

Antioxidants, Oxidation and the Liver

What are Antioxidants?

The term “antioxidant” is everywhere these days. From green tea to chocolate and even coffee, the media has become inundated with advertising professing the benefits of antioxidant rich-foods. And while most people are aware that antioxidants are good for their health, many are much less certain about what they actually do in the body and why they are so important. In a nutshell, antioxidants are just what their name suggests – they are agents that prevent oxidation. But what then, is oxidation and why is it so important to prevent it from occurring? In a chemical sense, oxidation is a reaction in which electrons are transferred from one molecule to another molecule or “oxidizing agent”.¹ Molecules that have been oxidized have therefore lost some of their electrons. There are many normal functions and processes in the body that require oxidation reactions. In fact, it has been hypothesized that free radicals played a crucial role in the origin of life.² However, these reactions can also have harmful repercussions.

The Danger of Free Radicals

When a molecule loses an uneven number of electrons it becomes a “free radical”. Free radicals are highly unstable and reactive molecules that possess an unpaired electron in their outermost shell. These molecules react within a very short time with another molecule in order to gain or lose another electron. However, this produces another free radical, and kicks off a chain reaction of free radical production that can have deleterious effects.^{1,3}

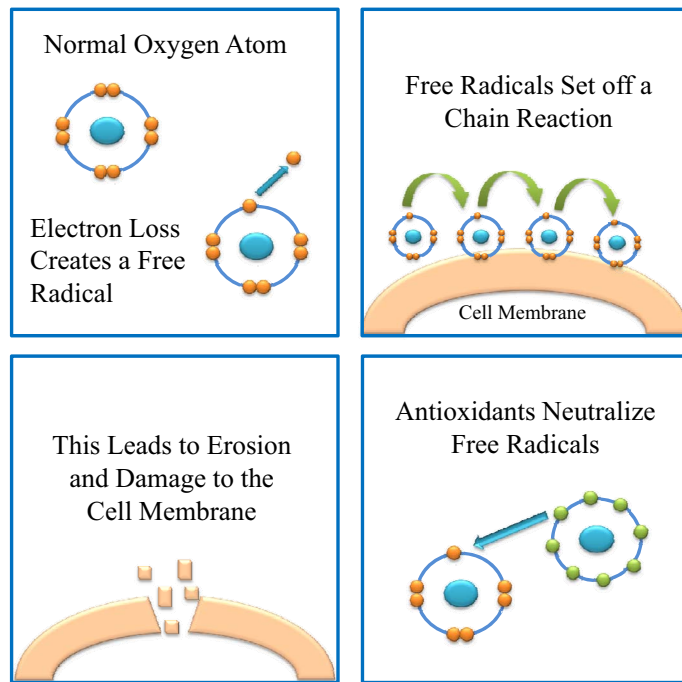


Figure 1. Antioxidants and Free Radicals

One of the most common, and most damaging, types of free radicals are reactive oxygen species (ROS) or oxygen free radicals, and include a number of chemically reactive species derived from oxygen,^{1,3} such as the superoxide anion and the hydroxyl radical. These highly reactive molecules readily react with other molecules in the body, initiating a cascade of free radical producing reactions.^{1,3} However, the production of ROS is required for the generation of cellular energy, creating a difficult situation for the body, which must first produce these molecules, and then eliminate them before they can cause damage.

High levels of ROS are associated with oxidative stress, a dangerous condition that can result in a great deal of damage to the molecules and organs of the body. Because of this, ROS are also potentially toxic, carcinogenic and mutagenic. The damage caused by ROS has been linked to the development of cancer, atherosclerosis, heart disease and autoimmune diseases.³

ROS can attack various different molecules in the body, including DNA, lipids and proteins. DNA damage can result in replication errors and can play a role in the initiation of cancer. In fact, studies have shown a high prevalence of cancer in individuals exposed to chronic oxidative stress.^{4,5} Lipid peroxidation, or the oxidation of low density

lipoprotein (LDL) cholesterol is another well known and dangerous effect of ROS. Lipid peroxidation leads to blood vessel damage and has been associated with the development of atherosclerosis and heart disease.^{6,7}

The Antioxidant Solution

If ROS are so dangerous, the obvious question that arises is how this damage can be prevented? The answer to this question is simple - antioxidants. Antioxidants are vitally important molecules that play the role of free radical scavengers. They are molecules that, in one way or another, react with ROS or other free radicals, stopping the uncontrolled free-radical chain reaction in its tracks, and preventing additional damage and oxidative stress.^{1,3}

Antioxidants come in many forms, and act in many different ways. Some antioxidants, like vitamin E, vitamin C, beta-carotene and numerous flavonoids, are able to form stable radicals themselves that eventually undergo reactions to form non-radical products.¹ Others are enzymes or molecules involved in enzymatic reactions that convert the ROS into different, non-radical products.¹

Antioxidants in the Liver

Antioxidants play an especially important role in the liver. The liver is essential for a wide variety of functions including metabolism, detoxification, protein synthesis, glycogen storage and the production of some hormones. With the liver being a major site of cellular metabolism and energy production it is not a surprise that oxidative stress and free radicals are a serious problem for this critical organ. However, the liver is also a major site of detoxification and is well equipped for dealing with toxic and harmful substances, including free radicals.

The Importance of Glutathione (GSH)

The most important molecule for eliminating free radicals in the liver, and in the whole body for that matter, is glutathione (GSH). GSH is a molecule that is found in all healthy cells, and is composed of three amino acids – glutamine, cysteine and glycine.⁸ The production of GSH is catalyzed by the enzymes γ -glutamylcysteine synthetase (GCS) and GSH synthetase, and occurs in all cell types, although the liver is by far the greatest producer and exporter of GSH.¹

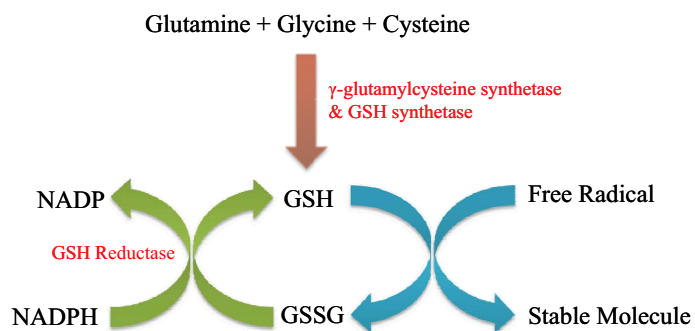


Figure 2. The production and recycling of glutathione (GSH)

GSH is considered the most important antioxidant in the human body⁸, and its role as an exceptional antioxidant is made possible by various important characteristics. First of all, GSH is able to scavenge free radicals both directly and indirectly through a variety of different enzymatic reactions.^{8,9} Its antioxidant capabilities are thus extremely potent and versatile. Furthermore, when oxidized, GSH forms the glutathione radical, but this then reacts with another glutathione radical to produce GS-SG, a stable, non-radical compound. This reaction prevents additional damage and quickly halts the chain of free radical production. GS-SG can then be recycled back into GSH by the enzyme glutathione reductase.⁹

GSH is not merely an antioxidant; it is one of the most important molecules in the body, and is involved in numerous different cellular reactions and processes.⁸⁻¹⁰ In addition to its antioxidant role, GSH, along with the enzyme glutathione-S-transferase (GST), is an essential component of the Phase II enzyme detoxification system in the liver. Together GSH and GST react with various toxic and potentially carcinogenic waste products or molecules, detoxifying them, and protecting the body.^{8,9} GSH is also involved in the proper utilization of lipids, glucose and amino acids by the liver¹¹, the removal of formaldehyde from the body¹², normal cellular growth¹³, proper immune function¹² and sperm production.¹⁴

GSH Deficiency and Liver Health

It is apparent that GSH is a vitally important molecule for the maintenance of both liver and whole body health.

Roles of Glutathione⁸

Antioxidant Defense

- Scavenging free radicals and other reactive species
- Removing hydrogen and lipid peroxides
- Preventing oxidation of biomolecules

Metabolism

- Synthesis of leukotrienes and prostaglandins
- Removal of formaldehyde
- Involved in Phase I and II detoxification of toxins
- Utilization of cellular lipids, glucose and amino acids
- Storage and transport of cysteine

Regulation

- Intracellular antioxidant status
- Signal transduction and gene expression
- DNA and protein synthesis, and proteolysis
- Cell proliferation and apoptosis
- Cytokine production and immune response
- Mitochondrial function and integrity

Deficiencies in GSH place the body in a situation of oxidative stress, increasing the likelihood of cellular damage and disease.¹² GSH imbalances have been linked to a variety of conditions and disease, including cancer, neurodegenerative disorders, cystic fibrosis (CF), HIV and aging.¹²

In regards to the liver specifically, a great deal of research has shown that GSH is intimately linked to liver health. The situation can be fairly complex, however, as low GSH levels can be either the cause or the resulting effect of a condition.¹² For example, inherited disorders in GSH metabolism can significantly disrupt liver function, and can even lead to death.¹² Nutritional amino acid deficiency can also result in depleted GSH.⁸ Alternatively, certain conditions, like excessive alcohol consumption, can deplete glutathione, putting serious stress on the liver.¹²

Overall, there are two primary mechanisms by which GSH can be depleted. First, excessive production of ROS can lead to GSH depletion. Excessive ROS production is often associated with inflammation, a

common manifestation of many disease conditions.¹⁵ GSH can also be depleted by the metabolism of large quantities of drugs or other chemicals. For example, acetaminophen overdose is known to deplete GSH. GSH is required for the detoxification of substances like acetaminophen, so high levels can lead to GSH depletion.¹⁵

In the case of alcohol-induced liver disease, ethanol consumption puts a strain on the liver's detoxification systems while also inducing inflammatory pathways and promoting lipid peroxidation.^{16,17} It is therefore not surprising that alcohol consumption has been shown to deplete GSH levels in the liver. It has also been associated with reduced activity of the enzyme glutathione reductase, which is essential for the regeneration of GSH from GS-SG.¹⁶ The earliest stage of alcohol-induced liver disease is a fatty liver, which then can progress to alcoholic hepatitis and finally, cirrhosis and eventually death.

Glutathione depletion and oxidative stress have also been implicated in the pathogenesis of non-alcoholic fatty liver disease (NAFLD).¹⁷ NAFLD is often associated with obesity, diabetes and high cholesterol levels, and follows a progression similar to that of alcohol-induced liver disease.^{18,19} Abnormal antioxidant status has been reported in 90% of NAFLD patients, and glutathione levels have been found to be reduced by as much as 57%.¹⁷

Oxidative stress can induce a number of extremely damaging events in the liver. Hepatotoxicity by oxidative stress may be achieved through a direct attack of ROS on essential biomolecules, resulting in a loss of biological function and cellular death. It may also occur through the activation of proinflammatory molecules or Kupffer cells.¹⁷ Kupffer cells are a special type of macrophage that reside only in the liver, and are involved in the liver's response to toxic compounds.²⁰ Prolonged activation of Kupffer cells (by toxic agents, for example) stimulates the cells to release inflammation causing compounds and ROS. This results in an exacerbation of liver injury.²⁰ Recent research also suggests that chronic activation of Kupffer cells can result in the release of molecules that promote excessive liver cell proliferation, thereby contributing to the development of liver cancer.²⁰

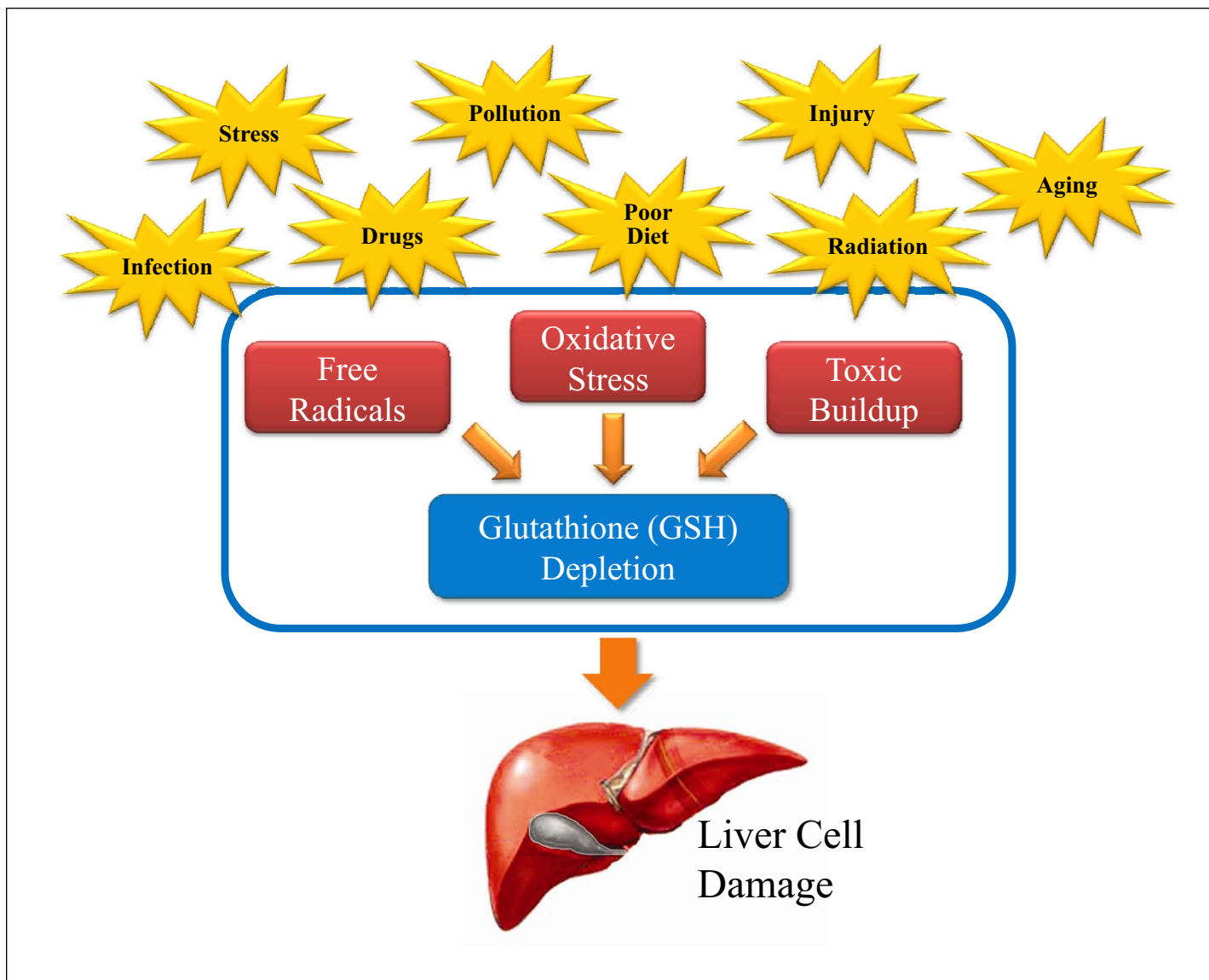


Figure 3. The Causes and Effects of Glutathione Depletion

The Problem with Glutathione Supplementation

The importance of adequate GSH is undeniable, and supplementing with this essential antioxidant would seem to be a very good idea. There is a problem, however. Although oral GSH supplementation seems to be effective in rodents²¹, research has shown that it has very poor bioavailability in humans. In one study, a single administration of a 3g dose of glutathione did not significantly increase circulating glutathione levels.²²

The problem seems to occur somewhere between the digestive tract and the liver. Research suggests that GSH can be effectively absorbed by the body from the gastrointestinal tract via a specialized uptake mechanism. However, once absorbed GSH in the

bloodstream is broken down by enzymes before it reaches the liver.²³ It is possible, therefore, that oral GSH may still be beneficial for conditions of the intestinal tract, like Crohn's disease, but not for other conditions requiring GSH supplementation.²³ In one trial, for example, a 300 mg/day dose of GSH administered to cirrhosis patients for 28 days did not improve GSH status.²⁴

N-Acetyl-Cysteine (NAC) as a Solution

The good news is that there is another way to increase GSH levels. GSH is composed of three amino acids – cysteine, glutamine and glycine.⁸ Research has shown that the availability of cysteine is rate-limiting for GSH synthesis.²⁵ For instance, human subjects given a cysteine-free diet for 10 days showed a significant decrease in GSH synthesis and turnover, compared to when they received a cysteine-containing diet.²⁵ These

findings suggest that cysteine is most likely to be in short supply, thereby limiting cellular production of GSH, and that supplementation with cysteine could potentially boost GSH levels. L-cysteine is an extremely unstable molecule, and is not suitable for supplementation.²³ N-Acetyl-Cysteine, on the other hand, is a safe and orally bioavailable source of cysteine.^{23,26} Following absorption in the intestines, NAC is converted to cysteine and can be used to increase GSH production.

NAC supplementation has been shown to effectively replenish GSH in depleted patients.²³ NAC supplementation has been demonstrated to have clinical benefits to HIV patients, and was associated with a significant improvement in their depleted GSH levels.²⁷

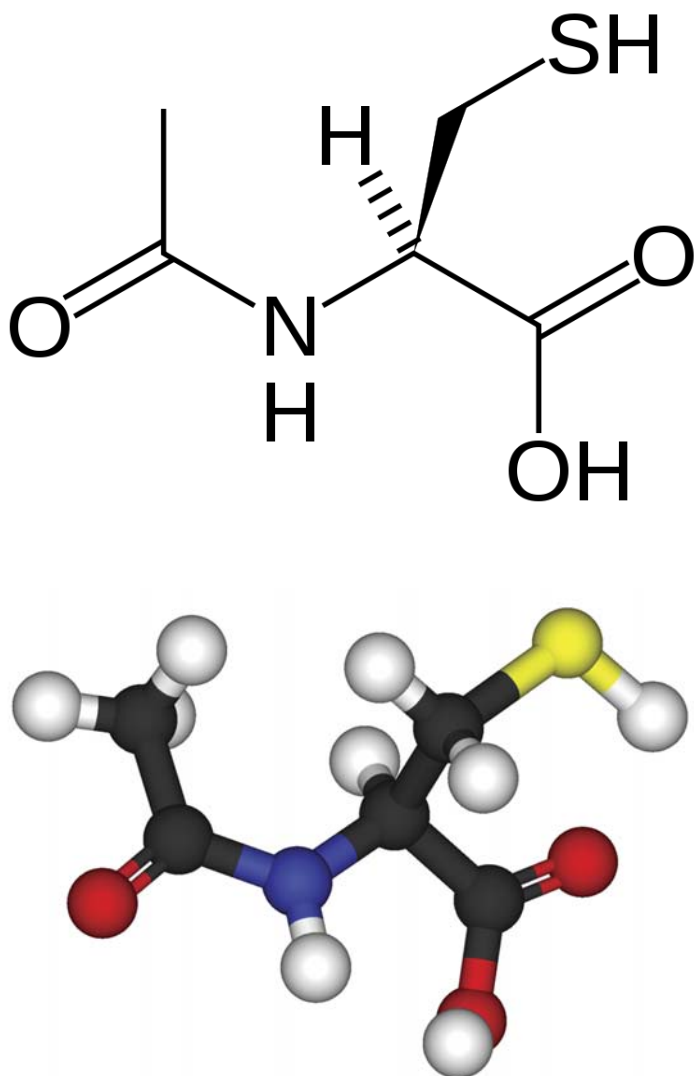


Figure 4. The Chemical Structure of N-Acetylcysteine

In terms of liver health, NAC is well-established as a treatment for acetaminophen poisoning. When given within 8 hours of overdose, 140 mg of oral NAC per kilogram of body weight was protective and reduced the incidence of hepatotoxicity regardless of initial acetaminophen concentration.²⁸ Furthermore, no deaths were clearly caused by acetaminophen among patients in whom NAC therapy was begun within 16 hours.²⁸ NAC's actions in preventing acetaminophen induced hepatotoxicity has been linked to its antioxidant effects and its ability to replete GSH.²⁹

NAC is also beneficial in cases of liver failure not related to acetaminophen. In 170 children with acute liver failure NAC was found to be a safe and effective treatment option. Children receiving NAC had a shorter length of hospitalization, a greater survival with the native liver and a better survival rate after liver transplant than those not receiving NAC.²⁹

In addition to its ability to increase GSH production, NAC is also beneficial for respiratory conditions, and has been shown to help reduce mucous in the lungs, reduce cough severity and improve pulmonary function.³⁰ NAC also has cardiovascular benefits, and research has demonstrated that it is able to lower levels of the toxic amino acid homocysteine by 45%³¹ and lipoprotein(a) levels by 70%.³² Finally, NAC appears to have anti-cancer properties as well. In vitro studies have shown that NAC has direct anti-carcinogenic effects. In animal and cellular studies NAC was found to selectively protect normal cells, but not malignant ones, from chemotherapy and radiation toxicity.^{30,33}

Conclusions

Antioxidants are vital for protection from free radical damage, whether the free radicals are produced through biochemical processes or as the result of a disease condition. The liver is particularly vulnerable to free radical damage, which occurs when the most important liver antioxidant, glutathione, is depleted. Oxidative stress in the liver can lead to liver cell death, inflammation of the liver, liver diseases and possibly even liver cancer. Increasing glutathione levels is critical, especially in conditions where it has been depleted. NAC supplementation is by far the safest and most effective method to naturally increase glutathione levels, thereby enhancing the health of the liver and the entire body.

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