoid receptors (19), skin is considered as one of the major retinoid-responsive tissues.

Vitamin C is a water-soluble, powerful antioxidant that has been shown to attenuate UV irradiation-mediated damages in the skin (20). Vitamin C significantly suppresses the UV light-triggered production of free radicals, protecting cells from oxidative stress (21). It has an additional role in wound healing by increasing pro-collagen and collagen synthesis (22, 23), which stimulate the formation of the skin barrier. In efficacy studies on human skin, vitamin C significantly increased epidermal moisture content, improving skin hydration (24).

Vitamin D is synthesized from 7-dehydrocholesterol by two key enzymes, 25-hydroxylase (CYP27A1) and 25-hydroxyvitamin D_3 1-α-hydroxylase (CYP27B1), in human skin following UVB irradiation (25, 26). A primary biological role of $1,25D_3$ in skin is the stimulation of antimicrobial defense through increasing levels of cathelicidin antimicrobial peptide (CAMP), an innate immune element (27). In addition to antimicrobial activity, vitamin D_3 significantly inhibits the proliferation of keratinocytes (28). Vitamin D appears to modulate inflammation, angiogenesis, and wound healing through regulation of CAMP production (29, 30).

Vitamin E is a lipid-soluble, membrane-bound antioxidant in multiple tissues (31). Since the level of vitamin E can be depleted even after exposure to a single dose of UV irradiation, it is a sensitive oxidative stress maker in human skin (32). A number of studies have shown that vitamin E treatment modulates UV irradiation-mediated free radical damages in skin; *e.g.*, lipid peroxidation (33), photoaging (34, 35), immunosuppression (36), and photocarcinogenesis (37). Vitamin E significantly suppresses collagen breakdown by inhibiting MMP-1 expression (38). In addition to antioxidant properties, vitamin E could downregulate features of skin inflammation; *i.e.*, attenuating production of inflammatory prostaglandin, pro-inflammatory cytokines, cyclooxygenease-2, and NADPH oxidase (39, 40), suggesting the use of vitamin E as an anti-inflammatory agent in skin.

Carotenoids are widely used as skin protectants and supplementation with carotenoids has been

shown to protect against UV-induced erythema.

Their recruitment, both through food, both in supplementation, allows the deposit of the carotenoid pigment in the epidermal layers, and then gives the skin a safe and efficient protective tool.

A great number of plants and plant extracts are studied for their antioxidative action. Flavonoids like Rutin and phenolic compounds like Hesperidin derivates also have antitumor, antiviral and antibacterial activities, and antiradical and antioxidative activities (41).

Phenolic compounds are characterized by presenting in its chemical structure an aromatic ring linked to a hydroxyl group, which has a great ability to donate electron and hydrogen, this explains their exceptional antioxidant activities (42). Among the characteristics of polyphenols of Green tea the following deserves special attention: chemopreventive and therapeutic activities in cancer treatment; prevention of lipoperoxidation in mammals; prevention of adverse effects caused by UV radiation, with a reduction of oxidative damage and reduction in metalloproteinase production (42).

Skin and mucosal surfaces of mammalian species are populated by millions of bacteria that impart diverse metabolic effects (43).

These host-associated microbes play a well-established role in homeostasis in the gastrointestinal (GI) tract (44, 45).

There is now substantial evidence linking various gut microbiota and local immunity networks with systematic effects on the immune system (46-48).

Disruption of the normal balance between microbial communities in the intestine is associated with allergic, autoimmune, metabolic, and neoplastic pathologies in the GI tract and other distant tissues (49).

An exaggerated paradigm of an organ distal from the bowel that could benefit from probiotic consumption is the skin. Interestingly, research data from both mice and humans suggest that dietary supplementation with probiotic lactic acid bacteria has beneficial effects in the skin (50). The importance of probiotic effects on skin extends beyond obvious cosmetic aspects to broader host health. Indeed, the appearance of the skin and its appendages has been considered by medicine traditions worldwide as a clinical sign of good health (50).

John H. Stokes and Donald M. Pillsbury were among the first to propose the use of probiotic Lactobacillus acidophilus cultures to treat acne vulgaris. In recent years, aspects of a gut-brain (emotional states)-skin link have been validated via modern scientific investigations. It is evident that gut microbes and oral probiotics could be linked to the skin, and particularly acne severity, by their ability to influence systemic inflammation, oxidative stress, glycaemic control, tissue lipid content, and even mood. This intricate relationship between gut microbiota and the skin may also be influenced by diet, a current area of intense scrutiny by those who study acne (51).

Minerals, including zinc, copper, and selenium, also have an important role in maintaining skin health. Zinc is an essential cofactor of numerous metalloenzymes. Its main function is to protect the skin against photodamage by absorbing UV irradiation, limiting penetration of radiation into skin (52).

Copper is known to stimulate the maturation of collagen, thus is critical in improving skin elasticity and thickness (53).

While it also plays a role in melanin synthesis enables pigmentation of skin and hair (54).

Lastly, selenium protects the skin from UV irradiation induced oxidative stress by stimulating the activities of the selenium-dependent antioxidant enzymes, glutathione peroxidase and thioredoxin reductase, that are present in the plasma membrane of epidermal keratinocytes (55, 56).

Iron deficiency can promote hair loss and nail brittleness, Sulfur, makes efficient disulfide bridges of keratin and it regulates the synthesis.

It 's very difficult to consider aging as a normal physiological process, much less to that of the skin, the body's business card. Whole Skin undergoes, over time, a series of increasingly irreversible changes: a load epidermal cell turnover slows down and the thickness decreases. These modifications affect hydration that is severely compromised. The Derma, the part below the Epidermis, rich in fibroblasts, GAG (fundamental substance and hyaluronic acid) and blood vessels, does not escape to the degenerative process. The fibroblasts slow down the synthesis of the fibers (Collagen, Reticular, Elastin), so the aged fibers are not replaced with new vital structures; Hyaluronic acid slowly decreases and with it the ability to retain water. Therefore occurring phenomena of fibrosis that result, clinically, with the wizened appearance, dull complexion, loss of elasticity and the appearance of wrinkles.

In a pilot open-label study, it has been investigated the effect of a dietary supplement, BioCell Collagen® (BCC), which contains a naturally occurring matrix of hydrolyzed collagen type II (approximately 1.5 to 2.5 kDa) and low-molecular-weight hyaluronic acid and chondroitin sulfate, in 26 healthy females who displayed visible signs of chrono and photoaging in the face. Daily supplementation with 1 g of BCC for 12 weeks led to a significant reduction of skin dryness/scaling (76%, $P = 0.002$) and global lines/wrinkles (13.2%, $P = 0.028$) as measured by visual/tactile score. Additionally, a significant increase in the content of hemoglobin (17.7%, $P = 0.018$) and collagen

(6.3%, P = 0.002) in the skin dermis was observed after 6 weeks of supplementation. At the end of the study, the increase in hemoglobin remained significant (15%, P = 0.008), while the increase in collagen content was maintained, but the difference from baseline was not significant (3.5%, P = 0.134). This study provided preliminary data suggesting that dietary supplementation with BCC elicits several physiological events which can be harnessed to counteract natural photoaging processes to reduce visible aging signs in the human face (57).

According to Riccarda Serri (58), dermatologist also founded Skineco, The International Association of ecodermatologists, pointed out that specifically it is very important to keep photo-aging under control, and in this respect we could mention, as stated by Dr Serri, the interesting section "The Latest on Skin Photo-protection," in which S. Gonzales provides an in-depth summary of the rationale for sunscreens, antiradicals, and active photo-protection, useful for preventing "visible solar scars," a good definition of photo-aging. However, photoprotectors and antioxidants can be a useful strategy for the control of physiological aging, but not as a daily step equal for every kind of skin, but it has to be personalized by a dermatologist on each patient.

A nutritional approach should not be underestimated: "The chapters dedicated to the importance of dietetic supplements ("Aging Skin and Food Supplements: The Myth and the Truth," E. Berardesca) or amino acids in "building" collagen, the most present protein in our skin ("Nutrition and Skin. Collagen Integrity: A Dominant Role for Amino Acids," by F. S. Dioguardi), prove to be most interesting.

New findings on the role and interactions that foods, the gut microbiota and nutraceuticals can have tell us that a proper nutritional balance supported by specific supplements can be a strategy to fight skin aging.

III-millennium medicine carefully and strictly follows the nutritional process and increasingly finds evidence of "good interactions" between health and beauty.

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EJAMED and SOLGAR MASTERCLASS 2017

With this special issue, EJAMeD would like to celebrate the 70th anniversary of the foundation of Solgar International, a prestigious NY-based company working all over the world with distinctive consistency in the area of nutraceuticals, and the 25th anniversary of Solgar Italia Multinutrient S.p.A., their first-rate partner for Italy and Switzerland.

The ideal of a beauty that "comes from within" is the common thread that underpins the expertise of modern dermatology and all those disciplines that, at the same time and synergistically, pursue the same ideal beauty.

Among the qualified professionals and scholars who with their articles have made this special issue possible, we would like to thank the speakers at Solgar Masterclass 2017- *Beauty and Bendessere*, namely:

Vittorino Andreoli, MD, PhD Member of the New York Academy of Sciences, Giovanni Scapagnini, Department of Medicine and Health Sciences, School of Medicine, University of Molise, Campobasso, Italy, *Immaculata De Vivo, BD*, *PhD*, Harvard Medical School, Harvard T.H. Chan School of Public Health, Boston (MA, USA), *Arrigo Selli, MD, Maria Concetta Romano, MD, Ivo Bianchi, MD, Filippo Ongaro, MD, Emanuele Bartoletti, MD, Attilio F. Speciani, MD*, *Lucia Bacciottini*, *PhD in Applied Pathophysiology* , Biologist Nutritionist, *Pierluigi Gargiulo, MD*, *Mario Vignoni, MD*, *Gianluca Pazzaglia. MD.*

Special thanks go to Professor *Richard Passwater PhD*, who for over twenty years has directed the Solgar Nutritional Research Centre, the pride and joy of the highest level of nutraceutical research, and to Professor *Peter Rohdewald, PhD*, Institute of Pharmaceutical Chemistry, University of Münster, Germany.

New trends in wellness and wellbeing.

Vittorino Andreoli, MD, PhD

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Wellbeing is a state of life we all can achieve, even (though it sounds like a paradox) when we are ill. As opposed to medicine and drugs, it is not something we resort to when we want to get rid of damage, make a problem disappear, it is a perception of our being that, as such, must be constantly fed.

To do this, as Vittorino Andreoli says, we must consider «man as someone who can live better, through a science that provides the tools to achieve such goal». In his book "La nuova disciplina del bendessere™ - Vivere il meglio possibile", he lays the grounds for this new science, for which he has coined a new name, «bendessere», meaning the study of man as a whole where the wellbeing of the body can be separated from that of the mind and from social wellbeing.

With the approach described here, everyone can not only monitor the signs of wellbeing in their own life but can put in practice anything that pursues it, at any age, depending on its needs. In a fascinating and suggestive journey that touches on multiple dimensions – from relationships to beauty – Andreoli leads us to find a peaceful, rewarding life imbued with «a humanism that, aware of our weaknesses, tries to understand in what conditions everyone can live better» and look «for the meaning of life, for the basics, which does not mean success or money»." The Center of Research for Bendessere has its application and basis through the practical manual "Bendessere and Nature" by the author psychologist Giada Caudullo, who leads the Center of Study for Bendessere.

The second book of Andreoli, i. e. "Le forme della bellezza – Viaggio nell'arte del bendessere™", emphasises how important liking oneself and being liked is for one's self-esteem.

Beauty is not just an abstract concept. It belongs to all of us, and every day it is expressed by the way we decorate our body as well as the behaviours we practise in our relational life, in our gestures, in our words. Vittorino Andreoli takes us through a journey to find the right definition of a beauty that goes beyond the prevalent criteria, and even as far as to understand ugliness. An outstanding tapestry of thoughts talking with a selection of works, through which the great psychiatrist tells the experience of beauty in all its dimensions: the world, reason, sacredness, feelings, thoughts and attitudes, such as discretion, elegance, and mystery.

From looks to smiles, from hands to the «region of Eros», this journey to the discovery of the beauty in us reveals the multitude of nuances of an ever-changing reality, from the first «creative» urges of rock painting to the enigmatic look of the Mona Lisa, through to the vibrant sensuality of the Nursing Madonna.

While today's prevalent beauty is «superficial», fleeting, unable to fulfil man's deepest needs, the idea is to overcome such deadlock by linking beauty to the so-called bendessere, the new science invented and upheld by Andreoli, which aims at improving man's life, «wellbeing as being well, living well»."
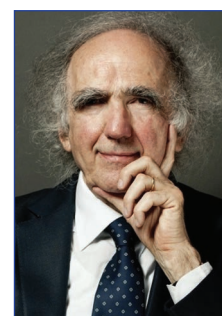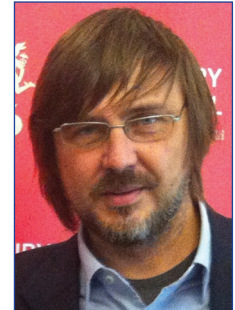
Vittorino Andreoli MD, PhD

Nutraceuticals for skin health: an update on polyphenols.

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Skin aging depends by both intrinsic and extrinsic factors. Among the latter, solar UV exposure represent the main cause of photoaging. The quality of skin aging has a great social relevance because its aesthetic impact, but also in terms of global health system, being skin cancer the most diffused type of tumor. Nutrition, providing a consistent amount of active compounds, is a direct factor affecting well-being, health and proper skin condition. Natural derived polyphenols have recently attracted considerable attention because of their skin photoprotection effects. They have been shown to modulate different molecular targets, impinging on several signalling pathways, and showing pleiotropic activity on cells and tissues. In the skin context they display anti-oxidant, anti-inflammatory and immunomodulatory activities and also control dermal extracellular matrix remodeling. In the present review, we will give an update on the recent scientific advances in the field of dermatology about the efficacy and mechanisms of action of some of the most used polyphenols.

Giovanni Scapagnini
MD, PhD

Lifestyle choices and impact on aging: the role of telomeres.

Immaculata De Vivo, BD, PhD

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Telomeres are dynamic nucleoprotein structures at the ends of linear chromosomes that maintain the genomic integrity of a cell. Telomere length shortens with age due to incomplete replication of DNA ends with each cell division (1). Telomere attrition can also occur through damage incurred by reactive oxygen species released in the inflammation process or chemical alterations to purines and pyrimidines from oxidative stress. Once telomeres shorten to a critical length, a proliferation block is encountered where the cell either undergoes programmed cell death (apoptosis) or ceases to divide (cellular senescence).

Telomere length can be considered a biological clock that limits the lifespan of a cell and an organism. Mutations in known telomere maintenance genes have been shown to cause inherited congenital disorders, such as dyskeratosis congenita, that typically exhibit very short telomeres (2).

Cases with short telomeres often have reduced lifespans. Accelerated telomere shortening has also been implicated in complex diseases that cannot be wholly explained by genetics such as cardiovascular disease, type 2 diabetes, lupus, and ulcerative colitis (3, 4). Studies have shown that lifestyle choices alter the rate of telomere shortening, impacting an individual's health and longevity.

Certain lifestyle factors, such as smoking, body mass index (5, 6), and psychological stress (7) have been found to correlate with accelerated telomere shortening, likely via

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increasing DNA damage through oxidative stress. Recently, studies have identified lifestyle factors that can potentially protect telomeres. People who lead a healthy lifestyle by increasing physical activity (8), practicing meditation (9), adhering to the Mediterranean diet (10) and multivitamin use have longer telomeres than those who do not. These studies highlight the influence of lifestyle factors on key biological mechanisms of health and aging and the importance of identifying modifiable risk factors in preventative health.

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# Human constitutions: functional and aesthetic expressions.

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Classing human beings based on their Constitution means revealing the peculiar features and explain the reason why some people suffer from some diseases and other people don't (1). Such framework is pursued by all traditional medicines, both Western and Eastern (2), and has been a guideline for non-symptomatic treatments since time immemorial. Not only the great doctors of the past (Empedocles of Agrigento, Hippocrates, Galen, and so on) but also the greatest names of modern psychology and medicine (Steiner, Jung, Eysenk, Murri, Pende, and so on) have built their theories and therapeutic approaches on the concept of Constitution (3, 4). However, in the light of today's scientific advancements, the definition of Constitution should be updated and completed by notions of genetics and epigenetics (5). An updated definition could be: *"an individual's Constitution is the variable set of physical and psychic features he/she has at a specific time in his/her life"*.

It should be noted that such framework is not a mere philosophical-nosological exercise but has clinical and therapeutic relevance to Natural Medicine (6), which by definition tends to have a global approach to the individual. It is actually known that mineral supplements have structural influence and impact on the Genetic Constitution, vitamins and fat supplements have metabolic influence and affect the Phenotypic Constitution, amino acid and herbal supplements have influence on hormones and neurotransmitters and can impact on an individual's temperamental expression and psycho-physical response (7, 8).
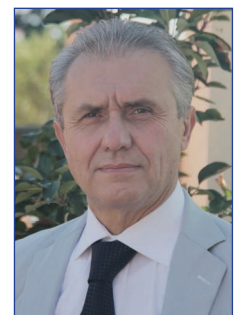
Ivo Bianchi MD

As a matter of fact, while the classic constitutional framework only included the observation of an individual's physical and psychic features, a more modern approach must be completed by considerations about the autonomic nervous system, as well as the endocrine and neurological systems (9), on which a modern supplementation made up of multiple classes of natural substances can be based upon.

The trial I conducted, with the help of sophisticated software (*medicloud*), developed in partnership with the magazine Scienza Natura, has a strictly clinical approach. Five hundred patients were ranked by constitution (1), and important medical considerations came to the fore even at a preliminary review of the patients' data:

- The highest number of patients, which means a higher morbidity rate, was found to have a Melancholic Constitution, which confirmed what Galen already stated 2000 years ago: *a negative psychological condition results in a higher morbidity rate* (10).
- Different constitutions are a predisposing factor to a variety of conditions (11, 12); in particular, a review of such data found that:

a. a Melancholic Constitution is associated with autoimmune problems as well as major depressive problems.

b. a Biliary Constitution is associated with a tendency to psychophysical exhaustion, often leading to viral diseases and neurovegetative symptoms.

c. a Phlegmatic Constitution is associated with a hypo-metabolic tendency that often results in hypothyroidism, asthenic obesity, dyslipidemia.

d. a Sanguine Constitution is associated with congestive and inflammatory problems. Such individuals are often diagnosed with metabolic syndrome, hypertension, asthenic obesity and several levels of inflammation.

- Depending on their Constitution, specific patients responded to specific supplements (8, 12). A review of the data shows that a general therapeutic protocol could be potentially used, once the patient's Constitution has been identified. Such guidelines can be helpful above all at a preventative stage, and each supplement should be adjusted the patient's current state.

It must be kept in mind that not only people but also all therapeutically-active substances can be ranked by Constitution. Our trial showed that there is a basic supplementation that can help each Constitution (concentrated omega 3 for a sanguine constitution, cinnamon and lipoic acid for a phlegmatic constitution, a pool of antioxidants and amino acids for a melancholic constitution, relaxing herbal extracts and serotoninergic amino acids for a biliary constitution) as well as specific natural substances, the effectiveness of which has been scientifically proven, for the peculiar symptoms of each Constitution (Rhodiola, Curcuma, Quercetin, and so on).

A beauty-protecting strategy has constitutional implications too; what fights the typical dryness and proneness to deformity of the ageing melancholic individual (Hyaluronic acid formula, and so on) could be unsuited to the decay of the sanguine individual, whose dysmetabolic drift often needs to be curbed (phytosterols, and so on) (8).

The underlying concept is therefore that, even in the area of aesthetics and anti-aging, there is no magic wand, there is no one-size-fits-all formula. Just recently, many celebrities spoke up about the detrimental effects of botulin injections and fillers that seem instead to have reduced other celebrities' wrinkles. So, there is no perfectly positive or perfectly negative treatment; there is an effective treatment insofar as it is customised to the individual's Constitution and responsiveness.

To conclude, we could say that it is not easy to identify an individual's Constitution (5), which also changes over time, sometimes as a result of specific events, so physical, instrumental and bio-humoral data need to be thoroughly examined. Some aids, such as the Medicloud software or the Neurovegetative Poster, have been designed for this purpose and can be extremely helpful. Once the individual's current Constitution has been identified, and depending on how imbalanced it is, a whole range of corrective measures can be taken, with behaviour, diet, music playing a key part too... However, specific supplements play a key role (8), because, if properly selected and measured out, they can affect virtually all of the symptoms of the constitutional imbalance and can bring an individual to that perfect

place midway between the two extremes that is called health.

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Living life fully: the new science of living well and longer.

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Living long is a conquest only if the years added to our life are a time of strength, vitality, health and joy. The current medical approach is indeed successful in curing diseases but not so much in enhancing health (1,2,3). Antiaging medicine offers a new strategy which has as prime objective the expansion of healthspan and the compression of disease to the latest stages of life (4,5,6, 7).

In order to accomplish such an ambitious goal one fundamental step is necessary: to start the fine-tuning of functionality at a much earlier stage of life, when the person is in a condition of complete health (8). This seems to be the only feasible approach to counteract the epidemic increase of chronic diseases associated with aging (9). Nutrition has a key role in regulating many epigenetic pathways involved in aging and chronic diseases. Instead of focusing on a specific diet, in antiaging medicine the attempt is to provide the body with all the nutrients needed to optimize it's metabolic machinery. Nutrition, food supplementation, physical training and emotional balance are the four core elements of an epigenetic regulation that can slow down aging and reduce the incidence of chronic degenerative diseases (10, 11).

Therefore, the objective of antiaging medicine is not to increase longevity but eventually to maintain adequate quality of life throughout the entire lifespan of a person. Today the scientific and methodological knowledge is sufficient to start using this preventive approach while science will continue to offer us new and more sophisticated diagnostic and therapeutic tools. Remaining passively anchored to old ideas and approaches is an enormous risk not only for the health of single individuals for the one of the entire society.

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New directions in Aesthetic Medicine in connection with "New Longevity".

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Over the last few years, the average age has constantly increased, and this of course has made people more aware of their own wellbeing and quality of life.

In this scenario, a key role is played by Aesthetic Medicine, since its main purpose is to support patients through different stages of life and assist them with healthy, controlled, general and skin ageing.

More and more patients are going to Aesthetic Doctors not just for 'a little' correction of their imperfections, but also to ask for a medical prescription that can help them improve the quality of their life according to their age, in the attempt to preserve the best physical and psychic conditions over the years. The true purpose of Aesthetic Medicine is actually to provide patients with a tailored health and behavioural programme for life that must include healthy eating, physical exercise based on age and ability, fighting stress and cosmetic skin protection, which made Aesthetic Medicine a sort of Preventive Medicine.

The rise in life expectancy and the resulting rise in the number of old patients in Society has brought to the fore a number of age-related health problems, so that there an increasing need to deal with a higher number of geriatric conditions. In addition to such focus on old people's health, there is increasing awareness of the medical-aesthetic treatment of skin aging.

Just little more than 40 years after the birth of Aesthetic Medicine, there are now a lot of treatments and techniques in Italy to reduce the signs of aging. In addition to the well-known bio-stimulation (1–3) and filling treatments, regenerative medicine methods, the new frontier of aesthetic and anti-aging medicine, are gaining ground.

Furthered by recent trials on fat tissue and the discovery of stem cells (4–5) in its stromal vascular fraction, as well as by advancements in the use of platelet-rich plasma, bio-stimulation has changed. While beforehand it only used biocompatible heterologous substances, now bio-stimulation can be performed with perfectly autologous substances (6–10), i. e. substances taken from the patient, processed and then injected back into the patient.

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In my lecture I will describe my experience as an aesthetic doctor, focusing on the treatment of over-60s, looking at the results of each treatment and following the changes that happen at different stages of life.

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The knowledge of food-related inflammatory cytokines (BAFF and PAF) as innovative tools for the achievement of well-being and beauty.

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Food is the primary source of energy, necessary for everyday activities and for the achievement of well-being, although often considered as an enemy. The term "food intolerance" has been frequently used incorrectly, often in a non-scientific and outdated way. Over the last years, our research group has been studying food-related inflammation and has realized that wrong eating habits can induce or maintain conditions or specific symptoms, including type 2 diabetes, obesity, colitis, autoimmune diseases, inflammatory skin diseases and even aging. The nutritional control of food-related inflammatory cytokines (BAFF, B Cell Activating Factor and PAF, Platelet Activating Factor) allows any doctor to reduce the speed of aging, to improve the timing of wound healing and to control many oxidative processes, supporting any kind of therapy with specific actions in aesthetic medicine, in aesthetic surgery.

In 2010, Lied GA (1) defined the strict correlation between the intake of certain foods and inflammation, by measuring the levels of BAFF after the ingestion of a reacting food. In 2016, Finkelman (2) in JACI stated that there is a balance between IgE and IgG in the knowledge of food antigens, and this balance determines different effects: protection, ana-
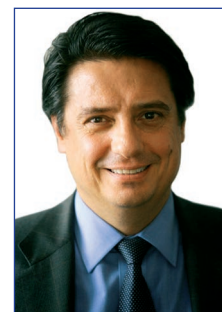
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phylaxis, simple detection and storage in memory, confirming that food-specific IgG are not antibodies "against" food but only the sign of excessive or repetitive eating, as defined also by Ligardeen (3) in 2012.

Our group (4), with the independent research of Duke University (NC - USA), defined the existence of 5 Food Groups with antigen similarities and identified both BAFF and PAF as important biomarkers of food-related inflammation. Kang (5), in 2016, showed that food-specific IgG, forming immune complexes, promote B cell memory and activate the production of BAFF from sensitized APC (Antigen Presenting Cells) thus sustaining an inflammatory response. These aspects are the scientific basis of a specific test, available in Europe under different brand-names, which measures the levels of inflammation, defines the food eaten in excess and suggests a personalized diet, which allows anyone anyone to reduce inflammation and its symptoms, as stated by Speciani and Piuri (6) in the Journal of the American College of Nutrition.

Many scientific papers, published over the very last years, have highlighted the role of BAFF and PAF and nutrition in the induction and maintenance of many skin diseases. BAFF has achieved a primary role in determining the causes of inflammatory skin diseases and autoimmune diseases, as well as Hashimoto thyroiditis (7) and Arthritis (8).

This scientific evidence shows that a personalized nutritional diet, together with the integrative help of specific supplements play a key role in the modulation of food-related inflammation, confirming the importance of nutrition as a valid and necessary tool to prevent diseases and to guarantee a rebalance of health, for the achievement of beauty and well-being.

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Beauty in female movement.

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Many disciplines of the various women's physical activities enhance the beauty, but only in the classical dance movement is properly aimed at the beauty in itself, so much to do the dance a real art. Achieving such grace needs the absolute dedication of a lifetime to accomplish art movement of bodies without perceiving the slightest effort.

Proper nutrition, not simply adequate energetic intake, is needed to achieve optimal dance performance. However, little scientific research exists concerning nutrition in dance, and so, to propose nutritional guidelines for this field, recommendations need to be based mainly on studies done in other physically active groups. Dancers may be at increased risk of poor micronutrient status due to their restricted energy intake; micronutrients that deserve concern are iron, calcium, and vitamin D (1).

Nutritional supplements that may help in achieving specific nutritional goals when dietary intake is inadequate include multivitamins and mineral, iron, calcium, and vitamin D supplements, It is important that dancers seek dietary advice from qualified specialists, since the pressure to maintain a low body weight and low body fat levels is high, especially in styles as ballet, and this can lead to an unbalanced diet and health problems if not correctly supervised. Early adolescence is period of rapid growth. Blood loss that occurs with menstruation in adolescent girls adds to the increased iron requirement of adolescence.

Dancers with possible decreased iron absorption may be affected by celiac disease which may result in nutrient malabsorption and iron-deficiency anemia (1, 2) or also may be used to follow a vegetarian or vegan diet with inadequate sources of iron: because iron from plants (nonheme iron) is less efficiently absorbed than that from animal sources, the US Food and Nutrition Board (FNB) of the Institute of Medicine (IOM) estimated that the bioavailability of iron from a vegetarian diet was only 10% versus 18% from a mixed Western diet. Therefore, the recommended dietary allowance of iron for individuals consuming a completely vegetarian diet may be 1.8 times higher than the recommended dietary allowance for non-vegetarians (3). Yet, a vegetarian diet does not appear to be associated with an increased risk of iron deficiency when it includes whole grains, legumes, nuts, seeds, dried fruit, iron-fortified cereal, and green leafy vegetables (see Sources) (4). The absorption of heme iron is less influenced by other dietary factors than that of nonheme iron (5). The absorption of nonheme iron is strongly influenced by enhancers and inhibitors present in the same meal (5).

Enhancers of nonheme iron absorption can be: i) vitamin C (ascorbic acid), that strongly enhances the absorption of nonheme iron by reducing dietary ferric iron (Fe^{3+}) to ferrous iron ($Fe2^{+}$) and forming an absorbable, iron-ascorbic acid complex (6); ii) other organic acids, like citric, malic, tartaric, and lactic acids, that have some enhancing effects on nonheme iron absorption (7).

Inhibitors of nonheme iron absorption are: i) phytic acid (phytate), present in legumes, whole grains, nuts, and seeds, that inhibits nonheme iron absorption, probably by binding to it. Small amounts of phytic acid (5 to 10 mg) can reduce nonheme iron absorption by 50%; the absorption of iron from legumes, such as soybeans, black beans, lentils, mung beans, and split peas, has been shown to be as low as 2% (3); food preparation, including soaking, germination, fermentation, and cooking, can help remove or degrade phytic acid (5); ii) polyphenolic compounds, that are present in coffee, black tea, and herbal tea, can markedly inhibit the absorption of nonheme iron (8); this effect may be reduced by the presence of vitamin C (5, 9); ii) soy protein: such as that found in tofu, they has an inhibitory
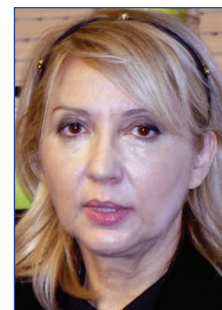
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effect on iron absorption that is only partly related to its phytic acid content (5, 9).

Iron supplements are indicated for the prevention and treatment of iron deficiency and iron deficiency anemia. A number of iron supplements are available, and different forms provide different proportions of elemental iron. In particular, iron bisglycinate, a highly absorbable form of iron, is a metal amino acid chelate, i. e. the product resulting from the reaction of a metal ion (iron) with an amino acid (glycine) with a mole ratio of one mole of metal to two moles of amino acids to form coordinate covalent bonds. Iron in this chelate is absorbed by active transport at jejunal dipeptide absorption sites. When such a chelate is ingested, intestinal uptake is significantly greater than for corresponding amounts of ingested inorganic metal salts (10). Nutritional supplements containing iron bisglycinate may help in achieving specific nutritional goals with chelated Iron can be particularly strategic in nutrient malabsorption and iron-deficiency anemia (11).

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Beauty in male movement.

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… Then, Atreus' son, wide-ruling, mighty Agamemnon, stood up before them, incensed, spirit filled with huge black rage. Eyes blazing fire…
Iliad, I, 101-104

Alexander Bain (1818–1903) stated that any behavioural study had to be based on neurophysiological grounds; he is the one who said that the mind is totally prey to bodily conditions. According to such author, movement comes before feeling, and feeling in turn comes before thinking. And this complex system especially affects and adjusts relationships (1 - 2).

The male body is movement, it is non-verbal language.

Powerful, even in its downcast or aging dimension.

But while well-defined muscles, especially in men, as the embodiment of youth and strength, are not necessarily associated with good health, they still stand as an intensive,

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effective form of communication (3).

The pursuit of such value can lead one to radicalise the beauty of the moving body: if exercise is too stressful, then cortisol levels will increase and the proteins stored in the muscles will decrease accordingly. The dramatic loss of calorie intake too, though usually resulting in a loss of fat and weight, will remarkably reduce the lean muscle tissue.

Conversely, the body, especially the male body, can and must achieve its greatest definition in a relational communication that gives purpose to its movement.

The subjective feeling of one's tone and one's Self can produce an extremely rewarding sense of wellbeing.

No longer a mere anatomical body, which is after all just an object; the body becomes a *lived body* that is evidence of our own being in the world, as an interface between us and the outer world (4).

A body, then, that communicates and lives in an unending relationship with the world that surrounds it in space and time.

An icon of beauty and wellbeing.

At last, it is the messenger of our deepest intentions, it speaks of our life, of the way we relate to the world; this relationships is revealed by gestures, movements, words and tones of voice, the expression of tiredness and pleasure (5).

Giving a personal, unique and unrepeatable shape to the movement of the body creates beauty and can only be born of the emotion and feeling that everything tends to a choice, that rests on organic grounds but is revealed as we decide which motor and intellectual direction our behaviour should take.

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How adult skin speaks of our childhood.

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Skin conditions, such as atopic dermatitis, may recur in adults too, and they usually affect the patient's life since childhood (1). Based on my professional experience, of which the following case is an example, the use of probiotics and prebiotics in medical practice has recently been proved to have an excellent safety profile (2). As modulators of the immune system, they can be used in skin inflammations, such as atopic dermatitis. Probiotics and prebiotics seem to be also effective in reducing the incidence of atopic dermatitis in infants, while their role in the treatment of acne, infected skin lesions and photoprotection is promising, even if larger trials need to be carried out.

A patient that I want to bring as an example is a 38-year-old man, an only child of a diabetic father who died of a heart attack and a frankly overweight mother suffering from mixed dyslipidaemia, pharmacologically treated with glycaemia-lowering drugs. The patient suffers from sleep disorders and it seems he never slept perfectly well till he was 4. Constantly un-

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derweight, since birth he has been suffering from atopic dermatitis, which, poorly responding to the usual, recurring topic treatments, occasionally attacks his skin. In his teenage years, the patient suffered from recurring migraine at night, panic attacks associated with agoraphobia, and fear of heights: situations that also affected his interpersonal relationships. During the clinical interview, he also mentioned basically normal bowel movements, more prone to diarrhoea than to constipation, normal urination and marked sensitivity to cold. He has always suffered from postprandial fullness, often accompanied by an annoying drowsiness. He mentions occasional heartburn associated with anxiety and a sugary diet.

Then, the patient mentions that his dermatitis, fairly well controlled over the years, remarkably recrudesced 5 years ago after a traumatic car accident, which markedly interfered with his emotional sphere. After taking his history, I implemented my clinical practice by describing and recommending eating rules based on a correct sequence of foods, so as to help digestion and absorption, and reduce ready-made foods full of colourings, additives and added sugar (4). I recommended him to eat meals that contain unsaturated fatty acids and omega-3/6/9 supplements associated with probiotics to rebalance his intestinal flora (2 – 3). In particular, many studies have found that it is important to rebalance the water content of the stratum corneum and the hydrolipidic film of the skin. The typical dry skin of patients with atopic dermatitis is associated with poor epidermal hydration and low levels of lipids in the skin surface. Food supplementation with unsaturated fatty acids, such as omega-3, omega-6 and omega-9, may rebalance the skin ecosystem and control the inflammation (3). To finish the picture, we can say that supplementation with alpha-lipoic acid and cinnamon (Cinnamomum zeylanicum) has been added to improve sugar metabolism and control insulin resistance. 12 weeks later, the patient reported normal bowel movements, restorative sleep, a well-balanced feeling of hunger, and a remarkable reduction in itchiness and skin erythema (3).

A proper diet, a correct sequence of meals and selected foods, and prescribed supplements are key to solving chronic cases of atopic dermatitis (5 - 6).

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The Health Hormone.

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For nearly 30 years I have been dealing with the prevention of breast cancer. I soon realised I was not preventing anything at all, at most I was just working on diagnoses that might hopefully be early ones. It didn't take me much longer to find that breast is an organ and it falls ill only once the rest of the body has fallen ill. Therefore, I quickly came to believe that to prevent breast diseases one had to try to keep the whole body in good health.

Actually, I firmly believe that there are not many different diseases, but there is just good health as opposed to poor health, and that different forms of a disease are merely the organic expressions of poor health and the affected organ is simply the weakest one, probably because of some constitutional factor.

So, in a broader sense, one should wonder: how does one fall ill?

One falls ill when the "health hormone" has gone!

There's no such hormone, of course, but there is a combination of hormones, which, when they align, as if they were a planetary or alchemic combination, we feel really energetic, efficient, motivated, in other words, in good health! Likewise, there is another combination of hormones, that is specific to illness and that makes us feel tired, sluggish and really makes us ill, even with major diseases.

According to the allostatic model, homeostasis is the distinctive aptitude of human organisms to maintain their features as environmental conditions change, by adjusting themselves. Homeostasis is achieved through allostasis, which is "the ability to keep a physiological system stable through change"(1). Homeostasis is disrupted by sources of stress, which are aptly called stressors.

Physiological response and Pathological response

Stressors induce a physiological response in the body, which is perfectly normal. This is what happens with acute stress, with short-lived stress, which is known as acute allostatic load. If stress is too mild, the body does not respond to it; if stress is too intense, it can overwhelm us and even kill us; if stress is instead just a little above our abilities, it induces a so-called "hormetic" response.

It is an adaptive response that makes us stronger and, if the stress reoccurs, it can cope with it. A classical example is when you try to build muscle with weights. If the weight is too light, you will have no effect; if the weight is too heavy, you won't even be able to lift it; if it is heavy enough, there and then you will only feel your muscles have swollen. But, the day after, you will feel shattered, which means your muscle fibres have been damaged. Wait a few days, though, and they will get stronger than before and, if you go back to the gym, they will be able to lift the same weight without any problem.

But, to achieve such a situation, a hormetic stimulus must have a definite duration and must not be repeated until our body has recovered. If stressors, even milder ones, are constantly repeated over time and add up, they become a chronic allostatic overload and induce a pathological response instead of a physiological one.

The body responds in one of these ways:

0 inflammation
1 oxidative stress
2 catabolic physiology
3 insulin resistance

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Inflammation

Inflammation is the typical physiological defence mechanism of the immune system. Supposing you cut yourself with a knife that has germs on its blade. Substances are instantly released that dilate your blood vessels, thus slowing down the blood flow in that area (vasodilation). This enables the white blood cells in the blood to stop and fight against the attacker.

In addition, the white blood cells produce cytokines, proteins that send messages (a bit like sending a text message on your mobile phone!). Cytokines are produced in a specific sequence during inflammation: first the inflammatory cytokines, then the anti-inflammatory ones.

The inflammatory cytokines cause fever, loss of strength and appetite, and trigger blood coagulation, causing thrombi to form, and make the liver produce fibrinogen (the material the thrombi are made of) and CRP (the inflammation-specific C-Reactive protein), which is actually used as a marker for inflammation in blood tests. Therefore, the inflammatory cytokines are the source of the general symptoms that go with an inflammation, which break out in a specific area of the body (in this case, the place where you cut yourself) and then spread everywhere.

Then, the anti-inflammatory cytokines, triggered by cortisol, a hormone produced by the adrenal glands (small glands located above the kidneys, one per side), step in and put out the inflammation. Sometimes, the inflammation will kill the bacterium and heal the wound. So, the inflammation will end and everything will go back to normal (1 – 2).

Oxidative stress

Inflammation induces oxidative stress, that is the production of free radicals, and free radicals in turn induce inflammation. Free radicals are released very time a molecule, e.g. oxygen, wrenches an electron away from another molecule, which in turn steals it from another molecule, in a chain reaction that may involve biological molecules that are very important for health, such as fats in cellular membranes, proteins or even DNA (2).

Catabolic physiology

Now, imagine that our organism cannot kill the bacterium and/or we cut ourselves always in the same place, maybe for professional reasons. In this case, the two stressors (cutting and bacteria) would not be removed and the inflammatory process, therefore, could not end.

Unfortunately, in killing the germ, the inflammatory cells will also kill some of our healthy cells nearby. That's not too bad if the germ is defeated: it will have been a small price to pay for winning. But, if the inflammatory process fails to kill the germ, too many healthy cells would go lost, so, at some point, despite not winning, cortisol would still be produced to reduce the inflammation and contain the damage.

Reducing, not putting out, mind, because the two stressors are still alive, as we saw before. In other words, this is a lose-lose situation. The result is a chronic inflammatory response with chronic overproduction of cortisol (1 – 2).

You can replace the cut and the germ with any stressor in one of the three categories, emotional, nutritional or inflammatory. For instance, unresolved, recurring emotional stress will induce the same changes: it will trigger the inflammation and overproduction of cortisol. The cortisol, in turn, will trigger a physiological catabolic response. Metabolism is split into two categories: catabolism, which breaks up the organic matter to produce energy, and anabolism, which uses energy to build the cellular components, such as proteins or DNA.

Both catabolism and anabolism are essential and must be able to alternate with each other within our organism, and neither should be allowed to prevail. If anabolism prevails, tumours may set in; if catabolism prevails, we lose muscle and our skin gets as wrinkly and sagging as that of old people.

Cortisol triggers catabolism, and an overproduction of cortisol triggers a catabolic physiology, i.e. one that is mainly stuck to catabolism. The reason is that the purpose of a catabolic physiology is to release the energy required to assist the immune system (1 – 2).

Insulin resistance

The purpose of insulin is to bring the nutrients into the cells; therefore, insulin resistance, i.e. resistance to the effect of insulin, sets in to prevent the body cells, except the immune cells, stocking up on energy-boosting nutrients for catabolism. Therefore, the other cells will have comparatively few nutrients around, and the organs will suffer. In other words, the catabolic process aims at releasing the energy stored in our body that is sent to the immune system through insulin resistance (1).

Catabolism at work

First, sugars will be released, causing glycaemia to rise, including the smaller glycogen stores and the muscle cell proteins, which will be split into amino acids, then converted to glucose through the so-called neo-glycogenesis (i.e. glucose produced anew from proteins). The amino acids from the muscles are converted to cytokines too, also to assist the immune response.

The fate of muscle proteins is shared by the proteins of the cells of the gastrointestinal barrier, which gets thinner, causing digestive problems, more proneness to infections, and hypersensitivity to some foods.

The release of the amino acids, which, as the name suggests, are acidic substances, induces latent acidosis, which causes the depletion of the buffer systems of the body and the loss of precious minerals through urine (mainly potassium, magnesium and calcium). Then, protein catabolism will have repercussions on the brain too, reducing the production of neurotransmitters, and on the liver, reducing the synthesis of detoxifying enzymes.

Lastly, as to fats, LDL cholesterol will increase, HDL cholesterol will decrease, and fatty acids in the blood will increase, still in the attempt to divert energy to the immune system. Therefore, such rise in the level of cholesterol (just like the rise in glycaemia) has a specific purpose, and, far from being treated with statins (drugs that reduce the blood level of cholesterol), it will correct itself as soon as the chain of events that caused it to rise is solved.

Hormonal involvement

As far as hormones are concerned, insulin resistance induces hyperinsulinaemia, which aims at breaking such resistance; hypercortisolemia causes a loss of progesterone (cholesterol is first converted to pregnenolone, then to progesterone, which in these circumstances is diverted to convert into cortisol) and vitamin D (also produced from the cholesterol diverted to cortisol, as we saw); the inflammation and the cortisol cause the enzyme aromatase to convert testosterone to oestrogens. Again, a high level of cortisol blocks the conversion of the thyroid pro-hormone T4 to the real, active thyroid hormone T_3, to prevent over-catabolism: cortisol first speeds up catabolism, then curbs its excesses. Along with catabolism, anabolism slows down too; this means that all of metabolism will slow down.

The situation, then, will be like this:

0 high level of insulin
1 high level of cortisol
2 high level of oestrogens
3 low level of progesterone
4 low level of testosterone (and low level of Growth Hormone, GH)
5 low level of thyroid hormone T_3
6 low level of vitamin D
7 slow metabolism

A veritable hormonal "sturm und drang", with far-ranging repercussions. For instance, the oestradiol/progesterone imbalance will trigger women's hormonal conditions; a testosterone deficiency will trigger men's hormonal conditions; a low T3 will slow down all of metabolism; vitamin D deficiency has a lot of consequences, including osteoporosis. Just the opposite of a "health hormone" ! (1 – 2)

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