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Bibliographic Review

Glutathione: the master antioxidant

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Keywords

Oxidative stress, redox balance, ozone, glutathione, phytochemicals..

Abstract

The alteration in the balance between reactive oxygen species (ROS) generation and antioxidant buffering capacity creates oxidative stress in the body that leads to disease on the body systems. The ROS are the operating agents to create an oxidative state of the body. There are biological mechanisms to tamper these agents and to reach a redox equilibrium, for promotion of health and avoid sickness. Some mechanisms of the oxidative stress will be discussed on the paper. The glutathione is a tripeptide, it the first line of defence against oxidative damage, being very important for the protection of the cells from oxidative damage and maintaining redox homeostasis. The synthesis, the function, the consequences of the depletion and the supplementation of glutathione will be presented. The contribution of phytochemicals as antioxidants will be briefly discussed and examples of useful plants will be presented..

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1. The oxidative Stress

Oxidative stress is an alteration in the balance between reactive oxygen species (ROS) generation and antioxidant buffering capacity[1,2], causing alterations in the cellular metabolism and its regulation and damaging cellular constituents.[3,4] Oxidative stress plays a central role in the sustained immuno-inflammatory responses that accompany various ailments.[2]

Antioxidant is defined as any substance that present at very low concentrations compared with the oxidable substance and prevent or retard their oxidation.[5] ROS can be defined into two groups (Figure 1):[5]

- Free radicals, which are atoms, molecules, or ions capable of independent existence that contain one or more unpaired electrons in the most external orbital (identified with an upper dot)
- Non-radicals with high chemical reactivity.

Free radicals	(symbol)	Non-radicals	(symbol)
Superoxide anion	$O_2^{\bullet -}$	Peroxide hydrogen	H_2O_2
Hydroxyl radical	$^{\bullet}OH$	Peroxinitrite	ONOO-
Lipid radical	$L^{\bullet}$	Hypochlorous acid	HClO
Nitric oxide	$NO^{\bullet}$	Ozono	O_3
Alkonyl radical	$RO^{\bullet}$	Singlet oxygen	$^1O_2$
Alkylperoxy radical	$ROO^{\bullet}$	Alkyl hydroperoxide	ROOH
Glutathiyl radical	$GS^{\bullet}$		

Figure 1 - Classification of the reactive oxygen species.[5]

ROS are produced during metabolic and immune system function, where molecular oxygen (O_2) can unpair and leave free radicals which are highly unstable and reactive, leading to the formation of ROS.[6] This makes them highly unstable and reactive with other molecules to produce more stable species.[1] The mitochondria (electron transport of the respiratory chain) produce over 90% of cellular ROS[3], but inflammation and transition metal ions are involved.[5] The metabolism of drugs and xenobiotics, cigarette smoke and environmental pollutants and radiation, known as avoidable sources, can cause imbalance on the redox state.[5]

The enzyme systems that produce ROS are cytochrome P450, the mitochondrial respiratory chain, xanthine oxidase, uncoupled endothelial nitric oxide synthase, heme oxygenase, myeloperoxidase, lipoxygenase, cyclooxygenase, and NADPH oxidases.[1]

So, oxidative stress is basically a disruption between the antioxidant defense system and the generation of oxidants that happen acute or chronically[5], and when a cell is not stressed, the processes that generate ROS are well counterbalanced by antioxidant systems.[3] Since ROS are constantly generated during normal cellular metabolism[7], this means that if there is a change on the balance of prooxidant /antioxidant system in favour of prooxidant there will be damage to the cellular macromolecules.[1] If cells are not capable of coping with the intensity of oxidative stress, this can cause lipid, protein and DNA damage that culminate in death via necrosis or apoptosis.[1,7] That can happen under pathological conditions where ROS generation may be sharply increased, overwhelming the capacity of the cellular antioxidant defense system.[7]

Figure 2 shows the acute and the chronic reaction and evolution of the oxidative stress. Under normal circumstances, the ROS values varies over a considered normal range. A stressing event increases its levels and under some situations the levels might not return to the previous (normal) levels and the system will be up regulated on a new higher (bad) level called quasi-stationary level. The upregulation of the antioxidant potential may result in the restoration of ROS levels back into the initial range, but on a chronic oxidative stress due to a prolonged increase in ROS levels. In this case, the chronic state may lead to cardiovascular and neurodegenerative diseases, diabetes mellitus, cancer, and aging.[3,8,9] Cardio-tolerance to oxidative stress reduces with advancing age due to the antioxidant levels, particularly enzymatic antioxidants, contributing to the development of CMD. In rat, heart metabolic and functional tolerance toward oxidative stress decreases with age.[10]

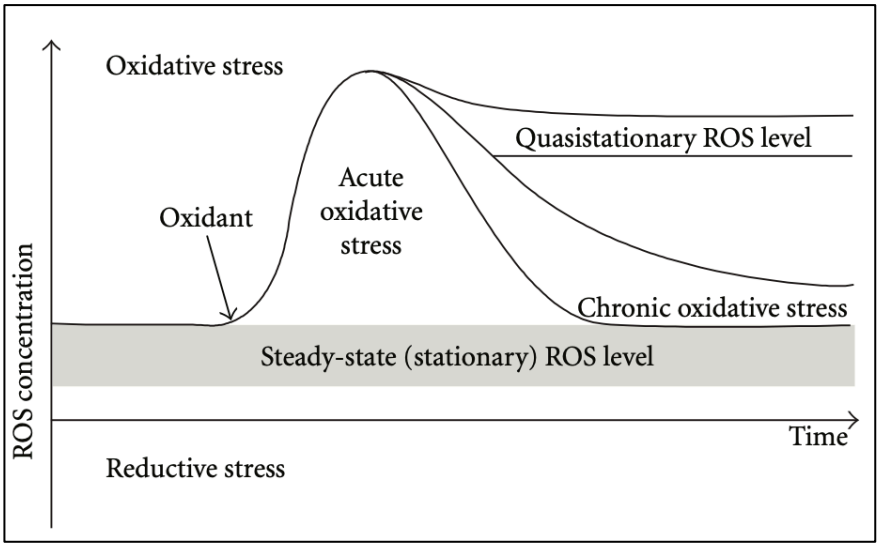

Figure 2 - The dynamics of reactive oxygen species in biological systems.[3]

The excessive generation of ROS are responsible for cardiovascular diseases, including atherosclerosis, myocardial ischemia and cardiomyopathy, myocardial infarction, which can lead heart failure[7], and also for neurologic diseases, cancer, chronic inflammation, chronic rejection (transplant) and cystic fibrosis (figure 3).[5] However, at physiological levels, ROS functions as signalling mediators, regulates various physiological functions such as growth, proliferation, host defence and genomic stability, but at excessive levels it causes a deviation in the redox state.[1,3] ROS at low to moderate concentrations play a key homeostatic function, they protect the cell against ROS damage by stimulating antioxidant responses and maintaining or reestablishing redox balance.[1] Also, production of ROS by phagocytes (O_2.-), that then can be dismuted into H_2O_2 by the action of superoxide dismutases), is part of the mechanism of killing microorganisms.[11]

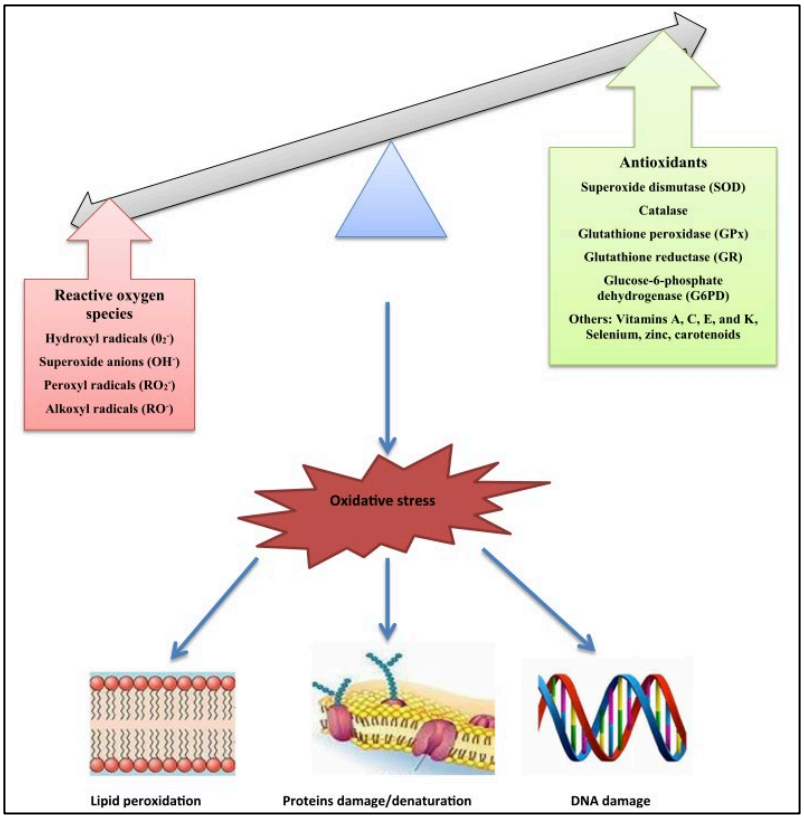

Figure 3 - Oxidative stress resulting from an imbalance between ROS generation and antioxidant system and its consequences on cellular macromolecules.[1]

The superoxide radical (O_2.-) is the first to be produced by most of the enzyme systems and then it can undergo rapid dismutation to hydrogen peroxide (H_2O_2), which mediates most signaling effects of ROS.[12] The H_2O_2 can be potentially hazardous if there are reduced metals present in the cells. H_2O_2 can react with ferrous iron (Fe^{2+}) and produce the hydroxyl radical ($^{\bullet}OH$), which can react with any molecule next to where it is produced.[11] It is quite dangerous wen it is produced close to a membrane, since it starts a free radical chain reaction that will damage the membrane. There is lipid peroxidation by $^{\bullet}OH$, resulting a lipid radical ($L^{\bullet}$) and water. Then, the $L^{\bullet}$ can react with oxygen to produce hydroperoxide radical (LOO), which then reacts with another lipid molecule, generating a lipid peroxide (LOOH) and so on.[11]

Other agents have an aggressive behavior inside cell, and these include inflammatory cytokines generated by the adipose tissue, where for instance the fatty acids cause insulin resistance in humans due to a decrease in insulin-stimulated muscle glucose transport activity[13], tumor necrosis factor-alpha (TNF-α), involved in the pathogenesis of nonalcoholic steatohepatiti[14], and interleukin-6 (IL-6).[5]

The human body include endogenous mechanisms that may function as antioxidants (local and systemically), and they can be complemented by the provision of exogenous antioxidants caried in by the diet or as a supplement.[2] The consumption of the exogenous antioxidants are very useful, since they can maintain or re-establish the redox homeostasis, either inhibiting the ROS production or indirectly by the enhancement of endogenous antioxidant systems, providing a prophylactic and therapeutic tool against many diseases.[2]

The cellular protection against oxidative and electrophile toxicities is provided by two types of antioxidants:[15]

(i) direct antioxidants, which are redox active, short-lived

(ii) indirect antioxidants activate the Keap1/Nrf2/ARE pathway resulting in transcriptional induction of a battery of cytoprotective proteins.

The defense against ROS can be enzymatic and non-enzymatic antioxidants:[1,5]

a) Enzymatic

- Superoxide dismutases (SOD) are the major antioxidant defense systems against $O_2^{\bullet}$-, and inhibit the oxidative inactivation of nitric oxide, preventing peroxynitrite formation and endothelial and mitochondrial dysfunction; it is the only enzyme that acts on a radical ($O_2^{\bullet-} + O_2^{\bullet-} + 2H \longrightarrow H_2O_2 + O_2$);[4]
    - Catalase - it is one of the most efficient known enzymes, decomposes H_2O_2 to oxygen and water;

- Glutathione peroxidase (GPx) - catalyzes the reduction of H_2O_2 to water, and lipid peroxides to their alcohols; requires the activity of glutathione reductase;
- Paraoxonases (PON) - PON-1 prevents the peroxidation of HDL and LDL, breaks down cholesteryl esters and lipoproteins seen in oxidized lipoproteins;
- Heme oxygenase (HO) - catalyzes degradation of heme to carbon monoxide, biliverdin, and free ferrous iron;
- Thioredoxin (Trx) - enhances the vascular redox and the nitic oxide synthase.

b) Non-enzymatic

- Bilirubin, uric acid, glutathione, vitamins C and E and polyphenols;
- Selenium, copper, zinc, iron, and calcium also contribute to the antioxidant buffering capacity, but iron and copper may act as pro-oxidants by catalyzing the production of hydroxyl radicals from O_2^- and H_2O_2.

2. The glutathione

Glutathione (GSH) was discovered in 1888 by Rey Pailhade[5] with the name *hydrogénant le soufre*, and later in 1929 Hophins F G proved to be GSH. It is 133 years old. Glutathione peroxidase was discovered in 1957 by Mills G C. In 1969, E. M. and N. S. Kosower coined it as "Lest I forget thee, glutathione", reflecting the state of GSH science on the 60^{ths}. Later, Helmut Sies, in 1999, proposed a new slogan: *inevitable GSH*, because, on his words, *practically all major biologic processes involve the thiol redox state*, reflecting the paramount importance of GSH on the body homeostasis.[16]

Glutathione is an endogenous component of cellular metabolism, it is a tripeptide consisting of γ-glutamine-cysteine-glycine (Figure 4).[16-20] Precursors of GSH include cysteine, N-acetylcysteine, glutathione monoethyl ester, and oxothiazolidine 4-carboxylate (OTC).[21] For this reason, it is not an essential nutrient, because it can be synthetized from those amino acids.[5]

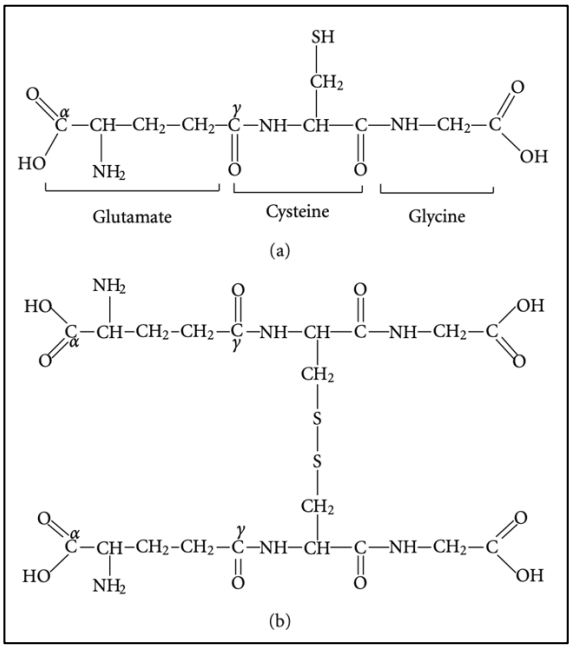

Figure 4 - Glutathione: a) reduced form (the cysteine residue is the key functional component of glutathione, providing); b) oxidized form (cysteine residues form the intermolecular dipeptide bond)[20]

Glutathione is the most abundant low molecular weight non-protein thiol in most cells.[11,20-24] The term *thiol* refers to compounds containing sulfur and the sulfurcontaining amino acids (SAAs) are methionine, cysteine, cystine (the disulfide form of cysteine)[11], homocysteine, homocystine, and taurine. N-acetylcysteine and glutathione can be synthetized from SAAs.[21] GSH lacks the toxicity associated with cysteine, which allows it to be a good cellular “redox buffer”.[16]

Inside the cells GSH exist in reduced state (GSH), about 90% of the total, and in oxidized state (glutathione disulfide, GSSG), that consists of two reduced glutathiones bound at the sulfur atoms (Figure 4).[5,16,19,20] GSH may be oxidized directly by oxidants, such as hydroxyl radical (HO•) or peroxynitrite (ONOO−).[20] The ratio of GSH to GSSG determines cell redox status of cells and an increased ratio of GSH/GSSG is indicative of oxidative stress.[5,20] Healthy cells at rest have a GSH/GSSG ratio >100, while the ratio drops to 1 to 10 in cells exposed to oxidant stress.[19]
The GSH exported by the liver is a supply for other organs, like the kidney, for example.[20] That is, GSH (and GSSG) can also be found outside the cells, for example in the thin layer of fluid covering the air spaces, where gas exchange occurs, secreted by epithelial cells, which is important for smokers or people that inhale particles or other oxidants (nitrogen dioxide and H_2O_2, for example).[11,20] Also, macrophages provide an additional source of reactive species in pulmonary epithelial, and it is useful to have high levels of GSH also in the lungs.[20] Important role of GSH is also in intestinal epithelial cells that contact with the exterior, and the main function here is the detoxification of injurious external agents to prevent damage to the organism.[20]

Methods of measurement

The development of methods can nowadays measure precisely and accurately the reduced GSH concentrations: enzymatic, spectrophotometric, fluorometric, and HPLC-based methods. The invention of electrospray interface, enabling the coupling of HPLC to mass spectrometers, has helped in the development of a method with increased specificity and sensitivity in comparison to the spectrophotometric- or fluorescence- based detection methods.[25] A procedure used for measuring GSH and GSSG now is high performance liquid chromatography.[11] The concentration of GSH is on average 12 mM in the mammalian cells, a value quite higher comparing with other published values.[5] In most cells the GSH concentration is about 1–2 mM [11] or higher concentration levels (5 mM), as much as concentrations of glucose, potassium, and cholesterol[19], but in the hepatocytes the concentration is of 10 mmol l^{-1}. [11] The intracellular concentration varies from 0,5 mM to 10 mM, and the extracellular concentrations has from one to three lower values.[11] In most cells and tissues, the estimated redox potential for the GSH/GSSG couple ranges from −260 mV to −150 mV.[11]

Regulation of transcription of GSH-related genes

GSH has a role in signal transduction, in gene expression, and in apoptosis.[26] It is known that GSH is a key component in the regulation of redox homeostasis. Regulation of the activities of GSH-related enzymes is under control of several cellular signaling systems ending on the production of the first enzyme for GSH synthesis, *γ*GLCL. In this process the Nrf2/Keap1 system is involved, particularly on the neutralization of oxidants and electrophiles. When the levels of these rise in the cell, also with activation of various protein kinases, there is disruption of the Nrf2/Keap1 association, resulting in Nrf2 stabilization and migration into the nucleus. Here, the interaction with the specific genes stimulates the transcription for the enzymes production.[3] GSH modulates signaling cascades and gene expression under conditions that must be rated as physiological rather than stressing.[26]

Synthesis

Glutathione it is produced exclusively in the cytosol using the amino acids by three ways and then it is actively pumped into mitochondria:[19]

- *De novo* synthesis (two steps) catalyzed by the enzymes glutamate cysteine ligase (GCL) and glutathione synthetase (Figure 5), which is primarily controlled by the cellular level of the amino acid cysteine, the availability of which (and also ATP) is the rate limiting step[5,16]
- Regeneration of oxidized GSSG to reduced GSH by glutathione reductase (requires NADPH)
- Recycling of cysteine from conjugated glutathione via GGTP (requires NADPH).

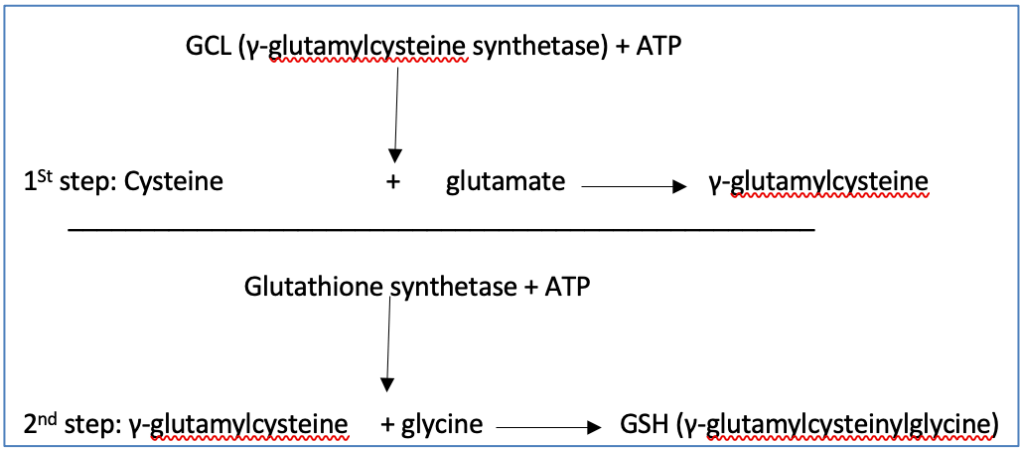

Figure 5 - The two steps synthesis of glutathione[5,11,20]

The insufficiency of sulfur amino acid will compromise the synthesis of GSH, greater than the hepatic protein synthesis, that will jeopardize the antioxidant process.[19] Functioning of T cells is dependent on intracellular glutathione concentrations and may also be affected by sulfur amino acid insufficiency.[17] Sulfur metabolism in humans is very complicated and has an important function in redox biochemistry.[27]

GSH is consumed by oxidation, conjugation, and hydrolysis and can be directly oxidized by reactive oxygen and nitrogen species or indirectly during GSH-dependent peroxidase-catalyzed reactions.[20]

After being synthesized, GSH can undergo transport across the plasma membrane and be involved on interorgan transport network.[16] The intracellular hepatic GSH, the major source of synthesis, it is an important pool of cysteine for other cells to produce GSH. This is excreted out of the cells into the blood, and it will travel to other cells, but before moving in the cell by specific amino acid transporters, GSH must be broken down by the action of two enzymes: γ-glutamyl transpeptidase and a dipeptidase. Once inside the cell, the GSH biosynthesis occurs.[11,16]

Functions

GSH is often referred to as *the master antioxidant*[28] and it is important for the protection of the cells from oxidative damage and maintaining redox homeostasis.[11,20,26,29] GSH is often considered to be a key player of the defense system the first line of defence against oxidative damage.[3,5] It is reported as a key player of the defense system. Enhanced ROS levels may require not only enhanced GSH action to maintain redox status, but also enhanced energy and material consumption to replace consumed GSH.[1]

It is carrier of an active thiol group (cysteine residue), it acts as an antioxidant, either directly by interacting with ROS and electrophiles, or by operating as a cofactor for various enzymes. The GSH is a cosubstrate of glutathione peroxidase (GPx) for the reduction of the hydrogen peroxide (H_2O_2) and of organic peroxides (LOOH - lipid peroxides).[20]

The function on the elimination of reactive oxygen and nitrogen species is of great importance. GSH may either directly bind some ROS species or serve as a source of reductive power for certain antioxidant systems.[3] GSH is an important antioxidant and reacts directly with ROS, RNS (NO•), and other reactive species, particularly HO•, HOCl, RO•, RO_2•,^{1}O2, and ONOO−, and it is also involved on the detoxification of products derived from the oxidation of lipids (malonic dialdehyde and 4-hidroxy-2-nonenal).[5,20] However, virtually all compounds known as antioxidants possess prooxidant properties.[3,29,30]

The cellular and mitochondrial levels directly are highly associated with health and longevity.[19] Its intracellular pool is of paramount importance for the defense against oxygen radical pathology.[19,23,24] and it is the substrate for GSH transferases and peroxidases, enzymes that catalyze the reactions for detoxification of xenobiotics and reactive oxygen species[18,22,23] and protects the body from reducing agents.[18] It is the sulfhydryl group (−SH) of the cysteine that is involved in reduction and conjugation reactions, which are the most important functions of GSH.[11] Glutathione directly scavenges diverse oxidants:[19]

- superoxide anion
- hydroxyl radical
- nitric oxide
- carbon radicals.

Glutathione catalytically detoxifies: hydroperoxides, peroxynitrites, and lipid peroxides.[19] Another way glutathione protects cells from oxidants is through recycling and a mild sparing effect of vitamins C and E.[19,31]

In the cell, GSH has other functions. In the mitochondria it regulates apoptosis versus necrosis[11], which is important, because the intracellular accumulation of GSSG due to oxidative stress is toxic to cells and triggers apoptosis[19] and in the nucleus, GSH is a key regulator of cellular division.[11] However, cells normally contain high glutathione reductase activity, which maintain most of the GSH in the reduced form.[11] Glutathione may function as an anticarcinogen by acting as an antioxidant or by binding with cellular mutagens[32] and through GSH conjugation carries on detoxification by binding electrophiles that could otherwise bind to proteins or nucleic acids, resulting in cellular damage and genetic mutations.[18] In fact, the elimination of many xenobiotic compounds can occur through conjugation with GSH.[11] Also, GSH is also used in the elimination of electrophiles (4-hydroxy-2-nonenal).[11]

Other functions of glutathione include protection from mercury and other toxic metals, protection from alcohol, and protection from persistent organic pollutants[5,19], regulation of cell growth, protein function, and maintenance of immune function[24], and helps in the reduction of the mucus and inflammation in the airway and on the detoxification of the lungs.[5] Many more functions can be referred: decrease on tumor necrosis factor, protein-C reactive, restore some hepatic functions, fights the acute and the chronic fatigue, gives good function to the crystalline lens, increases insulin growth factor (IGF-1) and DEHA and protects against diabetes.[5]

Joseph Pizzorno, on an excellent Editorial, summarizes some of the roles of glutathione:[19]

1. Direct chemical neutralization of singlet oxygen, hydroxyl radicals, and superoxide radicals
2. Cofactor for several antioxidant enzymes
3. Regeneration of vitamins C and E [5]
4. Neutralization of free radicals produced by Phase I liver metabolism of chemical toxins
5. Regulation of cellular proliferation and apoptosis
6. Vital to mitochondrial function and maintenance of mitochondrial DNA (mtDNA).

Volodymyr I. Lushchak indicates several additional functions in cells:[20]

1. a reserve form of cysteine
2. stores and transports nitric oxide
3. participates in the metabolism of estrogens, leukotrienes, and prostaglandins, the reduction of ribonucleotides to deoxyribonucleotides, the maturation of iron-sulfur clusters of diverse proteins,
4. involved in the operation of certain transcription factors (particularly those involved in redox signalling)
5. the detoxification of many endogenous compounds and xenobiotics.

Depletion of glutathione

Cellular GSH reserves can be depleted by three ways:[3]

- increasing GSH oxidation
- inhibition of biosynthesis
- inactivation of the genes encoding the enzymes of GSH synthesis.

There is a relationship between GSH, nutrition, oxidative stress, disease, and aging. Protein-deficient diets or fasting (secondary manifestation of AIDS, cancer, burns, chronic digestive diseases, alcoholism), aging, overdosage of paracetamol and chronic disease cause glutathione deficiency.[22,23]

GSH deficiency causes cellular risk for oxidative damage[11,33], some diseases appear to be exacerbated by decreasing GSH[11] and it has been postulated that decreased levels of reduced GSH would be a marker for increased susceptibility to oxidant injury.[25,28] GSH imbalance is observed in a wide range of pathological conditions including tuberculosis, HIV, diabetes, and cancer[3,28] and cardiometabolic disorders.[1,5,12,29] One third out of 74 patients with chronic disease had GSH levels below the lower limit of normal and GSSG levels were like those in control subjects.[25]

Aging appears to be accelerated because of a decrease in the antioxidant capacity of tissues reflected in a decreased plasma GSH level.[34] GSH has been related to the aging process and it has been described as an anti-aging medication.[23,28,35] Unfortunately, after the age of 20 years, the optimal levels decrease by 10% per year.[5] Several studies on aged people have been published. In one investigation, 176 healthy subjects were divided in five groups, the youngest was group 1 (n=25; 0.2-1 years old) and the oldest was the group five (n=60; 41-69 years old). The results showed that GSH levels and the GSH/GSSG molar ratio in groups 1 and 5 were significantly lower than the other groups ($p<0.001$) and the GSSG levels were significantly higher in these periods ($p<0.001$). GPx activity in group 5 was increased as compared to the other groups ($p<0.001$). The authors concluded that their findings showed that the glutathione redox system is affected by age and the oxidative stress increases during the aging process.[35]

To test the hypothesis that blood glutathione levels are lower in aging human subjects, Calvin A Lang and et al performed a study in 39 men and 130 women, 20 to 94 years old. The reference group was comprised of the 20- to 39-year-old subjects, whose blood glutathione levels were 547 ± 53.5 μg/10^{10} erythrocytes. The cutoff for the lower values of GSH was 440 μg/10^{10} erythrocyte. The authors found that the incidence of low blood glutathione content in the older subjects increased significantly, particularly in the 60- to 79-year-old group (452 ± 86.8 μg/10^{10} erythrocytes), which was 17% lower than the reference group ($p<0.001$). This was a concern for the authors, since these low glutathione levels in apparently elderly people have a decreased capacity for detoxification reactions mediated by the GSH.[36]

Julius M. et al studied a community-based sample of elderly (n=33) found higher GSH levels associated with fewer illnesses ($p<0.05$), better self-rated health ($p<0.01$), lower cholesterol ($p<0,05$), lower body mass index, and lower blood pressures. The authors stressed the association of higher glutathione levels with higher levels of physical health in a sample of community-based elderly.[37]

The Lang CA's group studied the physical and the mental health in 87 white women (range = 60 to 103 years old) over a 5-year period (three moments), and the results were compared with representative individuals in this region and with normal national data. The healthy subjects were in top physical and mental health and the subjects of all ages had very high blood total glutathione G(T) levels in waves I and II, but only normal levels in wave III. The authors concluded that their findings confirm that high blood G(T) concentrations and excellent physical and mental health are characteristics of long-lived women.[38]

In the lab, with rats, the results of P. Abete *et al.* showed that the cardiac release of oxidized glutathione (an index of the ability of the heart to inactivate oxygen metabolites) was significantly lower in hearts from rats of 6 and 12 months than in younger animals ($P<0.001$).[10]

The age-dependent change could be partly compensated by physical training because skeletal muscle appears to be able to deliver GSH into circulation with the adaptation of muscle to exercise training reflected in an increased plasma GSH level in the trained subject.[34] However, on the other hand, decreased plasma GSH concentration following physical exercise demonstrates increased GSH consumption in skeletal muscle resulting in a reduced export rate from muscle into plasma. The results of study of K. Gohil *et al.* showed that during prolonged submaximal exercise, GSH decreased 60% from control, and GSSG increased 100%, meaning that there is an increase of formation of active $O_2^{\bullet -}$ species during physical exercise.[39] At sports level, the high oxygen consumption during the intense physical activity causes a high production of ROS in the mitochondria during the respiratory process. This means that for the athlete in training, muscle catabolism or a decrease in plasma GSH are counterproductive.[21] Also, suboptimal intakes of sulfur amino acids during training may exert a proinflammatory influence because, at low levels of intake, cysteine is preferentially incorporated into protein rather than GSH. Because whey protein is rich on cysteine and methionine (this can be converted on cysteine), the athletes are advised to follow this kind of supplementation after training or competition.[21]

At ocular level the importance of GSH was proven in a study with 14 patients with early age-related macular degeneration (AMD), which were compared with 14 age- gender- matched healthy controls. In the AMD patients there was higher oxidized glutathione ($p= 0.024$) and lower redox index ($p= 0.043$) levels than the controls.[40]

Supplementation

Optimizing glutathione levels has been proposed as a strategy for health promotion and disease prevention.[29] The oral supplementation has been a matter of debate, it might be useless, because of the possibility of digestion by digestive proteases[29] and GSH alone does not adequately restore GSH levels because it is rapid hydrolyzed by the liver and intestines.[41] A Meister, in a review paper published back in 1991, wrote that glutathione deficiency induced by inhibition of its synthesis may be prevented or reversed by administration of glutathione esters which, in contrast to glutathione, are readily transported into cells and hydrolyzed to form glutathione intracellularly.[42] Oral supplementation for four weeks (500 mg twice a day) was given to 40 adult volunteers without acute or chronic disease. The results showed that there were no differences in oxidative stress biomarkers between treatment groups at baseline and the authors concluded that no significant changes were observed in biomarkers of oxidative stress, including glutathione status.[43] On the other hand, a six-month study randomized, double-blinded, placebo-controlled trial found that taking oral glutathione at either 250 or 1000 mg/day led to significant increases in the body stores of glutathione in 54 non-smoking adults in a dose-dependent manner and also decrease in the markers for oxidative stress. At six months, mean GSH levels increased 30-35 % in erythrocytes, plasma, and lymphocytes and 260 % in buccal cells in the high-dose group ($p < 0.05$).[44] Oral administration of GSH increases plasma glutathione levels[32] and it promotes an increase in hepatic GSH levels.[22,32] These apparently conflicting results warrant more studies on this subject because the present data seems to be inconclusive.

In the meanwhile, the oral dosages for GSH are 250 mg daily (range: 50-600 mg), and it can also be supplement via nebulizer twice day (600 mg).[45] The study of Ellaine W Flagg estimated the dietary intake of GSH with questionnaire applied to 69 white men and women. They found that the daily glutathione intake ranged from 13.0 mg to 109.9 mg (mean 34.8 mg) and those fruits and vegetables contributed over 50% of usual dietary glutathione intake and meats contributed less than 25%.[32]

GSH may be increased by supplying its amino acid precursor cysteine, in the form of prodrugs.[33] There are some interventions to increase GSH levels, and it can be done orally by supplementation with:

- GSH monoesters, diesters and triester[3,33,46]
- GSH precursors, such *N*-acetyl cysteine (NAC)[33,41,47]
- N-(2-mercaptopropionyl)-glycine[3,47]

Glutathione is synthetized from amino acids, so it makes sense to keep an intake of dietary protein at adequate amounts. It is not necessary for most people to supplement with protein to meet their daily requirement, but special populations involved on hard physical work, like athletes, seems reasonable, and whey protein seems to be a good choice. To investigate the effects of undenatured whey protein isolate supplementation for six months on plasma glutathione of Parkinson patients, fifteen patients received whey protein, and 17 received soy protein and served as a control group. Significant increases in plasma concentration of reduced glutathione and the ratio of reduced to oxidized glutathione were found in the whey-supplemented patients but not in a control group.[48]

The availability of L-cysteine is the rate-limiting factor for GSH synthesis.[22] However, cysteine is scarce in foodstuffs, and this makes this amino acid a limiting factor of the synthesis.[5] Also, cysteine is not usually utilized as a precursor, presumably due to its toxicity[3,5], but when consumed as a component of whey it can have some potential to increase GSH levels.[3] After oxidative stress induced by acute intoxication in rats, hepatic GSH markedly increased after whey protein supplementation and in the casein-supplemented rats the increase was negligible.[49] The study on the effect of soy- and whey protein-isolate supplemented diet on the redox parameters of trained mice showed that free radical concentrations and glutathione composition of the tissue indicated that whey protein supplementation of the regular diet was able to prevent oxidative stress regardless of training and the authors concluded that athletes consuming these supplements could training with higher intensity.[50]

The supplementation with N-acetylcysteine (NAC) can make some sense. Oral supplementation increased plasma cysteine levels, ultimately leading to increase on GSH levels.[41] A systematic review (twelve clinical trials), published 2017, showed that the data suggested statistically significant cognitive improvements following NAC treatment, that there may be some benefit to using NAC in certain populations, although the studies were variable concerning design and outcome.[51]

In study of Lisa D. Coles *et al*., five people with mild to moderate Parkinson's disease and three controls were included, a high dose of NAC (3 000 mg taken orally twice daily) for a period of four weeks. There was an increase of cysteine levels and antioxidant measures (catalase and GSH/GSSG) relative to baseline, but measures of lipid peroxidation were unchanged. Of note, symptomatic adverse events were reported by 3 of the 5 subjects with Parkinson disease. The authors reported that more studies are needed.[52] Although NAC is promising as a supplement to both boost glutathione levels and potentially mitigate some of the issues related to oxidative stress, the research is not conclusive.[29]

GSH can also be supplemented intravenously or intramuscular, after a sensitivity test has been done, because there are allergic reactions in few people.[5] It was found in a study, performed back to 1978, that glutathione and its analogues are completely hydrolyzed after entering the extracellular space (intravascular) and it must be resynthesized after reuptake of the constituent amino acids. After the intravenous injection of the labelled tripeptides, the radioactivity accumulated first in the kidney, indicating the place where degradation takes place.[53]

It has been advised the dose of 600 mg diluted in 100 ml of physiological serum (PS), to which 1g of vitamin C can be added, once or twice a week. For cancer patients undergoing chemotherapy, the dose can go up 1200-1300 mg, diluted in 200 ml of PS and the administration must be done slowly.[5] GSH was administered intravenous, 600 mg twice daily, for 30 days, in an open label fashion, to nine patients with early, untreated Parkinson disease. All patients improved significantly after GSH therapy, and the therapeutic effect lasted for 2-4 months.[54]
Other types of supplementations had been used with apparently good results: omega-3 fatty acids, vitamins B, C, E, alpha-lipoic acid, selenium, and phytonutrients.[29]

3. Phytochemicals and oxidative stress

Pphytochemicals are natural plant chemicals[55], which are bioactive non-nutrient plant compounds, with very important role on herbal medicine, or botanical medicine, or phytomedicine.[56,57] This practice involves the utilization of active ingredient parts of plants or other plant materials, or combinations[56], to provide preventive and therapeutic benefits since time immemorial.[55,57] However, although they have beneficial properties for health, they are considered essential for the organism.[55]

In the phytochemicals there are several chemical agents, classified as:[57]

- primary metabolites - perform important roles in the growth and development of plants (sugars, amino acids, proteins, purines, etc.)
- secondary metabolites - important for defense mechanisms of plants[2], which have been associated to the capacity of medicinal plants in the management of various diseases, and include alkaloids, terpenes, flavonoids, lignans, curcumins and so on[57]

Structurally, phytochemicals are divided into four classes:[55]

1. Terpenoids (isoprenoids) with an isoprene molecule as the structural unit. They are widely used because their aromatic qualities (essences of eucalyptus and flavor of ginger, for example) and on the production of parfums. The carotenoids belong to this group, and they are known for their colors, ranging from yellow to deep red.
2. Phenolic compounds consisting of at least one aromatic ring attached to one or more hydroxyl groups and they have antioxidant properties and radical-scavenging capacity.
3. Nitrogen alkaloids characterized by the presence of at least one atom of nitrogen, they are soluble in water. In humans they interact with neurotransmitters. Examples are theobromine and caffeine.
4. Organosulfur compounds or thiols containing sulfur element in their structure. They are present in garlic and vegetables of genus cruciferous.

There are phytochemicals with the potential to disrupt the link between ROS elevation and increased pathology and aging which is related to the inherent antioxidant activity, but, potentially more important, are from their indirect effects.[3] A great variety of diverse agents of natural origin have been found to activate the Nrf2 signaling. The phytochemicals are considered indirect antioxidants that activate the Keap1/Nrf2/ARE pathway resulting in transcriptional induction of cytoprotective proteins.[15] They often activate the expression of genes encoding phase I detoxification enzymes such as cytochrome P450. However, only a very small portion of consumed phytochemicals is absorbed in the gastrointestinal tract, usually much less than 1%.[3] For example, curcumin has a poor absorption and low systemic bioavailability that limits the access of adequate concentrations for pharmacological effects in certain tissues.[58]
depending on their chemical structure and functional groups (Figure 6) and Volodymer I. Lushak, in his review paper published in 2012, summarizes the phytochemicals that operate by affecting the Nrf2/Keap1 system:[3]

- Sulforaphane is found in broccoli and other cruciferous plants, and it is associated with Nrf2 activation via the direct modification of Keap1 cysteine residue(s). It is a gene inducer for the synthesis of antioxidant enzymes (GPx, GST, γGLCL), a potent phase II gene-inducing.[11]
- Curcumin is derived from the rhizomes of turmeric, a principal ingredient of curry powder[11], stimulates the expression of antioxidant and phase II detoxification enzyme genes and curcumin-induced expression is also mediated via Nrf2 activation in a ROS-related manner. Curcumin may mediate chemotherapy and chemopreventive effects on cancer[58]

- Epigallocatechin gallate (EGCG) is a major active polyphenol of green tea that exerts antioxidant, anti-inflammatory and chemopreventive properties.[8,59] EGCG inhibits expression of COX-2 and activation of mitogen-activated protein kinase.[59] Green tea, and its components mitigate cellular damage due to oxidative stress and it is supposed to enhance humoral and cell-mediated immunity.[8] The polyphenols are phenolic compounds in plants (there are more than 10 000 compounds), where the flavonoids are included (more than 4 000 different structures). These are subdivided on several groups, one of which is the flavanol group where EGCG belongs. The products act as reducing agents, hydrogen donators, single oxygen quenchers, superoxide radical scavengers and metal chelators among other antioxidant activities.[2,57]
- Allyl sulfides are major components of garlic that are capable of inducing phase II detoxification enzymes in a Nrf2-dependent manner.[2,15,60] These organosulfur compounds have strong antioxidant activity, preventing cells from stress oxidative via induction of the generation of glutathione, reducing the levels of ROS and scavenging free radicals.[61] They can be provided by ingestion of broccoli, cauliflower, brussels sprouts, garlic, and onion.[2,61]
- Resveratrol is found in grapes, bilberry, blueberry, other berries and on red wine.[62] It exerts antioxidant, anti-inflammatory, antiaging, and chemopreventive activities affecting cellular signaling. These activities are mediated, at least partially, by Nrf2 phosphorylation. The study performed by Anupam Bishayee *et al*. provided evidence that the supplementation of rats with resveratrol attenuated the oxidative stress and suppression of inflammatory response mediated by Nrf2.[62]
- Capsaicin found in hot chili pepper and ginger activates phase II detoxification enzyme expression in a Nrf2-dependent manner
- Lycopene is a natural carotenoid found in tomato and tomato products exerts chemopreventive activity in an Nrf2-dependent manner. It is a powerful antioxidant with great capacity to scavenge singlet molecular oxygen and peroxyl radicals, and also to act synergistically with other antioxidants.[2] It must be noted that the absorption of lycopene by the intestine is much more efficient from processed tomatoes. The study of C. Gärtner et al showed that Ingestion of tomato paste was found to yield 2.5-fold higher total and all-trans-lycopene peak concentrations ($p < 0.05$ and $p< 0.005$, respectively) and 3.8-fold higher area under the curve (AUC) responses ($P < 0.001$) than ingestion of fresh tomatoes[20]
- Carnosol also enhances the expression of phase II detoxification enzyme genes in an Nrf2-related manner

- Xanthohumol, a sesquiterpene from hop, also shows chemopreventive activity, inducing antioxidant and phase II detoxification enzymes and its action was linked with Nrf2 activation
- Ocimum sanctum leaf extract, that contains saponins, flavonoids and tannins, has strong antioxidant activity and free radicals scavenging property.[63,64]

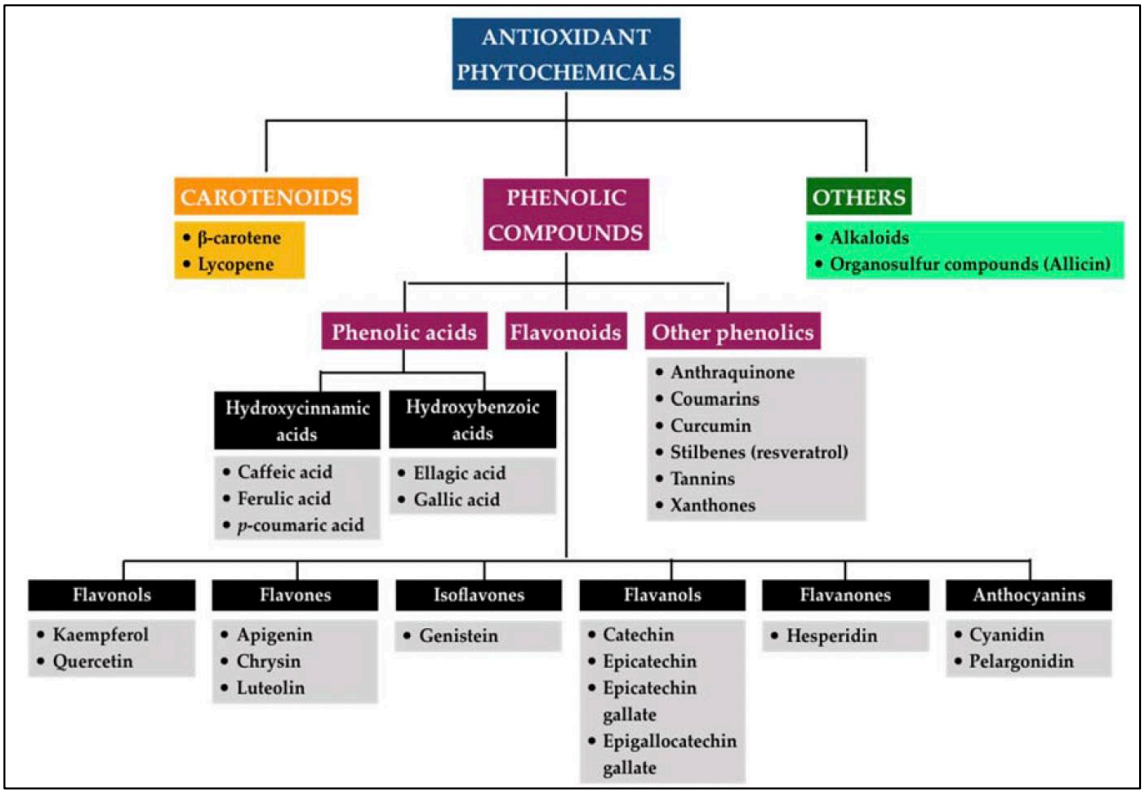

Figure 6 - Classification of antioxidant phytochemicals depending on their chemical structure and functional groups.[2]

Conclusion

The body lives under an equilibrium of the redox state and it is permanently under aggression and reaction to maintain the internal homeostasis. The stressful situations create acute answers that, if prolonged on time, they will be chronic and a cause of disease and premature aging, with consequences on the well-being. The oxidative stress is natural occurring process from the aerobic metabolism and, if under control or normal situations, it is a good tool for body health. However, where there is a long-lasting imbalance in favour of the oxidative stress the homeostasis will be broken and the aggression to the body will arise. Fortunately, the human body has natura defence mechanisms provided as soon as the aggression appears, and the oxidative stress is neutralized with the endogenous antioxidants. Several complex enzymatic and no-enzymatic mechanisms are involved in this process. Glutathione is an endogenous component of cellular metabolism, and it is sometimes named as *the master antioxidant* for its important function on the protection of the cells from oxidative damage and maintaining redox homeostasis. It is the key player on the body defenses. Glutathione it is produced exclusively in the cytosol from three amino acids, predominantly in the liver, and then distributed to all body. It acts as an antioxidant, either directly by interacting with ROS and electrophiles, or by operating as a cofactor for various enzymes. Oral supplementation, providing N-acetylcysteine, for instance, or intravenous are routes to keep GSH in good levels.

Finally, the consumption of phytochemicals is another strategy to fight the oxidative stress. They are natural plant chemicals with power to disrupt the ROS and maintain the homeostasis of the redox state. There are thousands of phytochemicals, classified according to the chemical structure and functional groups, provided by food.

The human body is an instable element of the earth, the balance between ROS and oxidants will determine health, longevity, and happiness. There are tools to keep this balance, the good and the right food is of paramount importance, but the other good health habits cannot be forgotten.

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NICO began to be described in 1979 by Ratner *et al* (Ratner, Person, Kleinman, Shklar, & Socransky, 1979),[2] however, it was from 1992 onwards that the term was incorporated and used

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