

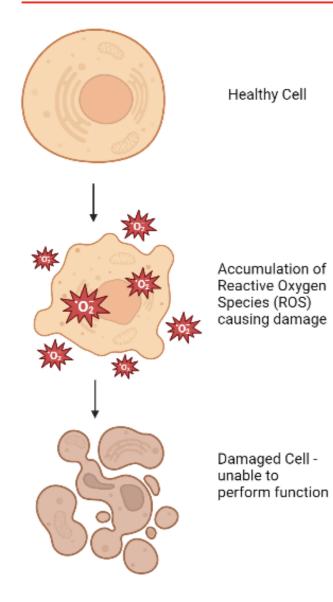
## THE EFFECTIVE SOLUTION TO GLUTATHIONE DEPLETION

www.continualg.com

**WHITE PAPER** 

# INTRODUCTION

Glutathione – the 'MASTER' antioxidant – is naturally produced within all cells of all oxygen using organisms.



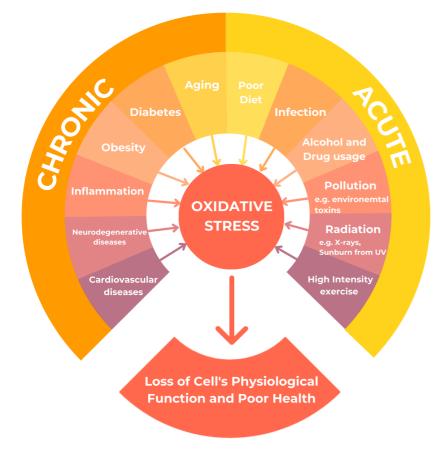
Oxidative reactions are necessary in fuelling various biochemical and metabolic processes in the human body, such as cellular respiration, which serves as a fundamental source of energy. However, these reactions often yield cytotoxic byproducts such as Reactive Oxygen Species (ROS).

In the absence of glutathione's antioxidant activity, ROS remain unneutralized within the cell, leading to damage of vital cellular components such as membranes, proteins, and DNA. This phenomenon, known as oxidative stress, disrupts the cell's physiological functions, potentially exacerbating symptoms associated with pre-existing medical conditions and compromising the capacity of the immune system.

Glyteine® is currently available in the Continual-G® range of dietary supplements.

# INTRODUCTION

The **aging** process and most **chronic disorders,** progressively diminish the capability of our cells to synthesize the precursor gamma-glutamylcysteine (GGC), Glyteine® is the proprietary form of GGC. GGC synthesis is the first step in glutathione production. Without sufficient glutathione our cells enter oxidative stress.



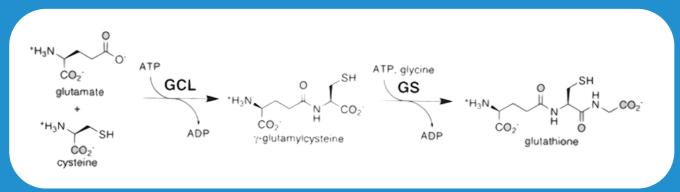
Additionally, our cells can be exposed to high concentrations of ROS and other free radicals from the environment and our lifestyle, whether this be in the form of smoke, alcohol, drugs, heavy metals, or a consequence of extreme exercise. Acute exposure to excessive levels of free radicals overwhelms our cellular reserves of glutathione, generating oxidative stress.

#### But what supplement options are there to improve our glutathione status?

While a variety of supplements claim to enhance glutathione levels. The gammaglutamylcysteine (GGC) in Glyteine® has the unique ability to enter INSIDE the body's cells, where it is immediately converted into glutathione. It provides the only option for effective relief from chronic and age-related oxidative stress that has resulted from the loss in capacity of our cells to produce enough GGC themselves. Glyteine® is also your only option for boosting your glutathione reserves to protect against any increased exposure to free radicals.

## **Introducing Glyteine:** Elevating Cellular Glutathione Levels for Optimal Health

Discover Glyteine®, the proprietary form of gamma-glutamylcysteine (GGC) which is the immediate precursor to glutathione. GGC is a naturally occurring peptide with a unique ability to boost cellular glutathione levels above homeostasis and provide relief from the ravages of oxidative stress. Glyteine® emerges as a powerful ally in supporting overall well-being.



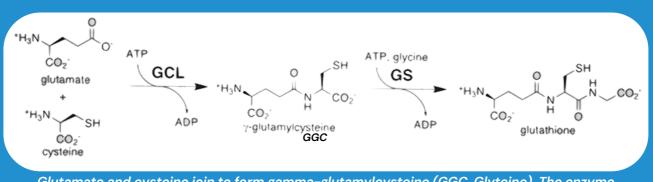
Glutamate and cysteine join to form gamma-glutamylcysteine (GGC, Glyteine). The enzyme glutathione synthase (GS), adds the amino acid glycine to Glyteine to form glutathione

### Homeostasis and Glutathione

Homeostasis is a fundamental concept in biology that ensures the body's internal environment remains stable and well-balanced despite external fluctuations. It involves a complex system of feedback mechanisms that constantly monitor and regulate various physiological variables to maintain optimal equilibrium set points for optimum cellular function and overall health.

Temperature homeostasis is a classic example of this concept. When the external temperature rises, the body responds by initiating cooling mechanisms such as sweating, while in colder temperatures, it triggers warming responses like shivering. These actions work together to keep the body's core temperature within a narrow range.

Similarly, glutathione production is part of its own feedback control loop. In a healthy state, the body's cells maintain a balanced production of glutathione, ensuring an effective defense against oxidative stress. However, disruptions caused by factors like diseases, aging, and lifestyle choices can lead to dysfunctional homeostasis which produces and maintains insufficient glutathione levels. In such cases, cells still produce GGC (glutathione's precursor) but not enough to fuel their glutathione needs for adequately countering free radicals. This results in prolonged oxidative stress within our cells that leads to cellular damage and loss of physiological function in the affected tissue.



Glutamate and cysteine join to form gamma-glutamylcysteine (GGC, Glyteine). The enzyme glutathione synthase (GS), adds the amino acid glycine to Glyteine to form glutathione

The unfortunate reality is the inadequacy of GGC production affects the ability of our cells to meet their glutathione needs for effectively preventing oxidative stress. Addressing glutathione depletion through supplementation with Glyteine® will help achieve the glutathione levels needed for overall good health.

#### **Protect your brain**

A compromised glutathione system in the brain is strongly correlated with oxidative stress and has been shown to be implicated in neurodegenerative diseases

#### **Boost your immunity**

The maintenance of a healthy homeostatic level of cellular glutathione is critical for supporting a functioning immune system.

#### Maintain your respiratory health

Many respiratory conditions are associated with glutathione deficiency.

#### **Protect your digestive system**

Our digestive system developed a sophisticated biochemistry to counter daily toxin exposure. The mucosal lining in our intestines, abundant in glutathione, acts as a crucial defense and supports gut barrier function.

#### Improve sports recovery

Boosting glutathione levels in cells and tissues supports physical activity and aids in post-activity recovery.

#### **Detoxify your body**

Glutathione neutralizes free radicals and reactive oxygen species (ROS), generated during energy production. It also aids in detoxifying harmful compounds like heavy metals and other environmental pollutants.

## **Outshining Competitors:** A Comparative Guide to Glyteine® and Other Supplements

Glutathione is one of the most studied molecules in healthcare. This is evidenced through more than 181,000 papers on glutathione listed on the website https://pubmed.ncbi.nlm.nih.gov. These studies underscore the significance of this peptide in promoting health and well-being.

Under normal physiological conditions, the concentration of glutathione within the body's cells is approximately **1,000 times higher** than that found in the surrounding extracellular fluid. This emphasizes that the body requires glutathione to be present WITHIN its cells in order to function.

Unfortunately, numerous supplements available in the market that purport to "boost glutathione" fail to acknowledge this essential factor, resulting in misconceptions and arguably deceptive claims.

## The Difficulties in Measuring Cellular Glutathione levels

It should be noted that there is a **lack of standardized analytical methods** for determining glutathione levels within the blood, plasma, tissue and cellular material.

Glutathione analysis is not conventionally performed as a standard blood sample test. This can be attributed to the fact that glutathione is considered a **high turnover** and dynamic molecule which can be both:

- readily **oxidized** into the disulfide form (GSSG)
- **broken down** into its constituent amino acids through the action of the endogenous enzyme, gamma glutamyl transferase (GGT).

This means that blood samples would require **specialized treatment and chilling** to preserve and stabilize the glutathione. There are many analysis methods available in the scientific literature, however none are particularly reliable or simple. Methods include HPLC, HPLC-MS/MS and enzyme-based assays.

Measuring glutathione in red blood cells (RBC) or plasma would seem like an acceptable method of determining glutathione (GSH) status, however, both present unique technical difficulties and are open to artifacts that can confound the interpretation of the results.

RED BLOOD CELLS (RBC)	BLOOD PLASMA
Lack a nucleus and most organelles do not model the oxidative stress experienced by other cells Pre-treatment of RBCs causes a release of iron which oxidizes glutathione to GSSG	Glutathione's major roles occur inside the cell, therefore extracellular glutathione in the plasma would not assist in measuring health status.

### **01.** Glutathione supplements

Supplements which have glutathione itself as their core ingredient have been a dominant player within the antioxidant market. However, this often inexpensive alternative comes with a functional cost, as their advertised benefits often include the misleading claim of:



#### "Increases glutathione levels available within the body's serum"

This is misleading as:

- The serum refers to the liquid component of the blood, which is an extracellular fluid exists on the outside of living cells
- Fails to address **cellular** glutathione levels meaning that the supplement is not explicitly entering the body's cells.
- Without cellular entry, the supplement cannot access and neutralize the ROS which promote oxidative stress

Given that the glutathione concentration in the extracellular environment is much lower than that found intracellularly by about a thousand-fold means that there is a significant negative concentration gradient which prevents further glutathione from passively entering cells from the plasma. Any glutathione taken orally will not be able to overcome this gradient and enter cells to combat oxidative stress.

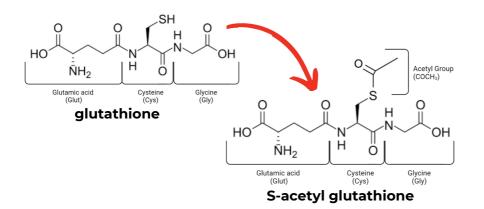
## Glyteine® is not subject to such a negative concentration gradient as the difference in concentration between our plasma and inside our cells is negligible.

Another issue with glutathione supplementation, more widely acknowledged in the literature, is that glutathione is easily broken down into its amino acid subunits. This is due to the enzyme gamma glutamyl transferase (GGT), which is most abundant on the cell surface of digestive organs such as the liver and pancreas. This enzyme effectively breaks down glutathione into its constituent amino acids that, though able to enter the cell, can do nothing to increase glutathione levels above homeostasis.

### 02. S-acetyl glutathione

Unfortunately, this flaw has been exploited – with S-acetyl glutathione claiming to **"add further protections to glutathione molecules to increase bioavailability and hence absorption"** whilst ignoring the aforementioned pharmacokinetic issue of an unfavorable glutathione concentration gradient – which prevents cellular entry.

S-acetyl glutathione supplements are glutathione molecules which are chemically altered through the addition of an acetyl (COCH3) group to the cysteine amino acid. This results in an **unnatural**, synthesized product which lacks clinical data on potential side effects; which other synthesized drugs are expected to conduct for regulatory approval.

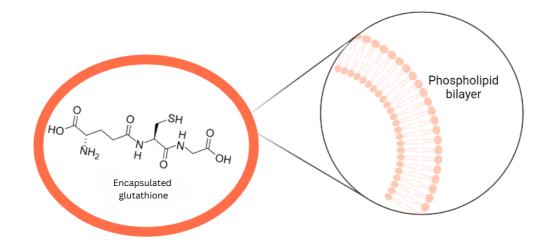


Claims of additional protection are misleading as there is no scientific or clinical evidence that shows:

- S-acetyl glutathione can increase cellular glutathione levels.
- Acetylated glutathione does not bind to the active site of the membranebound GGT enzyme and become degraded to its component amino acids.

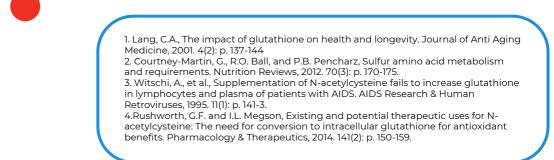
### 03. Liposomal glutathione

For this supplement, the glutathione is encapsulated in liposomes. Liposomes are made up of lipids, forming a membrane structure which mimics our body's cells. This strategy of encapsulation has been widely adopted across the pharmaceutical industry – most often used for hydrophilic drugs which have difficulty passing across cell membranes.



The theory of liposomal delivery is quite simple: Glutathione is packaged in liposomes, which resemble the membranes of our cells. When taken orally, these liposomes merge with the intestinal cell membranes, releasing glutathione into the cells.

However, the challenge lies in distributing glutathione to the rest of the body. Once fused with the intestinal cell membranes, the liposomes' journey ends. The extra glutathione **potentially** diffuses into the bloodstream, where there is no inherent molecular transport system for glutathione to move from our blood plasma into our cells; **meaning cellular glutathione cannot increase, and oxidative stress is not alleviated.** 



### 04. N-acetylcystiene (NAC)

We are aware that cysteine is one of the three building blocks that make up glutathione. However, this form of supplementation operates on the assumption: **'that there is an intimate connection between cysteine deficiency and low glutathione stores.'** 

#### This can be misleading as:

The typical American diet supplies often more than the required quantity of cysteine[1] and the other sulfur containing amino acid, methionine, which can be readily converted into cysteine in the liver[2]

Despite this abundance of cysteine in typical diets, the human body continues to show a decreased ability to produce enough glutathione to maintain homeostasis with age and during an array of pathologies – meaning the two factors are not as exclusively linked as consumers are led to believe.

Regardless of the lack of evidence surrounding cysteine deficiency, NAC treatment does not yield significant increases in glutathione levels in chronic diseases characterized by prolonged glutathione decrease, such as HIV/AIDS. Clinical trials and studies involving AIDS patients have shown limited efficacy of NAC supplementation in elevating glutathione levels within plasma and lymphocytes [3]. Similar disappointing results have been observed in other chronic conditions, including cystic fibrosis, contrast-induced nephropathy, and thrombosis[4].

### **05.** GlyNAC supplementation

The combination of Glycine with N-acetylcysteine has also been explored as a more recent supplement alternative. However, human clinical studies trialing this supplementation strategy [1] yield limited evidence of a beneficial cellular glutathione increase, using ambiguous methods such as:

#### 'Measuring glutathione levels in whole blood'

This is misleading as whole blood includes red and white blood cells and plasma. Whole blood measurements do not inform us whether the glutathione is inside cells, outside in the plasma or in both fractions.

Therefore, assessing glutathione levels solely in whole blood does not accurately reflect the glutathione status or antioxidant capacity of cells that are actively involved in metabolic processes and face the risk of oxidative stress.

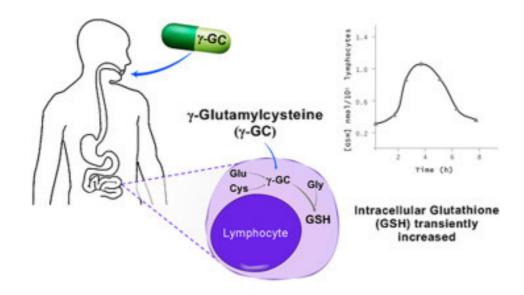
Human clinical studies measuring the bioavailability of Glyteine® used white blood cells (lymphocytes) rather than red blood cells and whole blood. Unlike red blood cells, lymphocytes contain both a nucleus and mitochondria. This approach allows for a more accurate understanding of the glutathione requirements and the potential impact of Glyteine® on cellular functions in a broader range of cells within the body.

### 06. Glyteine®

Unlike competitors, the intermediate gamma-glutamylcysteine, (GGC) contained in Glyteine® is naturally present both in the body's plasma and within cells, granting it **high oral bioavailability** as it remains intact without undergoing degradation by extracellular enzymes.

Interestingly, there exists a greater proportion of GGC in the plasma than within the cells. This is due to the rapid binding of GGC to glycine once inside the cell through the enzymatic action of glutathione synthase. This creates a positive concentration gradient, facilitating the uptake of both endogenously produced GGC and supplemented Glyteine® from the plasma into the cell, where the antioxidant properties of glutathione are most needed.

This was proven to be the case in a human clinical study performed with healthy, non-fasting adults, which demonstrated that orally administered Glyteine® can significantly increase lymphocyte glutathione levels above homeostasis within hours of a singular dose [1].



### **06.** Glyteine®

In conditions such as aging [2] and chronic disorders [3], the body's cellular production of gamma-glutamylcysteine (GGC) is diminished due to a dysfunctional homeostasis or glutathione homeostasis is overwhelmed by an increase in free radical exposure. In both cases, taking GGC containing Glyteine® supplements increases the cell's capacity to produce additional glutathione. This, in turn, alleviates oxidative stress, which could otherwise lead to symptoms such as lowered immunity and inflammation.

Additionally, during acute cases of oxidative stress caused by events such as infections, high-intensity exercise, pollution, and radiation exposure, Glyteine® supplementation prepares the body's cells to handle surges in reactive oxygen species (ROS). By increasing GGC levels beyond standard homeostatic levels through Glyteine® supplementation, a proportional elevation in the synthesis of glutathione and its antioxidant action can occur in response to acute exposure to ROS sources.

[1] Ferguson, G. and W. Bridge, Glutamate cysteine ligase and the age-related decline in cellular glutathione: The therapeutic potential of  $\gamma$ -glutamylcysteine. Archives of Biochemistry and Biophysics, 2016. 593: p. 12-23.

[2] Cao, P., et al., Therapeutic approaches to modulating glutathione levels as a pharmacological strategy in Alzheimer's disease. Curr Alzheimer Res, 2015. 12(4): p. 298-313.

### Immunity

The immune system employs reactive oxygen species (ROS) as part of its defense mechanism against invading pathogens. When a pathogen is detected, specific immune cells, like neutrophils and macrophages, activate an enzyme known as NADPH oxidase. This enzyme generates ROS, which act as potent oxidizing agents capable of harming the cellular components of the pathogen, such as proteins and DNA. This oxidative burst caused by ROS is crucial in neutralizing and eliminating the invaders, playing a vital role in the body's immune response to infections. However, excessive ROS production can lead to oxidative stress, disrupting immune function and damaging healthy cells [1]. It is essential to tightly regulate ROS levels to maintain a balanced immune response.

Glutathione is a master antioxidant and plays a pivotal role in neutralizing these harmful levels of ROS, thereby preventing cellular damage and maintaining redox balance [2-5]. A growing body of research highlights the critical role of glutathione (GSH) in the immune system and how when it is at depleted levels it impacts on various disease processes, including infections, inflammation, and immune-related disorders.

As individuals age or face chronic degenerative diseases associated with inflammation or autoimmune conditions, their antioxidant defense systems, including glutathione, may become compromised. Especially, in sick or aging individuals, the production of ROS can start to exceed the cellular production of glutathione, leading to an imbalance and a cascade of oxidative stress, inflammation, and tissue injury [6,7]. This underscores the importance of maintaining sufficient levels of cellular glutathione for optimal immune function and overall health.

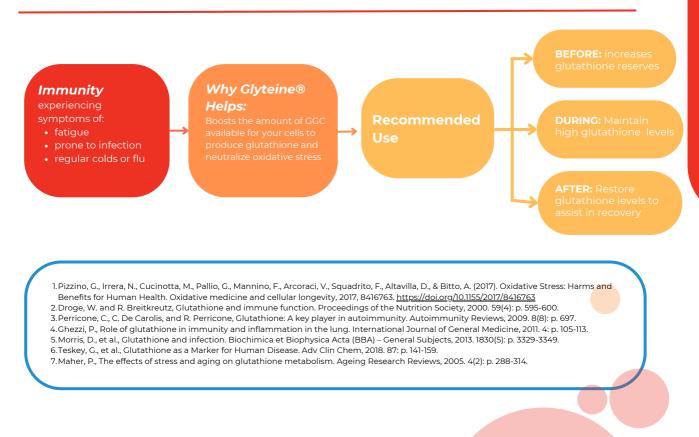
#### Immunity

Glyteine® offers a solution to the challenges associated with maintaining optimal cellular glutathione levels in the context of immune health.

By supplementing with Glyteine®, which contains the immediate precursor (gamma-glutamylcysteine, GGC) to glutathione, you can directly support the body's natural glutathione synthesis. Glyteine® enhances cellular glutathione levels, fortifies the antioxidant defense system, and promotes optimal immune responses.

Incorporating Glyteine® into your wellness routine offers a targeted approach to support your immune system, addressing the crucial role of glutathione in immune function. By boosting your body's natural glutathione synthesis, Glyteine® helps counterbalance the oxidative stress generated during immune responses, preventing excessive oxidative damage and supporting overall immune health.

#### How to make Glyteine® work for you:



### **Glutathione and Viral Infections**

Over the past decade, a wealth of evidence has accumulated, suggesting that individuals infected with viruses such as influenza experience chronic oxidative stress [1]. This poses a particular concern if glutathione levels are already low, as viral infections can further deplete glutathione, reaching a critical point where oxidative stress progressively damages tissues and leads to organ failure.

The availability of gamma-glutamylcysteine (Glyteine®) has revolutionized our ability to significantly increase cellular glutathione (GSH) levels within hours, a feat previously unattainable [2]. Consequently, it was nearly impossible to assess the effectiveness of glutathione augmentation as an antiviral strategy. Studies with N-acetylcysteine (NAC) and glutathione have yielded limited success [3-7].

There is also compelling evidence supporting the notion that increasing glutathione levels may serve as a prophylactic measure against viral infections. RNA viruses, including Covid-19/SARS CoV-2, are characterized by cellular redox disruption, leading to an excess of reactive oxygen species (ROS) and depletion of glutathione. This disruption creates an oxidative state that facilitates protein glutathionylation, where glutathione binds to cysteine residues. This process allows RNA viruses to regulate and control the activity of enzymes responsible for their replication cycle. It is hypothesized that increasing glutathione above homeostasis by Glyteine® administration will prevent an oxidative cellular environment and consequently disrupt the RNA virus replication cycle which will potentially prevent or treat infection.



### **Glutathione and Viral Infections**

A study published in 2003 demonstrated the anti-influenza properties of glutathione. Given that many viruses, including influenza, primarily affect the oral, nasal, and upper airway regions, they induce oxidative stress or conditions that deplete glutathione, thereby increasing vulnerability to such viral infections [8].

Oral administration of a single dose GGC has been demonstrated in a published human study to rapidly increase cellular levels of glutathione above basal levels (homeostasis) within hours [2]. Notably, neither glutathione itself nor the cysteine prodrug NAC have ever been shown in clinical studies to have such bioavailability. While N-acetylcysteine administration has shown some improvement in influenza symptoms, it does not appear to significantly affect the infection rate. This is not surprising, as N-acetylcysteine is not an effective means of increasing cellular glutathione levels above homeostasis and is only suitable for helping cells reach homeostasis during acute depletion [9-10]. Similarly, supplementation with glutathione itself proves futile.

Vitamin C (ascorbic acid) is renowned for its supportive role in the recovery from viral infections. It is interesting to note that glutathione is responsible for recycling cellular vitamin C, and conversely, vitamin C helps mitigate glutathione depletion [16]. However, unlike gamma-glutamylcysteine (Glyteine®), vitamin C cannot elevate cellular glutathione levels [2].

5. Fraternale, A., et al., Antiviral and immunomodulatory properties of new pro-glutathione (GSH) molecules. Curr Med Chem, 2006. 13(15): p. 1749-55. 6. Fraternale, A., et al., GSH and analogs in antiviral therapy. Mol Aspects Med, 2009. 30(1-2): p. 99-110.

7. Uchide, N. and H. Toyoda, Antioxidant therapy as a potential approach to severe influenza-associated complications. Molecules, 2011. 16(3): p. 2032-52.

Cai, J., et al., Inhibition of influenza infection by glutathione. Free Radical Biology & Medicine, 2003. 34(7): p. 928-936.
 Rushworth, G.F. and I.L. Megson, Existing and potential therapeutic uses for N-acetylcysteine: The need for conversion to intracellular glutathione for antioxidant benefits. Pharmacology & Therapeutics, 2014. 141(2): p. 150-159.

 10. Aitio, M.-L., N-acetylcysteine – passe-partout or much ado about nothing? British Journal of Clinical Pharmacology, 2006. 61(1): p. 5-15.
 11. Martensson, J. and A. Meister, Glutathione deficiency decreases tissue ascorbate levels in newborn rats: ascorbate spares glutathione and protects. Proc Natl Acad Sci U S A, 1991. 88(11): p. 4656-60.

I. Reshi, M.L., Y.C. Su, and J.R. Hong, RNA Viruses: ROS-Mediated Cell Death. Int J Cell Biol, 2014. 2014: p. 467452.

<sup>2.</sup> Zarka, M.H. and W.J. Bridge, Oral administration of y-glutamylcysteine increases intracellular glutathione levels above homeostasis in a randomised human trial pilot study. Redox Biology, 2017. 11: p. 631-636.

<sup>3.</sup> Sgarbanti, R., et al., Redox regulation of the influenza hemagglutinin maturation process: a new cell-mediated strategy for anti-influenza therapy. Antioxid Redox Signal, 2011. 15(3): p. 593-606.

<sup>4.</sup> De Flora, S., C. Grassi, and L. Carati, Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term Nacetylcysteine treatment. Eur Respir J, 1997. 10(7): p. 1535-41.

### **Aging and Age Associated Disease:** Osteoporosis

Osteoporosis is a medical condition characterized by weakened and porous bones, leading to an increased risk of fractures. In this condition, the bone density and quality decrease, making bones more susceptible to fractures even with minimal impact or stress.

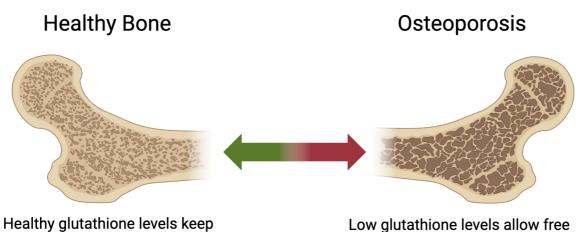
The bones in our body are continuously undergoing a process of remodeling, which involves the removal of old bone tissue and the replacement with new bone tissue. This process is typically balanced, with bone formation keeping pace with bone resorption. However, in osteoporosis, this balance is disrupted, and the rate of bone resorption becomes greater than the rate of bone formation, resulting in a net loss of bone mass.

Factors such as cellular changes, genetic damage, nutritional deficiencies (especially calcium and vitamin D), and oxidative stress contribute to this decline in bone health [2].

Oxidative stress occurs when reactive oxygen species (ROS) overwhelm the body's antioxidant defense system, including glutathione [3]. ROS levels rise with age and negative lifestyle factors like smoking and obesity, compromising tissue maintenance and disrupting the delicate balance between bone formation and resorption [4][5][6][7][8].



### Aging and Age Associated Disease: Osteoporosis



Healthy glutathione levels keep free radical production under control, protecting bones from damage by oxidative stress Low glutathione levels allow free radicals to build up, leading to oxidative stress and damage to bone cells

- 1. Sozen, T.; Ozisik, L.; Calik Basaran, N. An Overview And Management Of Osteoporosis. European Journal of Rheumatology 2017, 4 (1), 46-56. 2. Corrado, A.; Cici, D.; Rotondo, C.; Maruotti, N.; Cantatore, F. Molecular Basis Of Bone Aging. International Journal of Molecular Sciences 2020, 21 (10), 3679.
- 3. Xiu X, Wang Z, Ni Y, Yu Y, Wang G, Chen L. Suppression effect of N-acetylcysteine on bone loss in ovariectomized mice. Am J Transl Res. 2020;12(3):731-742.
- 4. Kamceva, G.; Arsova-Sarafinovska, Z.; Ruskovska, T.; Zdravkovska, M.; Kamceva-Panova, L.; Stikova, E. Cigarette Smoking And Oxidative Stress In Patients With Coronary Artery Disease. Open Access Macedonian Journal of Medical Sciences 2016, 4 (4), 636-640.
- 5. Matsuda, M.; Shimomura, I. Increased Oxidative Stress In Obesity: Implications For Metabolic Syndrome, Diabetes, Hypertension, Dyslipidemia, Atherosclerosis, And Cancer. Obesity Research & Clinical Practice 2013, 7 (5), e330-e341.
- 6. Cui, H.; Kong, Y.; Zhang, H. Oxidative Stress, Mitochondrial Dysfunction, And Aging. Journal of Signal Transduction 2012, 2012, 1-13.
- 7. Almeida M, Han L, Martin-Millan M, Plotkin LI, Stewart SA, Roberson PK, Kousteni S, O'Brien CA, Bellido T, Parfitt AM, Weinstein RS, Jilka RL, Manolagas SC. Skeletal involution by age-associated oxidative stress and its acceleration by loss of sex steroids. J Biol Chem. 2007;282:27285– 27297.
- 8. Domazetovic V, Marcucci G, Iantomasi T, Brandi ML, Vincenzini MT. Oxidative stress in bone remodeling: role of antioxidants. Clin Cases Miner Bone Metab. 2017;14:209–216.

### **Aging and Age Associated Disease:** Osteoporosis

Glutathione, the most potent antioxidant in the body, plays a crucial role in bone health. It not only neutralizes ROS but also recycles other antioxidants like vitamins C and E [9]. However, glutathione production decreases with age and is negatively influenced by stress, toxins, and illness [10]. Even intense exercise temporarily lowers cellular glutathione levels. While attempts to enhance glutathione through supplements or direct supplementation have had limited success, raising cellular glutathione above the body's natural set point (homeostasis) is crucial for achieving health benefits.

Vitamin D also plays a vital role in bone health, and its deficiency contributes to weak bone formation and osteoporosis [11]. Inadequate glutathione levels hinder calcium absorption and effective mineralization of the skeleton, further compromising bone strength and flexibility [12][13].

Elevating glutathione levels offers benefits for optimal vitamin D absorption, oxidative stress reduction, and bone health. Gammaglutamylcysteine (GGC) supplementation has shown the ability to raise cellular glutathione levels above homeostasis [14]. This holds promise as a preventive therapy for osteoporosis, addressing oxidative stress, and optimizing vitamin D and calcium absorption.

To summarize, addressing oxidative stress and optimizing glutathione levels are important considerations for promoting bone health and preventing osteoporosis. Supplementation with Glyteine®, which contains GGC presents a solution to increase cellular glutathione levels, reducing oxidative stress, supporting vitamin D and calcium absorption, and ultimately maintaining healthy bones.

9. Pizzorno, J. Glutathione!. Integrative Medicine: A Clinician's Journal 2014, 1 (13), 8-12.

- 11. Laird, E.; Ward, M.; McSorley, E.; Strain, J.; Wallace, J. Vitamin D And Bone Health; Potential Mechanisms. Nutrients 2010, 2 (7), 693-724.
- 12. Jain, S.K., R. Parsanathan, A.E. Achari, P. Kanikarla-Marie, and J.A. Bocchini, Jr., Glutathione Stimulates Vitamin D Regulatory and Glucose-Metabolism Genes, Lowers Oxidative Stress and Inflammation, and Increases 25-Hydroxy-Vitamin D Levels in Blood: A Novel Approach to Treat 25-Hydroxyvitamin D Deficiency. Antioxid Redox Signal, 2018. 29(17): p. 1792-1807

<sup>10.</sup> Mosoni, L.; Breuillé, D.; Buffière, C.; Obled, C.; Mirand, P. Age-Related Changes In Glutathione Availability And Skeletal Muscle Carbonyl Content In Healthy Rats. Experimental Gerontology 2004, 39 (2), 203-210.

<sup>13.</sup> Moine, L., M. Rivoira, G. Díaz de Barboza, A. Pérez, and N. Tolosa de Talamoni, Glutathione depleting drugs, antioxidants and intestinal calcium absorption. World J Gastroenterol, 2018. 24(44): p. 4979-4988.

<sup>14.</sup> Zarka, M.H. and W.J. Bridge, Oral administration of y-glutamylcysteine increases intracellular glutathione levels above homeostasis in a randomized human trial pilot study. Redox Biology, 2017. 11: p. 631-636.

### **Glutathione and Neurodegenerative Disease**

The aging process is often accompanied by an increased susceptibility to chronic diseases, and one influential theory explaining this phenomenon is the "free radical theory of aging" [1]. Extensively researched and widely accepted, this theory points to the crucial role of oxidative stress in the aging process.

As our lifespan extends, the risk of developing chronic diseases, especially neurodegenerative conditions [2], becomes more prominent, affecting brain health and cognitive function. Emerging evidence strongly links compromised glutathione (GSH) levels in the brain with oxidative stress and their involvement in neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear palsy, Huntington's disease, and multiple sclerosis [3,4,5].

Alzheimer's disease, the most common neurodegenerative disorder, leads to progressive memory loss and cognitive deficits. Currently, there is no cure, but the possibility of prevention or delaying its devastating effects remains hopeful. The disease is associated with the abnormal build-up of amyloid-beta protein in the brain, forming neuritic plaques that trigger inflammation and degeneration of surrounding cells, including microglial cells responsible for brain inflammation regulation.

In a healthy brain, microglial cells prevent neuritic plaque formation, but excessive amyloid-beta production can overwhelm this defense system, leading to a harmful cycle. Microglial cells respond to amyloid-beta clustering by releasing inflammatory messages and reactive oxygen species (ROS), which can damage neurons and initiate further microglial activity [6]. This vicious cycle worsens neurodegeneration, leading to loss of cognitive functions like organization of thoughts, learning, memory formation, and attention. Current strategies targeting amyloid-beta removal have not been effective in halting disease progression, as neural inflammation persists.

Maher, P., The effects of stress and aging on glutathione metabolism. Ageing Research Reviews, 2005. 4(2): p. 288-314.

<sup>1.</sup> Beckman, K.B. and B.N. AMES, The Free Radical Theory of Aging Matures. Physiological Reviews, 1998. 78(2): p. 547-581.

<sup>2.</sup> Lin, M.T. and M.F. Beal, Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature, 2006. 443(7113): p. 787-795. 3. Dringen, R. and J. Hirrlinger, Glutathione pathways in the brain. Biol Chem, 2003. 384(4): p. 505-16.

<sup>5.</sup>Currais, A. and P. Maher, Functional Consequences of Age-Dependent Changes in Glutathione Status in the Brain. Antioxidants & Redox Signaling, 2013. 19(8): p. 813-822.

<sup>6.</sup> Merighi S, Nigro M, Travagli A, Gessi S. Microglia and Alzheimer's Disease. Int J Mol Sci. 2022 Oct 27;23(21):12990. doi: 10.3390/ijms232112990.

### **Glutathione and Neurodegenerative Disease**

A promising prevention strategy involves gamma-glutamylcysteine (GGC), which increases cellular levels of glutathione, a powerful antioxidant protecting brain cells from amyloid-beta deposition, oxidative stress, and inflammation. Adequate glutathione levels in brain cells can mitigate inflammation early on, disrupting the cycle and preventing cognitive damage. GGC is clinically proven to rapidly increase cellular glutathione levels above homeostasis. Dysfunctional glutathione homeostasis has been implicated in various health conditions [7].

A study conducted on mice with early-onset Alzheimer's like pathology demonstrated a significant improvement in brain biochemistry and spatial memory following GGC (Glyteine®) supplementation [8]. These mice expressed a form of amyloid precursor protein, making them predisposed to the disease. The researchers tested their spatial memory using a Morris water maze, where the mice were placed in a water bath and timed on how long it took them to find a platform they could stand on. Over several weeks of training, the mice learned to remember the platform's location based on visual cues in the bath. Monitoring how long it took them to find the platform was a measure of their memory capacity over time.

Half of the mice received a Glyteine® containing diet from 3 months of age, while the others continued with their regular diet. By 6 months, the Alzheimer's predisposed mice on the diet without Glyteine® took an average of 50 seconds to find the platform, while those fed Glyteine® maintained an average of 32 seconds, similar to normal mice [8]. This result suggests that Glyteine® supplementation may have a positive impact on spatial memory in Alzheimer's disease.

GGC (Glyteine®) supplementation is a strong candidate prevention strategy to help prevent or delay this devastating cognitive disease – with long-term use and the regular transient increases in glutathione levels stopping the vicious cycle of oxidative stress, inflammation and neuron death perhaps even before it can begin.

 Chava B., Pocernich, D., Butterfield, A. (2012). Elevation of glutathione as a therapeutic strategy in Alzheimer disease, Biochimica et Biophysica Acta (BBA) -Molecular Basis of Disease, 182 (5), 625-630. <u>https://doi.org/10.1016/j.bbadis.2011.10.003</u>.
 Liu, Y., Chen, Z., Li, B., Yao, H., Zarka, M., Welch, J., . . . Braidy, N. (2021). Supplementation with y-glutamylcysteine (y-GC) lessens oxidative stress, brain inflammation and amyloid pathology and improves spatial memory in a murine model of AD. Neurochemistry International, 144, 104931. doi:10.1016/j.neuint.2020.104931

### **Cardiovascular Disease**

Cardiovascular disease (CDV) is the number 1 cause of death globally, taking an estimated 18 million lives each year. Whilst an increased risk of CDV is often genetic in origin, the effect of negative lifestyle choices such as excessive alcohol consumption, smoking and poor diet play a well documented role in the development of CVD.

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS), also known as free radicals, and the body's ability to neutralize them using antioxidants. ROS are naturally produced during metabolic processes, and they serve important roles in signaling and immune responses. However, excessive ROS production, often due to risk factors like smoking, high blood pressure, high cholesterol, diabetes, and inflammation, can lead to cellular damage and promote the development of cardiovascular diseases.

Complications in the cardiovascular system arise from elevated levels of free radicals which cause tissue damage and interrupt cellular signaling mechanisms [1]. Apart from lifestyle choices, there is a wide range of medical conditions that, by their nature, produce excessive free radicals, including diabetes, hypertension, stroke, and obesity. Whilst small bouts of increased levels of free radicals are normal and are indeed required for signaling purposes and immune responses, it is the oxidative stress caused by sustained and excessive free radical production that leads to deleterious health outcomes [2].



 Bajic, V.P., et al., Glutathione "Redox Homeostasis" and Its Relation to Cardiovascular Disease. Oxid Med Cell Longev, 2019. 2019: p. 5028181.
 Goszcz, K., et al., Antioxidants in Cardiovascular Therapy: Panacea or False Hope? Front Cardiovasc Med, 2015. 2: p. 29.

3. Li, H.G., S. Horke, and U. Forstermann, Oxidative stress in vascular disease and its pharmacological prevention. Trends in Pharmacological Sciences, 2013. 34(6): p. 313-319.

### **Cardiovascular Disease**

Means to prevent this sustained damage caused by oxidative stress have been studied extensively and are of major therapeutic interest [3]. Whilst there is an abundance of pharmacological means to control chronic diseases such as diabetes or hypertension, addressing excessive production of free radicals from all possible sources presents a major challenge.

As the principal intracellular antioxidant, glutathione has been extensively researched. This interest stems from numerous studies into chronic diseases in which elevated levels of free radicals cause sustained oxidative stress. Glutathione acts directly and indirectly to neutralize free radicals and several studies have reported that patients with heart disease have lower cellular levels of glutathione [3].



### **Glutathione Depletion in Mitochondrial Diseases**

Mitochondria, the energy powerhouses of our cells, are vital for aerobic respiration and the production of adenosine triphosphate (ATP) to fuel metabolic reactions [1,2,3]. However, mitochondrial diseases, arising from mutations in mitochondrial and nuclear DNA, can lead to impaired ATP production and an excessive generation of reactive oxygen species (ROS). These imbalances contribute to oxidative damage, disrupting cellular antioxidant defenses primarily involving glutathione. The resulting chronic glutathione deficiency further worsens oxidative stress, compromising nucleic acids, proteins, lipids, electron transport chain activity, and ATP production during oxidative phosphorylation.

Addressing mitochondrial diseases requires effective therapeutic strategies, as direct correction of underlying mutations remains challenging. Medical approaches primarily target oxidative stress, often utilizing dietary supplements. While coenzyme Q10 and vitamins B, C, E, and K are commonly prescribed, their efficacy in mitigating mitochondrial disease symptoms lacks robust evidence [4,5]. Supplementation with gamma-glutamylcysteine (GGC), the immediate precursor to glutathione, offers a promising solution. Glyteine® which contains GGC has demonstrated the potential to elevate intracellular glutathione levels above homeostasis [6]. This increased glutathione pool can help counteract the excessive flow of free radicals from dysfunctional mitochondria. Notably, GGC conversion to glutathione only requires 1 ATP molecule, making it a low-energy solution to overcome ATP deficiency in mitochondrial diseases. Glutathione synthesis, within a cell, from its three component amino acids requires 2 ATP molecules.

In summary, Glyteine® shows promise as a therapeutic intervention for mitochondrial diseases by efficiently elevating intracellular glutathione levels and addressing oxidative stress. Further research and clinical investigations are necessary to fully explore the efficacy and safety of Glyteine®, potentially opening new avenues for managing these complex and debilitating conditions.

- 1. Schapira, AHV 2006, 'Mitochondrial Disease', The Lancet, vol. 368, no. 9529, pp. 70-82.
- 2. Atkuri, KR, Cowan, TM, Kwan, T, Ng, A, Herzenberg, LA, Herzenberg, LA, Enns, GM 2009, 'Inherited disorders affecting mitochondrial function are associated with glutathione deficiency and hypocitrullinemia', Proceedings of the National Academy of Sciences, vol. 106, no. 10, pp. 3941-3945
- 3.Enns, GM, Moore, T, Le, A, Atkuri, K, Shah, MK, Cusmano-Ozg, K, Niemi, AK, Cowan, TM 2014, 'Degree of Glutathione Deficiency and Redox Imbalance Depend on Subtype of Mitochondrial Disease and Clinical Status', The Public Library of Science One, vol. 9, no. 6.
- Chinnery, PF, Turnbull, DM 2001, 'Epidemiology and treatment of mitochondrial disorders', American Journal of Medical Genetics, vol. 106, no. 1, pp. 94-101.
  Gorman, GS, Chinnery, PF, DiMauro, S, Hirano, M, Koga, Y, McFarland, R, Suomalainen, A, Thorburn, DR, Zeviani, M, Turnbull, DM 2016, 'Mitochondrial diseases', Nature Reviews Disease Primers, vol. 2, no. 26080, pp. 1-22.

7. Quintana-Cabrera, R, Fernandez-Fernandez, S, Bobo-Jimenez, V, Escobar, J, Sastre, J, Almeida, A, Bolaños, JP 2012, 'γ-Glutamylcysteine detoxifies reactive oxygen species by acting as glutathione peroxidase-1 cofactor', Nature Communications, vol. 3, no. 718, pp. 1-8.

C.Zarka, MH, Bridge, WJ 2017, 'Oral administration of y-glutamylcysteine increases intracellular glutathione levels above homeostasis in a randomised human trial pilot study', Redox Biology, vol. 11, pp. 631-636.

#### Glutathione and its significance in Athletic Performance and Recovery

Exercise plays a pivotal role in improving health and promoting overall well-being. However, it is not without its potential drawbacks. As exercise intensity increases, so does the rate of respiration, leading to proportionally heightened production of free radicals or reactive oxygen species (ROS). Consequently, this surge in ROS can induce oxidative stress, which can jeopardize the integrity of our cells and tissues [1].

Typically, the glutathione synthesized within our cells functions as an adept scavenger of ROS, effectively neutralizing free radicals such as superoxide and hydrogen peroxide before they can induce cellular damage. However, during periods of high-intensity or endurance exercise, cellular resources may struggle to cope with the increased demand for glutathione. This gives rise to a state of glutathione deficiency, wherein the body fails to fully neutralize all the ROS generated. As a result, we may encounter familiar symptoms, including reduced energy levels, fatigue, listlessness, and an overall feeling of being "run down," all of which can be attributed to the depletion of glutathione following exercise.

Despite the presence of various sports supplements targeting protein/energy deficits and electrolyte losses during the recovery phase, these approaches only address a portion of the problem. Protein and electrolyte levels can be rapidly replenished. However, until recently, the swift restoration of glutathione levels has posed a challenge that was not easily surmountable.

#### Glutathione and its significance in Athletic Performance and Recovery

#### **Glyteine® and enhancing performance:**

An athlete's optimal performance relies heavily on maintaining a consistent energy supply. High-intensity exercises elevate respiration rates, leading to the rapid depletion of glutathione supply and an increase in the production of ROS as byproducts of respiration.

Insufficient glutathione levels result in the accumulation of ROS within the mitochondria of cells, impairing energy production and, consequently, limiting athletic performance.



#### **Regular exercise:**

In line with the recommendations of sports scientists, incorporating antioxidants into exercise regimens serves as an effective strategy to counteract exercise-induced oxidative stress [2]. The inclusion of Glyteine® supplementation aids in optimizing energy production by enhancing mitochondrial efficiency. Remarkably, Glyteine® has the unique advantage of elevating cellular glutathione levels above the norm during resting states. This increased pool of glutathione serves as a buffer against the heightened ROS production that occurs during exercise, thereby allowing mitochondria to provide energy unhindered.

 Sen, C.K. and L. Packer, Thiol homeostasis and supplements in physical exercise. American Journal of Clinical Nutrition, 2000. 72(2 Suppl).
 Kerksick, C. and D. Willoughby, The antioxidant role of glutathione and Nacetyl-cysteine supplements and exercise-induced oxidative stress. J Int Soc Sports Nutr, 2005. 2: p. 38-44.

#### Glutathione and its significance in Athletic Performance and Recovery

#### A Powerful tool in sports recovery:

One of the key advantages of this supplementation is its ability to reduce inflammation and expedite tissue repair. By mitigating inflammation, the glutathione synthesized from Glyteine® aids in the recovery process, enabling athletes to recuperate more swiftly, train with increased intensity, and enhance their performance in subsequent training sessions. This natural competitive edge can be a game-changer for athletes seeking to maximize their potential.

Additionally, Glyteine® supplementation substantially fortifies the immune system, an attribute of paramount importance for athletes, given their elevated susceptibility to infections resulting from the physical stress endured during sports activities. Through strengthening the existing antioxidant defense systems in the body, Glyteine® supplementation contributes to a more resilient and healthier athletic profile.

By incorporating Glyteine® supplementation to support glutathione levels, athletes can reap these multifaceted benefits, optimizing their recovery, resilience, and overall athletic performance.

#### How to make Glyteine® work for you:

