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Enhancing the Skin's Natural Antioxidant Enzyme System by the Supplementation or Upregulation of Superoxide Dismutase, Catalase, and Glutathione Peroxidase

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Aaron Tabor and Robert M. Blair (eds.), *Nutritional Cosmetics: Beauty from Within*, 245–265, © 2009 William Andrew Inc.

Summary

The skin is the body's largest organ and its first line of defense. It protects against a variety of harmful pathogens and environmental factors such as UV radiation, air pollution, and extreme temperatures. Every square inch of skin contains approximately 20 blood vessels, 60,000 melanocytes (which provide pigmentation and guard against UV radiation), 650 sweat glands, and thousands of nerve endings. In order to preserve its effectiveness, the skin is sustained endogenously (protected from within) through an oxidant-antioxidant balance. Oxidation reactions are crucial factors in many metabolic processes; however, an excess of certain oxidants, classified as reactive oxygen species (ROS), can cause cell death and oxidative stress. Oxidative stress in turn induces cardiovascular disease, inflammation, and cancer, in addition to extensive skin damage. Endogenous and exogenous antioxidants, which inhibit oxidative stress, function to counter these effects in the body.

Examples of endogenous, enzymatic antioxidants include: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). SOD's role in maintaining the oxidant-antioxidant balance in the body, thus protecting the body against harmful free radicals, was first determined in 1968. Since then, numerous studies into the benefits of SOD's interactions with the free radical superoxide anion (O_2^-) have been conducted. SOD is essential in halting the proliferation of free radicals and in triggering the further antioxidant activity of CAT and GPx. Excess free radical production can be triggered by a variety of external factors (Fig. 13.1), each of which effectively disrupts the oxidant-antioxidant balance in the body.

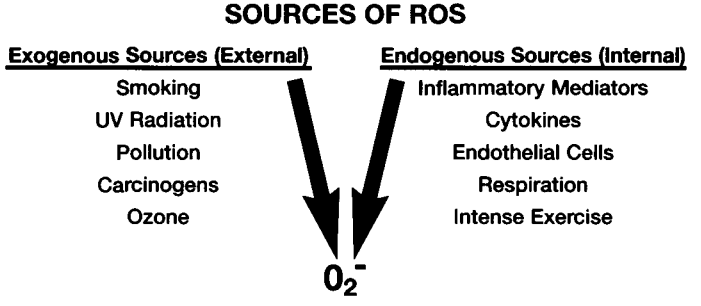

Figure 13.1 Endogenous and exogenous generation of ROS and antioxidant defense system.

These factors include: aging, exposure to UV radiation, smoking, high-intensity exercise, and pollution. Under such conditions, the body will experience oxidative stress.

Nutritional supplements may be effective in increasing the production of endogenous antioxidants. One compound, GliSODin®, has been shown to promote bioactive SOD, CAT, and GPx levels via oral supplementation. Studies have been performed on both humans and animals to demonstrate GliSODin®'s role in the maintenance of antioxidant levels, protection against inflammation, and overall skin health. In helping to preserve the oxidant-antioxidant balance, GliSODin® protects the skin and body from the ill effects of oxidative stress. Other supplements that provide the body extra amounts of the building blocks it requires to make these natural antioxidants, such as manganese, zinc, copper, and selenium, may also be an effective way to increase their presence in the body.

13.1 Introduction: Oxidative Stress

Oxidative stress is damage in a cell, tissue, or organ, caused by ROS. Free radicals and peroxides are generated during metabolism and exist inherently in all aerobic organisms. They are generated through chemical reactions involving both endogenous and exogenous molecules. Endogenous ROS production occurs as cells react with oxygen as part of cellular metabolic processes such as energy generation from mitochondria and detoxification reactions involving the liver cytochrome P-450 enzyme system. The by-products of these processes include hydroxyls, peroxides, and other damaging free radicals. Other endogenous biological indicators of oxidative stress are: lipid peroxidation, protein oxidation (glutamine synthetase activity and protein carbonyl levels), oxidative DNA damage, reduced mitochondrial function, and a decrease in levels of endogenous antioxidants in the heart, liver, blood, lungs, brain, and muscles [1].

Examples of exogenous sources of ROS are: cigarette smoke, environmental pollutants such as emission from automobiles and industries, ultraviolet (UV) radiation, asbestos, excess alcohol consumption, and bacterial, fungal, or viral infections. Oxidative stress can result in lipid peroxidation, which can lead to atherosclerosis (hardening of the arteries) and ultimately cardiovascular disease, damaged or prematurely aged skin, and cancer. Inflammation, which is the key manifestation of rheumatoid arthritis, metabolic syndrome, and diabetes, as well as neurodegenerative diseases like

Alzheimer's, may be accelerated by oxidative stress [2]. Also, oxidative stress may cause tissue toxicity and accelerated aging, which is especially evident in free radical damage to the skin. This process is similar to that of a freshly cut apple turning brown due to exposure to air.

Many of the listed exogenous sources of excess ROS lead to extensive skin damage. Smoking is one such example. In both the gaseous and tar phases of smoke, organic radicals are produced that react with existing molecular oxygen (O_2) to form dangerous free radicals such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and various hydroxyls. Smoking also increases the production of an enzyme that breaks down the protein collagen, which is what keeps skin elastic and supple. After the collagen molecules are broken down, they link back up again in a different way. This process is called cross-linking and causes the normally mobile collagen matrix to become stiff and inflexible. Ten minutes of smoking decreases the skin's O_2 supply for nearly an hour, because nicotine narrows blood vessels and prevents blood from circulating to the capillaries in the dermis. Eventually, the skin starts to exhibit characteristics of accelerated aging, such as premature wrinkles and sagging skin. Smokers are also 3.3% more likely to develop skin cancer [3].

Strong sunlight is another exogenous factor that contributes to skin damage, because it generates free radicals in the skin. The surfaces of the hands, face, neck, and arms are frequently exposed to light and are thus most susceptible to the effects of photoaging. Photoaged skin is characterized by laxity, deep wrinkles, uneven pigmentation, brown spots, and a leathery appearance. In contrast, chronologically aged skin that has been protected from the sun has reduced elasticity but is smooth and unblemished. Fisher et al. (1997) has shown that frequent exposure to UV irradiation leads to an increase in the levels of enzymes that degrade collagen and contribute to photoaging [4]. Sunlight also activates messenger molecules (cytokines), which create inflammatory products in skin cells. Additionally, skin cancer is more likely to develop in people with photoaged skin.

13.2 Role of Endogenous Antioxidants

An antioxidant is a substance that slows or prevents the oxidation of other chemicals in the body. It may be acquired through dietary means or through endogenous production. In order for its role to be completely understood, however, the concept of oxidation must first be discussed. Oxidation

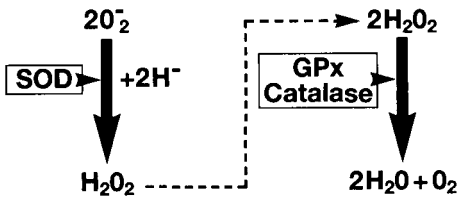

Figure 13.2 Roles of SOD, catalase, and GPx in the inactivation of the superoxide ion.

describes the process of stealing electrons from other atoms for stability. These reactions often involve the production of highly unstable free radicals, which can initiate the formation of multiple destructive ROS. This chain reaction begins with the reduction of O_2. Antioxidants are efficient and effective because they combine with O_2^- at the beginning of the free radical pathway, neutralizing the cascade, and thus preventing damage (Fig. 13.2). Antioxidants can be both nonenzymatic and enzymatic. Nonenzymatic antioxidants include lipid-soluble vitamin E, co-enzyme Q10, phosphatidylserine, and water-soluble vitamin C. Other nonenzymatic antioxidants are beta-carotene, uric acid, and glutathione (GSH). These antioxidants are exogenous and, once ingested, are consumed rapidly or excreted. This occurs because a stoichiometric relationship exists for most vitamins, carotenoids, and thiols.

For example, one vitamin C molecule may halt the progress of only one ROS. Under conditions involving excess production of free radicals, vitamin C must be rapidly consumed to replace lost supplies. Importantly, if the body relies on vitamin C to fight ROS proliferation, vitamin C is no longer available to perform its other crucial duties. These duties include: the production of collagen, increased immunity, fat metabolism, bone health, and the synthesis of certain neurotransmitters.

Endogenous enzymatic antioxidant supplies, on the other hand, are more potent than nonenzymatic antioxidants and are depleted far less quickly. Primary examples of these are SOD, CAT, and GPx (Fig. 13.3). When young skin is exposed to both endogenous and exogenous sources of oxidative stress, there are sufficient levels of ATP (cellular energy) for DNA repair and cell renewal. However, as the individual ages, levels of ATP and endogenous antioxidants are depleted. Additionally, SOD, CAT, and GPx are readily available to scavenge "rogue" ROS. However, supplies of ATP

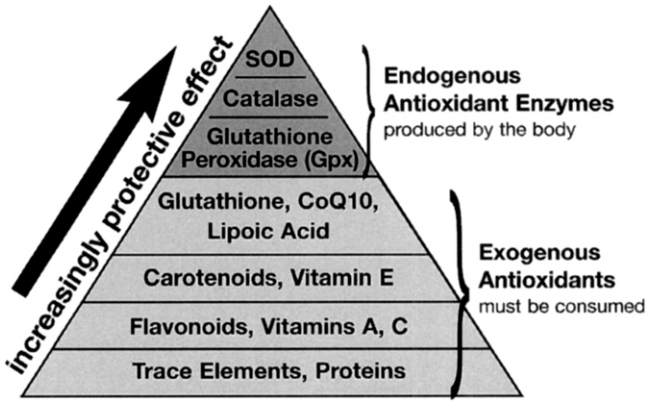

Figure 13.3 Compares the protective effects of the endogenous, enzymatic antioxidants SOD, CAT, and GPx to antioxidants obtained exogenously (i.e., food-sourced).

and endogenous antioxidants decrease with age and therefore supplementation may be desired in order for the body to maintain ROS equilibrium.

13.2.1 Catalase

The enzymatic antioxidant catalase (CAT) is essential in promoting skin health. It is inherent in most aerobic animal cells and is localized in the liver and red blood cells. It uses iron as its trace metal cofactor and is composed of four subunits, each containing a heme group that is responsible for CAT activity. Once SOD catalyzes the reduction of O_2^- to H_2O_2, CAT catalyzes the further reduction of potentially damaging H_2O_2 to O_2 and H_2O. Like SOD, CAT is an extremely efficient enzyme whose high recycling rate demonstrates its ability to detoxify H_2O_2 and to prevent the formation of carbon dioxide (CO_2) bubbles in blood. Good dietary sources of CAT include: clover, lentils, mung beans, radishes, and sunflower seeds.

CAT plays a significant role in skin protection. Rhie et al. (2001) demonstrated the difference between the effects of acute and chronic UV radiation on CAT levels [5]. For acute exposure, human skin samples from a sun-protected area of skin (buttocks) from 13 young Koreans were irradiated with two times the UV radiation necessary to induce sunburn (minimum erythematous dose, MED). Expression of CAT in the epidermis decreased after 24 hours, and was 80% that of the control levels after 48 hours, before

levels began to recover. Expression of the enzyme in dermal cells decreased to 72% of control levels after 24 hours, before beginning to recover.

The effect of chronic UV radiation was evaluated using skin samples from sun-exposed (forearm) and sun-protected (upper-inner arm) areas of 13 young (average age 22.8 years) and 8 elderly (average age 70.7 years) Korean subjects. Epidermal CAT activity was greater than dermal CAT activity by 194% and 450% in young and old skin, respectively. CAT activity in the sun-exposed (forearm) epidermal cells exceeded that of the sun-protected (upper-inner arm) cells by 155% and 115% for old and young skin, respectively. However, CAT activity was significantly lower in the dermal cells of the older subjects, being 66% and 51% that of the young skin levels, respectively. These results demonstrated that CAT activity is upregulated on chronic UV exposure during the photoaging period.

Furthermore, a study by Shin et al. (2005) reported that dermal fibroblasts (cells responsible for the synthesis and maintenance of the extracellular matrix) contained lower levels of CAT after photoaging, and subsequently higher H_2O_2 levels than intrinsically aged skin in the same individuals (12 young and 12 elderly Koreans) [6]. Treatment of these photoaged fibroblasts with CAT reduced H_2O_2 levels. A reversal of aging-dependent mitogen-activated protein kinase activities was also reported, coupled with an inhibition of matrix-metalloproteinase (MMP)-1 expression. This study showed that induction and regulation of endogenous antioxidant enzymes could prevent skin aging.

A study performed at the Webb-Waring Lung Institute investigated the effects of CAT on skin burn patients [7]. Prior studies have indicated that during the pathogenesis of lung injury following skin burn, production of ROS such as O_2^- and H_2O_2 increases. Researchers at the Webb-Waring Lung Institute also found that H_2O_2 activity and CAT activity are heightened in adult respiratory distress syndrome patients. Thus, a study was conducted to determine CAT activity in skin burn patients. It was found that rats subjected to skin burn demonstrated increased levels of H_2O_2 and CAT activity in the bloodstream.

In addition to its role as a biomarker in the progression of adult respiratory distress syndrome and skin conditions, CAT is a proven defender of cell health, specifically in its defense against the proliferation of malignant carcinoma cells and nonmelanoma skin cancer. An *in vitro* study was performed on mice to compare CAT levels in papillomas (benign skin

tumors) with those in carcinomas (malignant skin tumors) [8]. Researchers applied a carcinogen to normal mouse skin to induce a genetic mutation in epidermal cells. A tumor promoter was then introduced to the cells in which the mutation was activated, causing their proliferation. The proliferation of tumor cells resulted in the formation of benign papillomas. The last step of the protocol involved the application of further genetic changes, stimulating the papilloma cells to become malignant. It was found that the malignant cells contained depleted CAT levels and elevated ROS, specifically H_2O_2, levels. In such instances, H_2O_2 functions as a secondary messenger that activates signal transduction pathways leading to the formation of carcinomas. CAT's role in the prevention of malignant skin tumors is critical, because nonmelanoma skin cancer (over 1 million cases diagnosed every year) is the most common form of malignancy in the United States.

13.2.2 Glutathione Peroxidase

Glutathione peroxidase (GPx) is a tetrameric glycoprotein whose trace metal cofactor is selenium, which promotes antioxidant activity. Selenium plays a huge role in the function of GPx, particularly in the recycling of the protein glutathione (GSH), and can be found in cereals, meat, fish, eggs, and Brazil nuts. Localized in the liver and composed of cysteine, glutamic acid (glutamine), and glycine, GSH is a crucial cofactor in the function of GPx. It plays a role in many biological processes such as enzyme catalysis, protein synthesis, membrane transport, receptor action, cell maturation, and leukotriene synthesis. Using its powerful antioxidant capabilities, GSH defends the cell, specifically the cell membrane and the mitochondria, against harmful free radicals. In addition to its role as an antioxidant, GSH also functions in immune response and in DNA repair and protection. Of the many isozymes of GPx, which vary in cellular location and substrate specificity, GPx 1 is the most abundant and is concentrated in the cytoplasm of most mammalian cells. It uses GSH to reduce H_2O_2 to O_2 and H_2O, and lipid peroxides to their respective alcohols.

GSH is a proven defender against the harmful effects of UVB radiation, which has been linked to melanoma (skin cancer), the breakdown of collagen, and the reddening and burning of the skin. A study was performed on the epidermis of hairless mice to assess the effects of GSH depletion on sunburn cell formation [9]. One group of mice was treated orally with a irreversible inhibitor called buthionine S, R-sulfoximine (BSO) that depleted GSH levels by 10–15%. The other group was not treated with BSO and maintained normal GSH levels. Both groups were then exposed

to a moderate amount of UVB radiation. The members of the BSO-treated group were found to contain a much higher sunburn cell count than those of the control group. This study shows that endogenous GSH functions to protect skin cells against moderate levels of UVB radiation.

A 2001 study reported similar findings that the potential of human dermal fibroblast cells to protect against UVA-1 exposure was highly dependent on GSH [10]. Repetitive low-dose UVA irradiation of human dermal fibroblast cells led to a substantial and synchronous upregulation of GPx and SOD activity, and protected cells from a potentially toxic dose of UVA. The study also showed that the increase was dependent on sufficient levels of selenium, the metal cofactor of GPx. Interestingly, the increase in GPx peaks around 12 hours after UVA irradiation to approximately 120% of baseline levels, before decreasing to 80% of baseline.

A 2004 study by Wenk et al. [11] reported that GPx protected against ROS hydroperoxide–induced breakdown of connective tissue in the skin. Human fibroblast cell lines were exposed to UVA radiation. The cells were programmed to overexpress phospholipid-hydroperoxide GPx (PHGPx). In normal human fibroblasts, exposure to UVA led to a NF-κB–mediated increase in interleukin 6 (IL-6) levels, which induced a 4.5-fold increase in matrix metalloproteinase-1 (MMP-1). MMP-1 exhibits substrate specificity for collagens type I and III and is the most important metalloproteinase for the degradation of the extracellular matrix during photoaging. Importantly, no change in MMP-1 levels was recorded after UVA exposure for the cells overexpressing PHGPx.

In further experiments, Wenk et al. treated PHGPx overexpressing cells with phosphatidylcholine hydroperoxides, resulting in a 70% reduction in IL-6 levels, compared to normal cells treated with phosphatidylcholine hydroperoxides. This study was the first to report the effects of GPx on MMP-1 regulation [11].

Selenium, the metal cofactor of GPx, has also proven to be effective in defending the skin against UVB radiation. Past studies have revealed that the proliferation of ROS plays a significant role in UVB radiation–induced cell death. A study was conducted to analyze the effects of selenium in the protection of keratinocytes (main cell type in the epidermis, composing 90% of human epidermal cells) and melanocytes from UVB radiation in human skin cells [12]. Cell cultures that were exposed directly to UVB radiation, without prior selenium supplementation, experienced 80% cell

death among keratinocytes. Cells that received selenium supplementation before being exposed to UVB radiation experienced far less cell death among keratinocytes. Similar results were observed for melanocytes in both groups. The body contains an elaborate antioxidant defense system that guards against the free radical damage caused by UVB radiation; however, studies show that UVB exposure depletes SOD and catalase supplies, whereas GPx levels remain constant. As most selenium action is exerted through cytosolic GPx (GPx-1), this demonstrates that GPx plays a major role in protecting the skin against the carcinogenic effects of UVB radiation.

Additionally, GPx-1 is crucial in the prevention of oxidative stress. A 2003 study was conducted among 636 patients with suspected coronary artery disease to assess the cardiovascular risks associated with GPx-1 activity in the red blood cells [13]. It was found that there is an inverse relationship between levels of GPx-1 activity and cardiovascular disease. This study compared the number of cardiovascular injuries in patients to its corresponding level of GPx-1 activity, measured in hemoglobin units per gram. Results showed that the rate of cardiovascular injury in patients in the lowest quartile of GPx-1 activity (20.8%) was almost three times that in patients in the highest quartile (7.0%).

13.2.3 Superoxide Dismutase

SOD is a class of oxido-reductase enzymes, each of which contains copper and zinc, or manganese at its active site. Collectively, they have the fastest recycling rate of any known enzyme. Intracellular SOD-1 uses copper or zinc as its trace metal cofactor and is located in the cytosol of the cell. SOD-2 uses manganese as a cofactor and is located in the mitochondria and bronchial epithelium. Its expression is activated by both endogenous and exogenous oxidants such as inflammatory cytokines, hyperoxia (excess oxygen in the body tissues), and cigarette smoke. Extracellular SOD-3 also uses copper or zinc as a cofactor and is located in pulmonary fluids and the interstitial spaces in the lungs and is crucial in protecting against vascular damage caused by ROS [14].

SOD, CAT, and GPx are the body's lead antioxidant defenses and operate through a feedback mechanism. SOD catalyzes the reduction of the potentially harmful O_2^- to form H_2O_2. Rising H_2O_2 levels result in the gradual inactivation of SOD. As discussed, CAT and GPx catalyze the reduction of H_2O_2 to O_2 and water (H_2O); thus, SOD is conserved. In the same way,

SOD's reduction of O_2^- to form H_2O_2 results in the conservation of CAT and GPx [15]. SOD can also inactivate certain enzymes that control levels of free iron, which can lead to the formation of hydroxyl free radicals in the body.

SOD has numerous health benefits, especially in relation to inflammation and skin protection. SOD-3, for example, protects against oxidative fragmentation of type I collagen in the skin. Immunochemical studies indicate that SOD-3 interacts with type I collagen in the extracellular matrix of blood vessels and airspaces, thus preventing the breakdown of collagen due to oxidative stress [16]. In addition, by neutralizing O_2^-, SOD inhibits the activation of latent collagenases by O_2^-, whose function is to degrade collagen. Once collagen is broken down, the fragments activate chemotactic agents for neutrophils, causing tissue damage and chronic inflammation.

SOD functions by controlling the oxidative reactions that contribute to the pathogenesis of inflammation. Examples of these reactions include: the initiation of lipid peroxidation, the inhibition of mitochondrial respiratory chain enzymes and sodium/potassium ATP-ase activity in the cell membrane, and the inactivation of membrane sodium channels. SOD also halts the proinflammatory effects of O_2^-, which include: damage to the endothelial cells (causing increased microvascular permeability) and the promotion of chemotactic factors such as leukotriene B_4 and the concentration of neutrophils at inflammation sites.

Extensive clinical research into the effects of SOD on inflammation has been conducted. SOD was first used clinically on the degenerative joint condition osteoarthritis. Two early studies were performed confirming SOD's effects on osteoarthritis patients. In both studies, patients were injected with purified bovine SOD (Orgotein), and their progress was charted for varying periods. Significant decreases in pain and subsequent improvements in function, the use of aids, and the use of analgesics were recorded. No adverse side effects were observed [17,18].

SOD also plays a major role in protecting against vascular inflammation, which leads to atherosclerosis and cardiovascular disease. A study was performed on mice lacking apolipoprotein E (responsible for the catabolism of lipoproteins) to examine the effects of SOD-1 and CAT on atherosclerosis [19]. Because the mice were apolipoprotein E–deficient, they developed a build-up of oxidized lipids in the arterial wall, which

caused atherosclerosis. Both SOD-1 and CAT were overexpressed in these mice. It was found that the overexpression of SOD-1 together with CAT resulted in reduced levels of F2-isoprostanes (biomarkers of lipid peroxidation and oxidative stress) and the inhibition of atherosclerosis. This study shows that SOD contributes to overall vascular health by protecting against atherosclerosis and oxidative stress.

Several studies with an orally effective delivery of SOD have shown skin health benefits, including the inhibition of UV radiation–induced skin burn, quicker healing, and significant reduction in negative reactions to the sun, particularly for the sun-sensitive and for those with sun allergies [20–22].

13.3 SOD and Dietary Nutrients: GliSODin®

GliSODin® is the first bioactive SOD by the oral route. As the exposure of SOD to the acidic nature of the stomach causes modifications in its quaternary structure and results in a nonfunctioning enzyme, all past oral SOD treatments have been unsuccessful. In order to avoid the denaturation of SOD, GliSODin® combines SOD (derived from SOD-rich melon extract) with gliadin, which is a wheat-based protein that protects SOD during its passage through the gastrointestinal (GI) tract. Gliadin acts in the small intestine by adhering to the sidewall. It then releases the SOD progressively, protecting against intestinal inactivation by digestive enzymes, and enables its recognition by immune-active cells in the GI tract [18]. In was shown *in vitro* that gliadin in combination with SOD improves SOD release, as was demonstrated by the progressive release of SOD in an environment replicating digestive conditions [23]. Animal studies indicate that the oral administration of the SOD-gliadin complex increases the activity of SOD, CAT, GPx, and other antioxidant enzymes in plasma cells, red blood cells, and the liver. Additionally, orally absorbed SOD-gliadin leads to decreased levels of certain biomarkers, such as F2-isoprostanes, which are breakdown markers from the destructive actions of oxidative stress in the body.

A significant *in vivo* study of the effects of GliSODin® with respect to elevated antioxidant levels in the bloodstream was conducted on Balb/c mice [24]. The mice were divided into two groups; each was orally supplemented with standardized SOD melon extract over a period of 28 days. One group was supplemented with SOD alone and the other with GliSODin®. Several biomarkers of oxidative stress, including antioxidant activity, mitochondrial

depolarization in hepatic cells, and apoptosis in hepatic cells, were used to analyze the results of both groups. In the group administered with unprotected SOD, there were no significant changes in antioxidant levels. However, the GliSODin® group demonstrated a dramatic increase in the activity of circulating antioxidant enzymes. In addition, hepatic cells isolated from the mice receiving GliSODin® presented a delayed depolarization response and an increase in the resistance to apoptosis induced by oxidative stress. This study demonstrates that GliSODin® supplementation leads to definitive improvements in the antioxidant status of the cells and protects them against the detrimental effects of oxidative stress.

Human studies also demonstrate the role of GliSODin® in promoting the production of SOD and other endogenous antioxidants. Muth et al. (2004) conducted a randomized, double-blind, placebo-controlled trial to determine GliSODin®'s role in the protection against DNA damage induced by hyperbaric oxygen (HBO), or pure oxygen at a pressure of 2.5 atmospheres [25]. Twenty male volunteers were randomly assigned to receive either a daily dose of GliSODin® (1,000 IU) or placebo for 2 weeks, prior to HBO exposure for 60 minutes. A comet assay was then used to measure the resulting DNA damage from HBO exposure (Fig. 13.4). A significantly greater amount of damage was found in the placebo group, as compared to little or no damage in the GliSODin®-supplemented group.

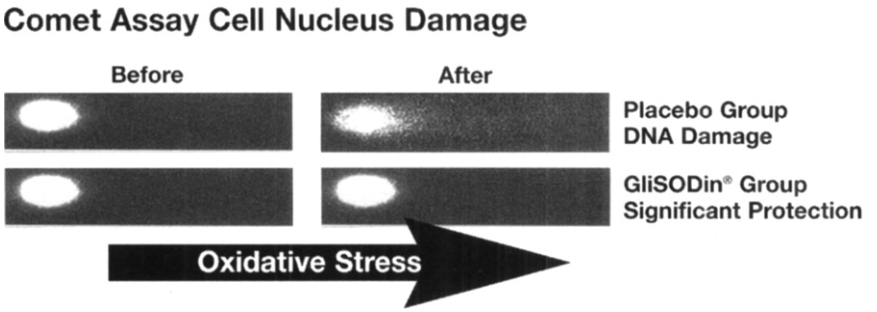

Figure 13.4 Effects of GliSODin® supplementation on DNA damage, measured by the comet assay—a microgel electrophoresis method that allows detection of DNA damage in individual cells. The upper row shows progressive and significant DNA damage in the placebo group, evidenced by the "comet's tail" after the spot. No such effects were observed in the GliSODin® group (1,000 mg/day), showing an intact cell nucleus with no DNA damage.

Additionally, urine levels of F2-isoprostanes, well-established markers of oxidative stress, increased by 33% from baseline in the placebo group but not in the GliSODin®-supplemented group. This study indicates that GliSODin® protects against DNA damage produced by HBO and thus confirms the antioxidant activity of the supplement.

GliSODin® also has proven anti-inflammatory effects. An *in vivo* study by Vouldoukis et al. performed on C57BL/6 mice analyzed these effects [26]. The mice were split into four groups and administered, respectively, with GliSODin®, gliadin alone, SOD from melon extract alone, or a placebo. After 28 days, peritoneal macrophages were activated by the proinflammatory INF-gamma and then collected after 24 hours. The ability of the macrophages to produce free radicals and cytokines such as the proinflammatory tumor necrosis factor (TNF-alpha) and the anti-inflammatory interleukine-10 (IL-10) was then measured. Of the four oral supplementations, only GliSODin® was found to protect cells from the effects of INF-gamma. This result was obtained by observing the significant increase in the production of anti-inflammatory IL-10 and consequent reduction of TNF-alpha in the GliSODin® group.

The impact of GliSODin® on antioxidant status and the vascular inflammatory process was evidenced by a study by Cloarec et al. (2007) [27]. Seventy-six patients at risk for cardiovascular disease were subjected to diet and lifestyle changes for one year, at which point the 34 remaining patients (42 dropped out due to the stringency of study) were randomly split into two groups. One group continued with the prescribed diet and lifestyle, while the other received an additional GliSODin® supplement (500 IU/day) for two more years. After two years, carotid artery intima media thickness (IMT), an indicator of atherosclerosis, was measured using ultrasound-B imaging. A significant reduction in the IMT in the SOD-supplemented group was found, as compared to an increase in the IMT of the nonsupplemented group. Moreover, malondialdehyde levels, a marker of lipid oxidation, were reduced by 34% in the GliSODin® group. This study demonstrates GliSODin®'s important role in cardiovascular health, reducing inflammation and providing improvement in the circulatory system.

13.3.1 GliSODin® and Skin Protection

GliSODin® supplementation is a valuable adjunct to sunscreen for skin protection, especially against harmful UV radiation. Unprotected skin can be severely damaged by constant exposure to the sun's UVB and UVA

rays. In addition to burning (erythema), UV radiation can damage collagen fibers and cellular DNA; they corrupt DNA molecules by causing malformations that can lead to mutations and unhealthy cell production. Three human studies demonstrate the benefits of GliSODin® with respect to protecting cells against UV radiation and photo-oxidative stress. The first study revealing the benefits of the antioxidant properties of GliSODin® for skin health was conducted as an open clinical trial conducted by 40 dermatologists in France. The trial involved 150 participants who were chosen based on their susceptibility to sunburn and other photo-oxidative stress reactions. 500 mg of GliSODin® were administered daily over 3–8 weeks of normal sun exposure (including use of sunscreen, SPF 20–100).

Study participants were split into three groups: 75 patients with significant flushing or reddening almost immediately during exposure; 60 patients who experienced negative sun reactions, including sun allergy; and 15 patients who experienced other irritating skin reactions such as pruritus and solar eczema.

Results after 4–8 weeks were as follows: of the 75 patients in the first group, 64 patients reported excellent tolerance, 6 patients had diminished burning, whereas 5 patients had no improvement; in the second group of 60 patients, 44 did not experience allergic reactions to the sun, whereas 6 had a reduced reaction and 10 had a reaction; and in the third group, all of the 15 participants had complete relief of their usual symptoms (Fig. 13.5). In summary, 86% of participants reported significant relief [20].

A follow-up randomized, placebo-controlled, double-blind trial by Mac-Mary et al. involved 49 healthy individuals: 10 phototype II, 19 phenotype III, and 20 phenotype IV [22]. Researchers used UV radiation to induce photo-oxidative stress on the inner forearms of the participants and measured the susceptibility of the participants to sunburn (minimum erythematous dose, or MED) and the resulting redness (actinic erythema). A UV stress test was then conducted before GliSODin® supplementation was administered. Participants received 500 mg doses each week for 4 weeks, and UV stress tests were conducted each week to measure progress. At the end of 4 weeks, an increase of 8 times in the minimum exposure to UV rays necessary to produce skin burn for phototype II participants was found in the GliSODin® group, compared to the placebo group (Fig. 13.6). Additionally, the induced redness decreased faster in the GliSODin® group compared to the control group (Fig. 13.7) [28]. The effects were more marked in the phototype II subjects than the other skin-type groups.

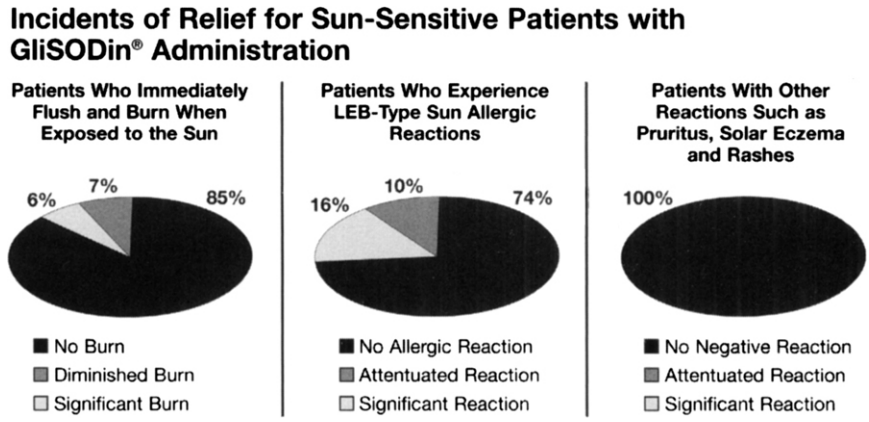

Figure 13.5 Effects of GliSODin® supplementation in 150 sun-sensitive patients divided into three groups; 75 Patients Who Immediately Flush and Burn When Exposed to the Sun; 60 Patients Who Experience LEB-type Sun Allergic Reactions; and 15 Patients With Other Reactions Such as Pruritus, Solar Eczema, and Rashes. This chart shows the scale of relief received in each group by percentage.

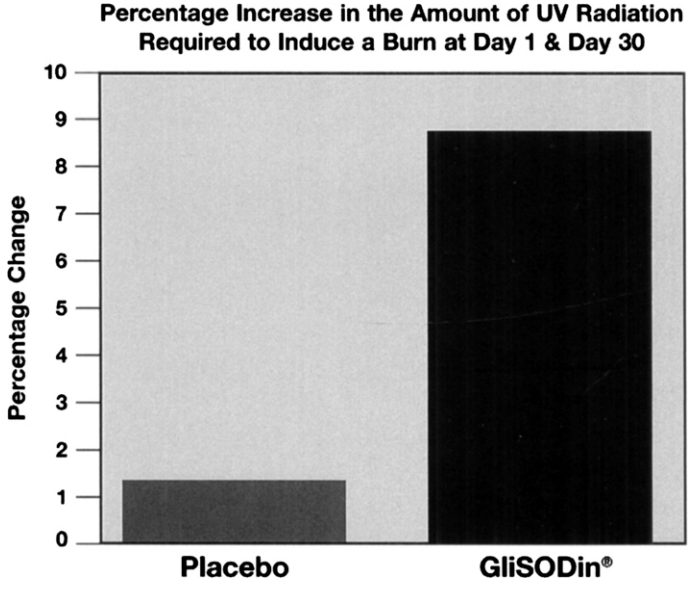

Figure 13.6 Compares the percentage increase of minimum erythematous dose (MED) of type II phototypes in GliSODin® group from day 1 to day 30, to that in placebo group.

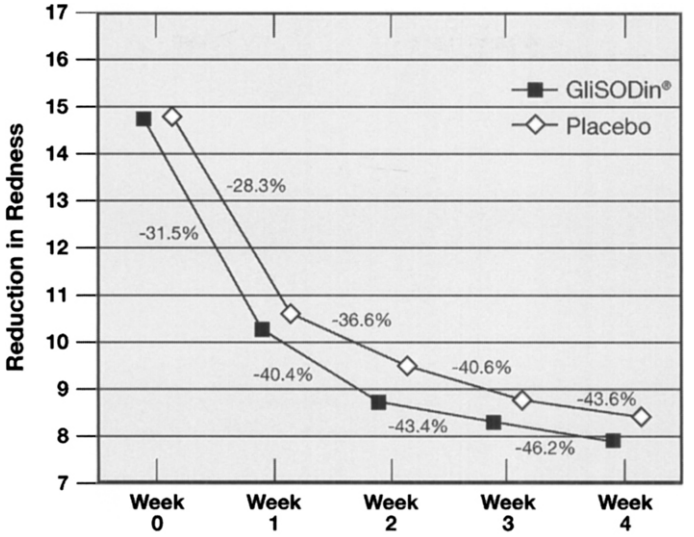

Figure 13.7 The redness produced by UV exposure was reduced in the GliSODin® group in type II phototypes, compared to placebo, suggesting a protective benefit. Figure generously provided by Dr. Humbert, Département de Dermatologie, Centre Hospitalier Universitaire St Jacques 2, Place St Jacques 25030 BESANCON CEDEX.

In addition, capillary regeneration was faster than in the control group. The number of capillaries in the GliSODin® treatment group increased by 37.7% after 4 weeks, compared to an increase of only 18.1% in the placebo group (Fig. 13.8) [28]. It was thus concluded that GliSODin® protects the skin from oxidative stress caused by UV radiation.

One of the first conclusive pilot trials of the role of GliSODin® in skin protection against UV radiation involved 15 patients who were chosen based on their high susceptibility to sunburn or sun disease. Administered daily were 500 mg of GliSODin® for 60 days of normal sun exposure. At the end of 60 days, patients reported significant reductions in sunburn, flushing, and skin rash [21].

13.4 Conclusions

ROS production is an inevitable consequence of metabolic processes in aerobic organisms. Under normal conditions, an oxidant-antioxidant balance—maintained by the defense mechanisms of endogenous, enzymatic

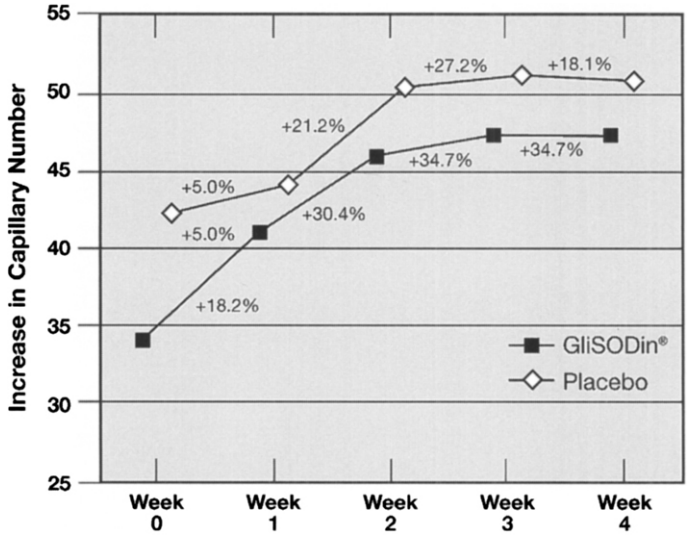

Figure 13.8 Increased evolution of capillary numbers after MED exposure during 4 weeks as a result of GliSODin® supplementation, compared to placebo, suggesting a protective benefit.

antioxidants such as SOD, catalase, and glutathione peroxidase—exists. However, an excess of ROS and the resulting effects of dangerous free radicals such as O_2^- can disrupt this balance and lead ultimately to oxidative stress.

O_2^- is the starting point of a cascade of free radical reactions, resulting in the unchecked proliferation of ROS. First discovered in 1968, SOD is the first antioxidant defense to mobilize against O_2^- and ensuing oxidative stress. For years, attempts at the oral administration of pure SOD to boost natural antioxidant levels failed, because SOD's delicate quaternary structure could not withstand the acidic nature of the GI tract. This issue was solved, however, with the advent of GliSODin®. GliSODin® combines SOD extracted from the cantaloupe melon with the wheat-based biopolymer gliadin, thus making SOD bioactive in the body. The SOD-gliadin combination increases SOD's efficiency, stability, and delivery during its passage into the bloodstream. Additionally, the role of GliSODin® in making SOD bioactive in the body promotes the activity of other crucial endogenous, enzymatic antioxidants such as catalase and GPx.

GliSODin® also produces anti-inflammatory effects. The results of the studies that have been discussed indicate that whereas SOD and gliadin

alone have little impact on the increase of IL-10 and the reduction of TNF-alpha, the SOD-gliadin combination is effective in this way. In addition to its anti-inflammatory capabilities, GliSODin® also protects skin from photo-oxidative stress caused by UV radiation. There are several UV studies whose results support this conclusion.

As methods to prevent oxidative stress and contribute toward healthy aging are considered, orally supplemented GliSODin® has been shown scientifically to offer a therapeutic means for the prevention and treatment of many conditions associated with inflammation and oxidative stress.

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