

**GliSODin®**

TECHNICAL PUBLICATION

LITERATURE REVIEW OF  
CANTALOUPE MELON SOD  
EXTRACT/ WHEAT GLIADIN  
BIOPOLYMER (GLISODIN®) AND ITS  
BENEFICIAL HEALTH ASPECTS

APRIL 2012

## *Summary*

The purpose of this technical publication is to review and update the science and health benefits reported to date for the superoxide dismutase enzyme extracted from cantaloupe melon and delivered in combination with wheat gliadin known as GliSODin.

Superoxide dismutase (SOD) constitutes part of the body's front line in antioxidant defenses, helping to maintain the physiological oxidant-antioxidant balance. However, this balance can be disturbed by a number of different factors, including aging, smoking, pollution, exposure to sunlight, infection and the subsequent immune response, and high intensity exercise. Under such conditions the body experiences oxidative stress, and this is linked with increased risk of chronic disease.

Oral supplementation with the enzyme in order to boost the body's antioxidant defenses has been ineffective due to the biochemical conditions experienced on passage through the gastrointestinal tract, which can degrade the enzyme and render it useless. This technical publication reviews the science related to GliSODin<sup>®</sup>, a trade name for SOD extracted from cantaloupe melon combined with wheat gliadin. Science is presented to demonstrate that combination with gliadin protects the SOD during passage through the

stomach and enhances absorption once inside the intestine.

An extensive section is dedicated to the proof of this concept, with results from *in vitro*, *in vivo* and human studies presented showing the increase in antioxidant status and reduction of markers of oxidative stress. It will also be shown that GliSODin's bioactivity in humans has been demonstrated with a dose of 500 mg in just 14 days' time.

Anti-inflammatory and immune system modulating effects for the SOD-gliadin combination have also been reported, and these are discussed in this publication, with particular attention paid to the mechanism(s) behind the effects.

Reduction of the production of reactive oxygen species associated with oxidative stress has many important health implications, with potential benefits for improved recovery after strenuous exercise, reduction of inflammation (reddening) in the skin on exposure to sunlight (UV radiation), improvement in heart health, and complications arising from diabetes. Sections in the review are dedicated to each of these areas.

This publication is presented in such a way as to be accessible to both scientists and non-scientists, with the intention of providing a concise and accurate review of the science behind the GliSODin<sup>®</sup> ingredient. Key references are presented throughout the review.

## Introduction

Production of reactive oxygen species (ROS) is a normal process in oxygen-breathing organisms. Under normal physiological conditions a balance between these species and the body's anti-oxidant defenses exists (**Figure 1**), but certain conditions, like smoking, pollution, exposure to sunlight (UV radiation), infection and the subsequent immune response, metabolism of sugars related to high intensity exercise, and aging, can increase the production of ROS like the superoxide ion ( $O_2^-$ ) and the hydroxyl ion ( $OH^-$ ), and disrupt the natural balance and lead ultimately to oxidative stress.<sup>[1]</sup> (**Figure 1**)<sup>[2]</sup>

The detrimental health effects that can result from prolonged exposure to oxidative stress include: DNA damage that can ultimately produce cancer, atherosclerosis (hardening of the arteries) that leads to cardiovascular disease, inflammation, rheumatoid arthritis, the metabolic syndrome and diabetes, and neurodegenerative diseases like Alzheimer's.<sup>[3]</sup>

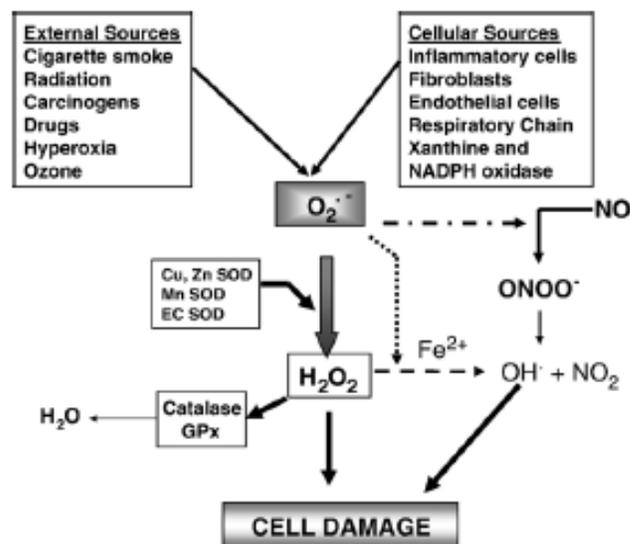
<sup>1</sup> Halliwell B., Gutteridge J.M.C., Cross C.E., "Free radicals, antioxidants, and human disease: where are we now?" *J. Lab Clin Med* (1992) Volume 119, Pages 598-620

<sup>2</sup> Rahman I., Biswas S.K., Kode A., "Oxidant and antioxidant balance in the airways and airway diseases" *European Journal of Pharmacology*, 2006, Volume 533, Pages 222-239

<sup>3</sup> Ding Q., Dimayuga E., Keller J.N., "Oxidative damage, protein synthesis, and protein degradation in Alzheimer's disease" *Current Alzheimer Research*. 2007, Volume 4, Pages 73-79

## *GliSODin - Definition and proof of concept/mechanism of action*

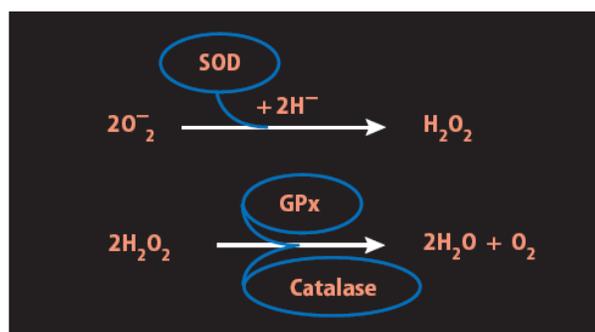
Superoxide dismutase (SOD), along with catalase and glutathione peroxidase, form the front line of the body's antioxidant enzyme defenses.<sup>[4]</sup> Since the superoxide anion is the starting point of the cascade of reactions of free radicals production. SOD, dubbed the "enzyme of life" on discovery in 1968, is the first antioxidant mobilized by the cell as defense against oxidative stress. The enzyme reacts with the superoxide ion and turns it into hydrogen peroxide ( $H_2O_2$ ). This is then catabolised by catalase and glutathione peroxidase to produce molecular oxygen ( $O_2$ ) and water ( $H_2O$ ).



**Figure 1:** Cellular generation of reactive oxygen species and antioxidant defense system.

<sup>4</sup> McCord J.M., Fridovich I., "Superoxide dismutase: an enzymatic function for erythrocyte hemocuprein" *J. Biol. Chem.*, (1969) Volume 224, Pages 6049-6055

These antioxidant enzymes have a distinct advantage over the antioxidants consumed from the diet or nutritional supplements, like the vitamins A, C, and E, carotenoids, and thiols since the enzymes are biological catalysts, reducing many times and more rapidly reactive oxygen species without being consumed themselves. By reacting at the beginning of the process, enzymes avoid the later occurrence of oxidized biological molecules. On the other hand, a non-catalytic or stoichiometric relationship exists for most vitamins, carotenoids and thiols, meaning a defined relationship exists – once vitamin C, for example removes a ROS more vitamin C must be consumed to replace that which has been lost.



**Figure 2:** Role of antioxidant enzymes in the inactivation of the superoxide ion.

Like most other protective mechanisms in the body, the production of SOD decreases with age,<sup>[5]</sup> while a cell's susceptibility to

<sup>5</sup> Di Massimo C., Scarpelli P., Di Lorenzo N., Caimi G. di Orio F., Ciancarelli M.G., "Impaired plasma nitric oxide availability and extracellular superoxide dismutase activity in healthy humans with advancing age" *Life Sciences*. 2006, Volume 78, Pages 1163-1167

oxidants increases, putting the cells under increasing oxidative stress.

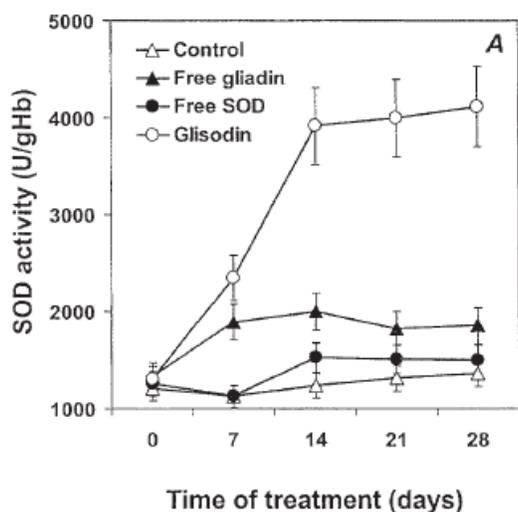
Oral administration of SOD and the other antioxidant enzymes present in many plant extracts is, under normal conditions, not effective. During passage through the gastrointestinal pathway the enzyme is denatured (deactivated) rendering it ineffective as an antioxidant. However, studies have shown that combining SOD with a wheat gliadin biopolymer system temporarily protects the SOD during its gastro-intestinal traffic. One explanation of this efficiency was presented by Clemente et al. who showed that gliadin increases the permeability of the intestinal by promoting the release of a zonulin, thereby allowing the transport of the macromolecule SOD across the intestinal barrier.<sup>[6]</sup>

The combination of SOD extracted from cantaloupe melon (*Cucumis melo* L.C.) with the wheat gliadin biopolymer (GliSODin<sup>®</sup>) significantly improves the delayed release of SOD as evidenced *in vitro* by the progressive increase of its activity in a medium mimicking the digestive conditions (**Figure 4**).<sup>[7]</sup>

<sup>6</sup> Clemente M.G., De Virgiliis S., Kang J.S., Macatagney R., Musu M.P., Di Pierro M.R., Drago S., Congia M., Fasano A., "Early effects of gliadin on enterocyte intracellular signaling involved in intestinal barrier function" *Gut* 2003, Volume 52, Pages 218-223

<sup>7</sup> Vouldoukis I., Conti M., Krauss P., Kamaté C., Blazquez S., Tefit M., Mazier D., Calenda A., Dugas B., "Supplementation with gliadin-combined plant superoxide dismutase extract promotes antioxidant defences and protects against oxidative stress" *Phytotherapy Research* 2004, Volume 18, Pages 957-962

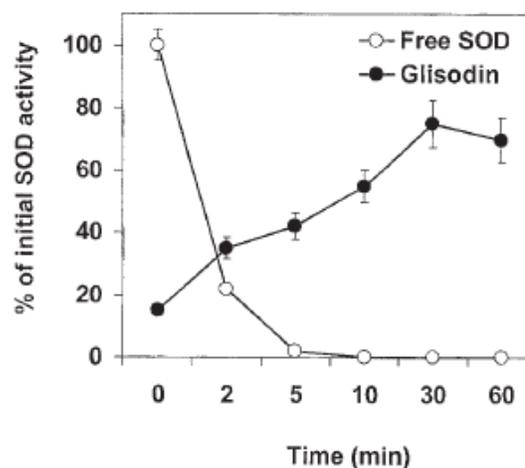
Vouldoukis et al. have shown *ex vivo* that prime activation of macrophages isolated from rodents with interferon-gamma (INF-gamma) subsequently challenged with IgG1/anti-IgG1 immune complexes leads to the significant production of superoxide anions.



**Figure 3.** Effect of a supplementation with GliSODin<sup>®</sup> on circulating SOD activity. Mice were fed for 28 days, with either a control diet or supplemented with (a) melon SOD extract (10 IU of non protected SOD), (b) gliadin (1 mg) or (c) GliSODin<sup>®</sup> (1 mg for 1IU).

This production may be regulated, in a dose-dependent manner, in macrophages originating from rodent previously supplemented with GliSODin<sup>®</sup>. These results prove the potent *in vivo* activation of antioxidant activities made by the SOD-containing melon extract/gliadin biopolymer combination.<sup>[8]</sup>

<sup>8</sup> Vouldoukis I., Lacan D., Kamate C., Coste P., Calenda A., Mazier D., Conti M., Dugas B., "Antioxidant and anti-inflammatory properties of a Cucumis melo LC. extract rich in superoxide dismutase activity" *Journal of Ethnopharmacology* 2004, Volume 94, Pages 67-75



**Figure 4.** Gliadin polymers delay the release of the melon SOD activity in a medium mimicking the digestive process. An identical amount (100 units) of melon-SOD extract was submitted free or combined with gliadin (GliSODin<sup>®</sup>) to conditions mimicking the digestive process, for 1 h at 37 °C.

An important proof of concept *in vivo* study by Vouldoukis et al. using Balb/c mice receiving the SOD melon extract orally, either alone or combined with wheat gliadin (GliSODin<sup>®</sup>) for 28 weeks showed that only the gliadin-SOD complex resulted in a significant increase in circulating antioxidant levels.<sup>[6]</sup> **Figure 3** illustrates circulating SOD activity in mice supplemented with free gliadin, free SOD, or GliSODin<sup>®</sup>, compared to control (no supplement).

Kick et al.<sup>[9]</sup> used the aortic cross-clamping technique with 18 pigs to induce ischemia-reperfusion process (HR), a well-established model of oxidative stress. After two weeks of supplementation with GliSODin<sup>®</sup> (1250 mg, nine pigs) or placebo (nine pigs), the

<sup>9</sup> Kick J., Hauser B., Bracht H., Albicini M., Öter M., Simon F., Ehrmann U., Garrel C., Sträter J., Brückner U.B., Leverage X.M., Schelzig H., Speit G., Rademacher P., Muth C.-M., "Effects of a cantaloupe melon extract/wheat gliadin biopolymer during aortic cross-clamping" *Intensive Care Medicine* 2007, doi:10.1007/s00134-006-0518-6

animals were subjected to aortic clamping to induce injury-related oxidative stress. Pigs were chosen to avoid confounding factors such as smoking and dietary habits, and because their tissue antioxidant profiles and susceptibility to oxidative stress are similar to humans.

At the end of the study, GliSODin<sup>®</sup>-supplemented animals had significantly lower levels of oxidative-stress induced DNA damage. Furthermore, the researchers found significantly lower levels of apoptotic (dead) cells in the spinal fluid of the swine, showing a marked protective benefit.

### *GliSODin<sup>®</sup> – Evidence from human studies*

GliSODin<sup>®</sup> was studied in a trial utilizing induced oxidative stress, thereby establishing its efficacy in humans. Similar results as the swine study were obtained in a randomized, double-blind, placebo-controlled clinical trial involving 20 men. Trials with such a design are considered the ‘gold-standard’ for scientific evaluation. Muth et al. assigned the volunteers (average age 31) to receive a daily dose of 1000 International Units (IU) of SOD (GliSODin<sup>®</sup>) or placebo for 14 days prior to being exposed to hyperbaric oxygen (HBO) – pure oxygen at a pressure of 2.5 atmospheres – for 60 minutes. DNA damage that results in exposure to HBO was measured using the

comet assay, and found to have significantly increased in the placebo group, while no significant changes were observed for the SOD-supplemented group. **(Figure 5)**

Moreover, the levels of F2-isoprostanes, well-accepted markers for oxidative stress, increased significantly in the placebo group (22.3 picograms per milliliter of plasma from the start of the study). No significant increases were observed in the SOD-supplemented group indicating significant protective benefit.

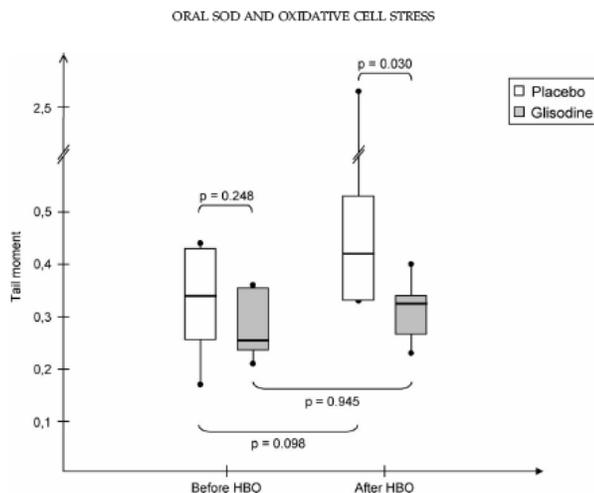
Muth et al. concluded that GliSODin<sup>®</sup> was able to protect against DNA damage induced by HBO and thereby proved the antioxidant activity of the supplement.<sup>[10]</sup>

Improvements in antioxidant status have also been observed in patients with HIV and AIDS, a population with reduced circulating antioxidant levels. Chenal et al. performed a double-blind clinical trial with 35 AIDS patients not receiving anti-retroviral therapy to receive placebo, non-protected SOD extracted from melon (*Cucumis melo*, 1000 IU SOD) or the SOD-gliadin combination (1000 IU SOD) every day.<sup>[11]</sup>

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<sup>10</sup> Muth C.M., Glenz Y., Klaus M., Radermacher P., Speit G., Leverage X., “Influence of an orally effective SOD on hyperbaric oxygen-related cell damage” *Free Radical Research*, 2004, Volume 38, Number 9, Pages 927-932

<sup>11</sup> Chenal H., Davit-Spraul A., Brevet J., Legrand A., Demouzon J., Cosson C., Dugas B., Montagnier L., Conti M., “Restored antioxidant circulating capacities in AIDS west african patients receiving an antioxidant nutraceutical *Cucumis melo* extract rich in superoxide dismutase activity,” *Abstract included at the XVI International AIDS Conference* Aug 2006



**Figure 5.** Results of the comet assay measuring human DNA damage that results from exposure to HBO. GliSODin<sup>®</sup> was protective while damage significantly increased in the placebo group.

At the end of 21 days of supplementation, patients receiving GliSODin<sup>®</sup> were found to have normalized circulating SOD1 activity and total antioxidant status, while no such effects were observed in the placebo or non-protected SOD groups. The researchers concluded that GliSODin<sup>®</sup> could improve antioxidant defenses.

Taken together these results from *in vitro*, *in vivo* and human studies show that GliSODin<sup>®</sup> is bioavailable when taken orally and can significantly improve the antioxidant status of an individual.

### ***Benefits of the antioxidant GliSODin for promoting immune health***

The combination of superoxide dismutase and gliadin (GliSODin<sup>®</sup>) was studied for its ability to regulate the immune response. Balb/c mice were fed a control diet supplemented with either free vegetal SOD (10

IU) or the SOD-gliadin combination (GliSODin<sup>®</sup>, 10 IU) for 28 days. Blood samples were taken every seven days and measures of circulating SOD, catalase or glutathione peroxidase indicate that their respective level only increased in the group receiving the GliSODin<sup>®</sup> combination.

Additionally, spleen cells isolated from mice in each of the groups showed that the mice supplemented with GliSODin<sup>®</sup> had increased production of type 1 helper T lymphocytes (Th1) and INF- $\gamma$  and IL-4. The immunoglobulin G (IgG) response – the predominant antibody used by the body to identify and neutralize foreign objects - was stimulated, while the response of IgE, the immunoglobulin associated with an allergic response, was only marginally affected.

Vouldoukis et al. proposed that the mechanism behind these effects was due to an activation of antigen presenting cells (APC), which results in the production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and nitric oxide (NO), both of which are reactive oxygen species and upset the oxidant-antioxidant balance. In response to this, production of the antioxidant enzymes catalase and glutathione peroxidase is induced. This results in a polarized adaptive immune system, highlighting the benefits of GliSODin<sup>®</sup> since this polarization is a sign of the natural equilibrium of antioxidants in the cells.<sup>[11]</sup>

Quality of life, a measure of overall health, daily activities, tiredness, and energy

levels, is an important issue for people infected with HIV and receiving highly active anti-retroviral therapy (HAART). Rahman et al. investigated the effect of receiving GliSODin<sup>®</sup>-containing supplements on quality of life and performance of 23 AIDS patients on HAART.<sup>[12]</sup> Subjects received two daily supplements of Resurgex, a blend of GliSODin<sup>®</sup> (500 IU), coenzyme Q10 (75 mg), and beta-glucans (100 mg) for up to 24 weeks.

Measures of Quality of Life (QoL) significantly improved in the patients, particularly for measure of overall health, daily activities, tiredness, and energy levels. Such improvements were supported by improvements in measures of performance status.

On a biochemical level, Rahman et al reported clinically important improvements in a number of metabolic and immunological functions, which would be tied to the improvements observed in performance and QoL.

These results indicated that supplements of GliSODin<sup>®</sup> could have significant benefits for the millions of people infected with the HIV-1 virus and receiving anti-retroviral therapy.

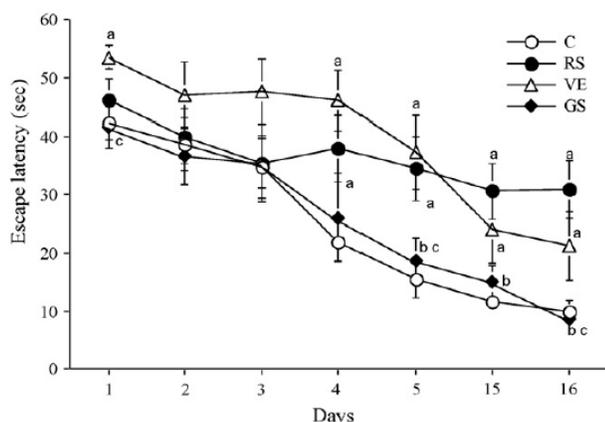
### *GliSODin<sup>®</sup> in neuroprotection*

Aging leads to suppression of brain functions such as learning and memory. The aging effect is accelerated by chronic stress, especially psychological stress. An experimental animal model with chronic immobilization stress significantly impairs spatial memory performance in immobilized animals. The effects of GliSODin<sup>®</sup> on stress-induced lipid peroxidation and impairment of spatial memory was investigated in rodents. Experimental mice were divided into four groups as follows: (1) control mice (C mice) fed in a normal cage without immobilization; (2) restraint-stressed (RS mice) fed in a small cage; (3) vitamin E mice (VE mice), mice were fed in a small cage with a diet supplemented with vitamin E; (4) GliSODin<sup>®</sup> mice (GS mice) fed in a small cage with a diet supplemented with GliSODin<sup>®</sup>. **[Fig. 6]** In the experimental model, mice were fed in narrow cages with 12 hour immobilization to generate psychological stress. Both GliSODin<sup>®</sup> and tocopherol protected against neuronal cell lipid peroxidation; however GliSODin<sup>®</sup> also prevented impairment of spatial memory, and significantly decreased latency of escape of the animal comparing to other experimental groups. The authors hypothesize that GliSODin<sup>®</sup> treatment may upregulate neurotrophic factors such as nerve growth factor (NGF) and insulin-like growth factor 1 (IGF-1) in the brain, enhance hippocampal

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<sup>12</sup> Rahman H., Rocco R., Latorre J., Tabassum V., “The effects of a specialized superoxide dismutase nutritional supplement for HIV patients on HAART” Millenium Biotechnologies.

neurogenesis and protect against stress-induced impairment of spatial memory induced by psychological stress.<sup>[13]</sup>



**Figure 6.** Spatial learning and memory in mice. The escape latency of GliSODin® (GS) receiving mice was significantly shorter than in the control groups (C, RS, VE).

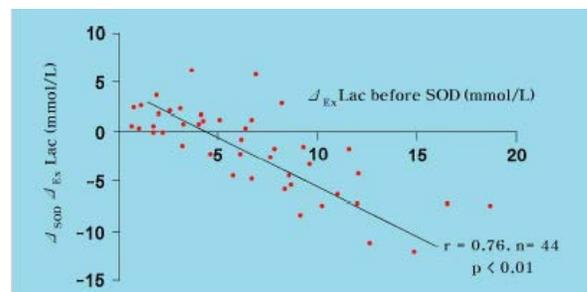
### ***Benefits of GliSODin® for physical performance & sport***

High intensity aerobic exercise can increase oxygen consumption by up to 20 – fold, overwhelming our antioxidant defenses and resulting in increased oxidative stress.<sup>[14]</sup> A study by Arent et al. at Rutgers University looked at the effects of pre-season training on performance capacity and oxidative stress responses of 22 soccer players randomly assigned to receive a supplement of Resurgex,

<sup>13</sup> Nakajima S. et al. Oral supplementation with melon superoxide dismutase extract promotes antioxidant defences in the brain and prevents stress induced impairment of spatial memory. *Behav Brain Res*, 2009 June 8;200(1):15-21.

<sup>14</sup> Marzatico F., Pansarasa O., Bertorelli L., Somenzini L., Della Valle G., “Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes” *Journal of Sports Medicine and Physical Fitness*, 1997, Volume 37, Issue 4, Pages 235-239

a blend of GliSODin® (500 IU), co-enzyme Q10 (75 mg), beta-glucans (100 mg), or an isocaloric equivalent daily for the duration of pre-season.



**Figure 7.** Effect of 4-week oral SOD administration on exercise-induced increase in plasma lactate

The group receiving the GliSODin® supplement showed improvements in measures of lactate production and “time to exhaustion”, and significantly greater reductions in oxidative stress-inducing lipid hydroperoxide levels than the control group. The researchers concluded that the GliSODin® may have meaningful effects possibly including improved recovery for people exercising at a high intensity<sup>[15]</sup>.

Hong et al. recruited 44 healthy individuals and assigned them to receive a daily SOD (GliSODin®) dose of 1500 IU for four weeks. Healthy volunteers were then submitted to cycling or treadmill exercise but assigned into two distinctive groups: the severe exercise group (27 subjects) or the moderate exercise group (17 subjects). Only volunteers ranging from the severe exercise group showed a

<sup>15</sup> Arent S.M., DiFabio D., Greenwood J., Pellegrino J., Williams C.A., “Nutritional supplementation in male college soccer players: effects on performance and oxidative stress” Rutgers University. 2004

significant reduction in the exercise-induced lactate production, after the four-weeks of SOD supplementation.<sup>[16]</sup> (**Figure 7**)

These studies indicate that supplementation with the gliadin-SOD complex could allow for quicker recovery for people undertaking strenuous exercise.

### ***Benefits of GliSODin® for Cardiovascular Health***

Researchers from France's National Association of Medical Prevention reported that supplementation with GliSODin®, in combination with diet and lifestyle changes, should significantly reduce the risk of cardiovascular disease by significantly reducing vascular inflammation, and may even positively impact on previous damage.

Cloarec et al. recruited 76 patients considered to be at risk of cardiovascular disease but free of any clinical symptoms of the disease and assigned them to diet and lifestyle changes for 12 months. Minor improvements in blood pressure, LDL-cholesterol (so-called 'bad' cholesterol) and body mass index (BMI) were reported. Due to the stringent conditions of the study 42 volunteers dropped out. The remaining 34 subjects were randomly divided into two groups, one to continue with the diet

and lifestyle recommendations only, and the second group received a daily supplement of GliSODin® (500 IU) for two more years.

Using ultrasound-B imaging to measure carotid artery intima thickness (IMT), a sign of hardening of the arteries (atherosclerosis), the researchers found a reduction in the progression of IMT in the SOD-supplemented group, compared to the diet and lifestyle only group. (**Figure 8**)

While no changes in antioxidant status was observed in the control group, significant improvements in antioxidant status as a result of SOD supplementation were observed. Furthermore, GliSODin® supplementation produced a 34 per cent reduction in malondialdehyde (MDA) levels – MDA is a reactive carbonyl compound and a major end product of lipid oxidation. A major part of the pathogenesis of atherosclerosis, and subsequently cardiovascular disease, is the oxidative modification of LDL-cholesterol.

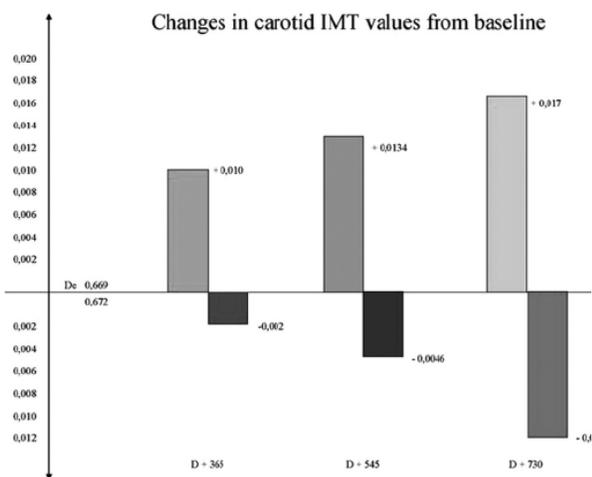
This study showed that supplementation with GliSODin® could impact antioxidant status and the inflammatory process, and had clear benefits against atherosclerosis, a major risk factor for cardiovascular disease.<sup>[17]</sup>

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<sup>16</sup> Hong Y., Hong S., Chang Y.H., Cho S.H., "Influence of an orally effective superoxide dismutase (glisodin) on strenuous exercise induced changes of blood antioxidant enzymes and plasma lactate," *presented at the American Association for Clinical Chemistry (AACC) National Meeting*, July 2004

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<sup>17</sup> Cloarec M., Caillard P., Provost J.-C., Dever J.-M., Elbeze Y., Zamaria N., "GliSODin®, a vegetal SOD with gliadin, a preventative agents vs. atherosclerosis, as confirmed with carotid ultrasound-B imaging" *European Annals of Allergy & Clinical Immunology*, 2007, Volume 39, Number 2, Pages 2-7



**Figure 8.** Changes of IMT at baseline, D365, D545 and D730. Upper columns: IMT in control group; lower columns: IMT in GliSODin® group. While the control group experienced significant thickening of IMT, the GliSODin® group experienced a significant reduction of IMT.

### *Alleviation of Reperfusion injury with oral administration of GliSODin®*

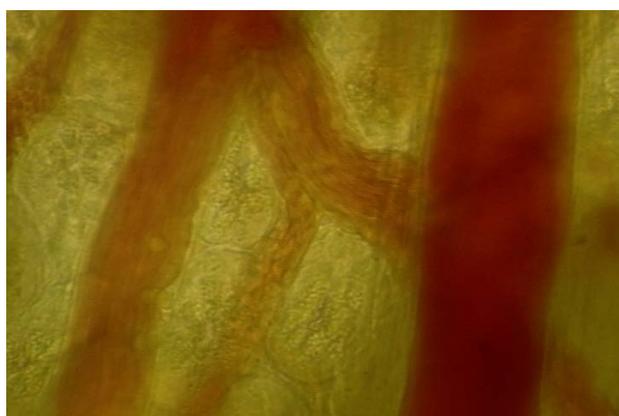
In the in vivo experiment with ICR mice, the cardiovascular effect of oral GliSODin® on gastrointestinal arterial blood circulation was evaluated. After 9 days of gavage supplementation with GliSODin® in a dose of 1.6 mg/kg of body weight, the anesthetized animals were subject to a ischemia-reperfusion injury of superior mesenteric artery by clamping the blood vessel for 15 minutes and then opening the clamp and restoring blood flow. By opening the clamp and restoring blood flow the blood vessel was subject to so called reperfusion injury.

Reperfusion injury is the tissue damage caused when blood supply returns to the tissue after a period of ischemia or lack of oxygen due to, e.g. artery clamping or blockage by blood clot. The absence of blood flow and oxygen

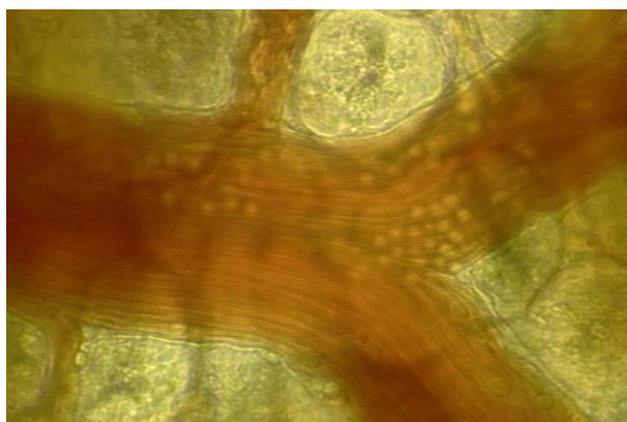
during the ischemic period creates a condition in which the restoration of circulation results in a massive formation of free radicals, which produce oxidative damage and inflammation of the affected vessel, i.e. mesenteric artery. The blood flow in reperfusion damaged artery is compromised and the white cells tend to adhere to the damaged epithelium impeding blood flow and tissue oxygenation. In the in vivo experiment a microscopic evaluation via camera of blood flow in the reperfusion-injury site with (A) and without (B) GliSODin® supplementation is presented in the attached photographs. The reperfusion injury site from animals receiving GliSODin® for 9 days (A) showed better passage of white blood cells through the damaged vessel in comparison to control mice with mesenteric artery showing attachment and aggregation of white blood cells to the epithelial cells lining the artery. (B) The results of this experiment show that a short term regimen with oral GliSODin® may alleviate tissue oxygen deprivation produced in the experimental model of cardiovascular event such as it may occur in stroke or cardiac arrest.

Reperfusion injury and its management play a critical role in recovery from stroke and from cardiac arrest. Repeated bouts of ischemia and reperfusion injury also are thought to be a factor leading to the formation and failure to heal of chronic wounds such as pressure sores and diabetic foot ulcers.

Picture (A) shows lack of attachment and aggregation of white blood cells in reperfusion-injury of mesenteric artery from animals which were pretreated with GliSODin<sup>®</sup> 1.6 mg/kg BW for nine days. Picture (B) shows attachment and aggregation of white blood cells in reperfusion-injury of mesenteric artery from control animals not pretreated with GliSODin<sup>®</sup>.<sup>[18]</sup>



A



B

### ***Benefits of GliSODin<sup>®</sup> for skin health***

A randomized double-blind clinical trial by Mac-Mary et al. showed that GliSODin<sup>®</sup> supplementation reduced skin-reddening when

healthy fair-skinned volunteers were exposed to UV radiation<sup>[19]</sup>. Fifty subjects were randomly assigned to receive a daily dose of GliSODin<sup>®</sup> (500 mg) or placebo four weeks. Subjects were exposed to UV radiation to induce sunburn on the inner-forearms, and the susceptibility of the participants to sunburn (defined as the minimum erythematous dose – MED) and a measure of the resulting redness (actinic erythema).

[Figure 9] shows that supplementation with GliSODin<sup>®</sup> resulted in an increase in the minimum exposure to UV rays necessary to produce skin burn for fair-skinned people (phenotype II), compared to placebo. The induced redness also decreased quicker in the

GliSODin<sup>®</sup>-supplemented group over the four-week period. These results confirmed the efficacy of the SOD-gliadin combination against the consequences of oxidative stress produced by exposure to UV radiation.

However, in a later study of similar design with Type II (fair-skinned) participants, GliSODin<sup>®</sup> significantly increased the MED in two weeks' time with a dose of just 250mg.<sup>[19]</sup>

These studies were built on two older studies; a pilot study of 15 patients who were susceptible to sun-burn supplemented with a

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<sup>19</sup> Mac-Mary M., Sainthillier J., Creidi P., Series J.P., Vix F., Humbert Ph., "Could a photobiological test be a suitable method to assess the anti-oxidant effect of a nutritional supplement (Glisodin<sup>®</sup>)?" *European Journal of Dermatology*, 2007, Volume 17, Number 2

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<sup>18</sup> Isocell Corp. Personal Communication 2012



**Figure 9.** GliSODin<sup>®</sup> induced increased in minimum erythematous dose (MED) compared to Placebo

daily dose of 500 mg GliSODin<sup>®</sup> for three to eight weeks of normal sun exposure; and an open clinical trial with 150 volunteers taking a daily GliSODin<sup>®</sup> supplement (500 mg) for 60 days. 86% of participants reported significant relief.<sup>[20, 21]</sup>

### *The role of GliSODin<sup>®</sup> in suppressing inflammation*

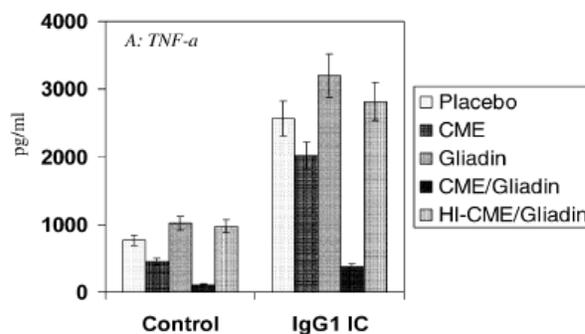
An *in vivo* study by Vouldoukis et al.<sup>[7]</sup> submitted C57BL/6 mice groups to various supplements for 28 days: placebo, gliadin only (1 mg), SOD only (5 IU), the SOD-gliadin combination (5 mg equivalent to 5 IU of SOD, GliSODin<sup>®</sup>), or heat-inactivated SOD-gliadin combination (5 mg) for 28 days and then given an intra-peritoneal INF- $\gamma$  injection (300 IU). Peritoneal macrophages were harvested 24 hours later and challenged with IgG1/anti-IgG1

<sup>20</sup> DermExpert Trial, "Evaluation Of Glisodin's Effect On Erythema Induced By UV Radiations," Intermediate Reports, February 2006

<sup>21</sup> Laverdet C., Pomarede N., Oliveres-Ghouti C., "Glisodin and Exposure to the Sun," an open study conducted in France on 150 patients by 40 dermatologists. Sponsored by ISOCELL Nutra, France. March 2005

immunocomplexes to amplify the inflammatory response. Only GliSODin<sup>®</sup> reduced the production of the pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF- $\alpha$ ) and promoted production of the anti-inflammatory cytokine interleukin-10 (IL-10), compared to the other treatments. (**Figure 6**)

This result also showed that it is necessary to preserve the enzymatic activity of the administered-SOD to retain the anti-inflammatory effect of GliSODin<sup>®</sup> since IL-10 production was not observed when GliSODin<sup>®</sup>



**Figure 6.** Effects of GliSODin<sup>®</sup> on production of pro- and anti-inflammatory cytokines

was previously heat inactivated.

The anti-inflammatory effects of GliSODin<sup>®</sup> are significant since chronic inflammation is associated with the onset and progression of many chronic diseases.

Moreover, Okada et al. reported that administration of the gliadin-SOD complex could prevent cancer progression, promoted by inflammation.<sup>[22]</sup> The researchers used

<sup>22</sup> Okada F., Shionoya H., Kobayashi M. Kobayashi T., Tazaxa H., Onuma K., Iuchi Y., Matsubara N., Ijichi T.,

C57BL/6 female mice implanted with a gelatine sponge (to promote inflammation) and injected with QR-32 tumor cells. The mice were then randomly supplemented with GliSODin<sup>®</sup>, SOD-only or gliadin only at a dose of 10 mg per kg of body weight. While tumor growth was suppressed in the GliSODin<sup>®</sup> group, the tumors in the mice supplemented with only SOD or gliadin were found to have significantly increased. The mechanism behind these effects was proposed to be related to reducing levels of the reactive oxygen species, the superoxide ion. It was also proposed that the melon SOD extract-gliadin combination may also be affecting immune response.

This result indicated that the antioxidant and anti-inflammatory properties of GliSODin<sup>®</sup> might have significant benefits for the prevention of tumor development and progression.

### ***Potential benefits of GliSODin<sup>®</sup> for diabetes:***

Diabetes is on the rise worldwide, with diabetic kidney disease just one of the many complications of the disease. It has been suggested that high glucose levels may result in increased oxidative stress, and thereby promote the development of diabetic kidney disease

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Dugas B., Hosokawa M., "Prevention of inflammation-mediated acquisition of metastatic properties of benign mouse fibrosarcina cells by administration of an orally available superoxide dismutase" *British Journal of Cancer* 2006, Volume 94, Pages 854-862

(diabetic nephropathy). To test whether oral administration of GliSODin<sup>®</sup> could positively impact on the pathogenesis of diabetic nephropathy, Naito et al. assigned diabetic and non-diabetic mice to receive a standard rodent diet and one group was supplemented with the cantaloupe melon extract-gliadin combination (0.08 per cent of the diet) for twelve weeks.<sup>[23]</sup>

No significant differences in blood glucose levels or body weight were observed between the diabetic mice in the SOD-gliadin-supplemented group and the control group. Both diabetic mice groups had higher blood glucose levels and body weights than non-diabetics.

In terms of kidney health, significant reductions in the levels of 8-hydroxydeoxyguanosin (8-OHdG), a marker of oxidative stress, were observed in the SOD-gliadin-supplemented group. Ha et al.<sup>[24]</sup> have reported that 8-OHdG formation is closely related to the development of diabetic kidney disease in rodents, and reductions in 8-OHdG were associated with improvements in kidney health amongst the test animals used by Naito et al.

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<sup>23</sup> Naito Y., Akagiri S., Uchiyama K., Kokura S., Yoshida N., Hasegawa G., Nakamura N., Ichikawa H., Toyokuni S., Ijichi T., Yoshikawa T., "Reduction of diabetes-induced renal oxidative stress by a cantaloupe melon extract/gliadin biopolymers, oxykine, in mice" *BioFactors* 2005, Volume 23, Pages 85-95

<sup>24</sup> Ha H., Kim C., Son Y., Chung M.H., Kim K.H., "DNA damage in the kidneys of diabetic rats exhibiting microalbuminuria" *Free Radis. Biol Med.*, 1994, Volume 16, Pages 271-274

This study suggested that the cantaloupe melon extract-gliadin combination might be a novel approach for preventing diabetic kidney disease by reducing oxidative stress.

### ***Benefits of GliSODin® in sports nutrition***

The double-blinded study included 19 members of the Polish National Rowing Team who were participating in a training camp. Subjects were randomly assigned to the supplemented group (n = 10), who received 2 capsules (total 500 mg) of GliSODin® extract once daily for 6 weeks, or the placebo group (n = 9). At the beginning and end of the study, subjects performed a 2,000-meter maximum effort test on a rowing ergometer. Blood

samples were taken before each test, 1 minute after completing the test, and after a 24-hr rest period. After supplementation, SOD activity was significantly higher (p = .0037) in the supplemented group at all measurement times, and post-exercise C-reactive protein was significantly lower (p = .00001) in athletes receiving GliSODin® than those in the placebo group [Table 1]. In conclusion, supplementation with an extract rich in SOD activity promoted antioxidant status and protected against increased inflammation in the serum of professional rowers.<sup>[25]</sup>

**Table 1**

Parameter	Before Supplementation			After Supplementation			Exercise	GliSODin	Exercise × GliSODin
	Preexercise	Postexercise	Recovery	Preexercise	Postexercise	Recovery			
CRP (mg/L)							<i>p</i> = .915	<i>p</i> < .001	<i>p</i> = .997
Sup	0.46 ± 0.31	0.49 ± 0.39	0.50 ± 0.37	0.18 ± 0.08*	0.21 ± 0.10*	0.23 ± 0.11*			
Pla	0.28 ± 0.26	0.54 ± 0.50	0.41 ± 0.39	0.61 ± 0.56	0.68 ± 0.62	0.66 ± 0.57			
TBARS (µmol/g Hb)							<i>p</i> < .001	<i>p</i> = .257	<i>p</i> = .638
Sup	1.77 ± 0.56	2.78 ± 1.11†	3.08 ± 0.89†	1.19 ± 0.31	1.89 ± 0.24†	1.71 ± 0.62			
Pla	1.51 ± 0.69	2.46 ± 0.69†	2.61 ± 0.78†	1.45 ± 0.59	2.47 ± 0.75†	1.77 ± 0.58			
CK (U/L)							<i>p</i> = .150	<i>p</i> = .261	<i>p</i> = .992
Sup	166 ± 86	204 ± 100	167 ± 79	75 ± 41	92 ± 46	146 ± 91			
Pla	210 ± 163	259 ± 177	224 ± 120	110 ± 88	130 ± 117	213 ± 132			
LDH (U/L)							<i>p</i> < .001	<i>p</i> = .515	<i>p</i> = .687
Sup	153 ± 68	242 ± 64†	195 ± 49	182 ± 51	262 ± 50†	185 ± 41			
Pla	128 ± 45	270 ± 43†	209 ± 37†	192 ± 78	279 ± 23†	205 ± 23			

Note. CRP = C-reactive protein; Sup = supplemented group; Pla = placebo group; TBARS = thiobarbituric-acid-reactive substances; CK = creatine kinase; LDH = lactate dehydrogenase.

\**p* < .05 relative to Pla group. †*p* < .05 relative to preexercise.

<sup>25</sup> Skarpanska-Stejborn A et al. Effects of oral supplementation with plant superoxide dismutase extract on selected redox parameters and inflammatory marker in 2000 m rowing-ergometer test. *Int J Sport Nutr Exerc Metabol* 2011;124-134.

## Conclusions

Production of reactive oxygen species (ROS) is a normal process in oxygen-breathing organisms. Under normal physiological conditions a balance between these species and the body's anti-oxidant defenses exists, but certain conditions can increase the production of ROS like the superoxide ion ( $O_2^-$ ) and disrupt the natural balance and lead ultimately to oxidative stress.

The superoxide ion is the starting point of the cascade of reactions of free radicals production. Superoxide dismutase (SOD), dubbed the "enzyme of life" on discovery in 1968, is the first antioxidant mobilized by the cell as defense against oxidative stress.

Oral delivery of the pure enzyme to boost the body's natural antioxidant defenses has been limited by the harsh conditions experienced in the gastrointestinal (GI) passage. However, combination of SOD extracted from cantaloupe melon (*Cucumis melo* L.C.) with the wheat gliadin biopolymer (GliSODin<sup>®</sup>) can significantly and progressively increase SOD stability and delivery during passage through the GI tract, as shown by results from *in vitro*, *in vivo* and human studies.

The anti-inflammatory and immune system modulating effects for the SOD-gliadin combination have also been discussed, with the results showing that, while no such effects are observed when SOD or gliadin alone are

administered, the SOD-gliadin combination is effective.

Such benefits can also be related to other conditions associated with oxidative stress, with significant health implications for improved recovery after strenuous exercise, reduction of inflammation (reddening) in the skin on exposure to sunlight (UV radiation), improvement in heart health, and complications arising from diabetes.

As strategies to inhibit oxidative stress and contribute towards healthy aging are considered, oral supplementation with GliSODin<sup>®</sup> has been shown scientifically to offer a therapeutic means for the prevention and treatment of many conditions associated with increased oxidative stress and inflammation.

In summary GliSODin<sup>®</sup> may offer important health benefits that go beyond its obvious internal anti-oxidant promoting action, in particular:

1. Cardiovascular effect preventing inflammatory damage to the lining of blood vessels,
2. Lowering circulating pro-inflammatory markers contributing to chronic degenerative conditions, and
3. Improving response to psychological stress and its detrimental effects on cognition and memory.