

Effects of Supplementation with Glutathione and its Precursors on Athlete Performance





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Received:  December 18, 2018; **Published:**  January 03, 2019

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Abstract

Glutathione (GSH) is a thiol-containing tripeptide consisting of glutamate (Glu), cysteine (Cys) and glycine (Gly), which plays central roles in the defense against oxidative damage and in signaling pathways. The cellular concentration of GSH and its oxidative state (GSSG), and their molar ratio are indicators of cell functionality and redox state. Glutathione is the most important antioxidant in human organism. Its level can be increased by supplementation with glutathione precursors. Furthermore, glutathione is the most popular drug (supplement) used by athletes and people remaining in physical activity. The increase in GSH body content can be achieved by supplementation with N-acetyl-L-cysteine (NAC), α -lipoic acid (ALA), whey preparation or L-glutamine. There are many scientific reports showing a key role of glutathione in treatment of many diseases. Furthermore, authors demonstrated improvement of the sport performance and physical condition of athletes by supplementation of glutathione and glutathione precursors.

Keywords: Glutathione; Glutathione Precursors; Oxidative Stress; Physical Activity; Athletes Performance

Abbreviations: ROS: Reactive Oxygen Species; NAC: N-acetyl-L-cysteine; ALA: α -Lipoic Acid; GCL: Glutamate Cysteine Ligase; GS: GSH Synthetase; RN: Reactive Nitrogen OS: Oxygen Species; IRS: Insulin Receptor Substrate; NS: Nutritional Supplements

Introduction

Intensive exercise is related to an increased generation of reactive oxygen species (ROS), the main cause of oxidative stress, as a consequence of higher energy demand [1,2]. In active tissues such as skeletal muscles, ROS activity results mainly from mitochondria damage and may impair the muscle force production. Therefore, many athletes consume antioxidant supplements to suppress muscle fatigue or to facilitate the process of recovery. Whether such antioxidant supplementation has beneficial effects on sport performance and health is a matter of controversy which seems to stem from considerable differences in study designs [3]. Some authors report that antioxidant supplementation, especially a prolonged one, may have deleterious effects on health and sport performance, e.g. by down regulating natural ROS quenching mechanisms or hampering organism adaptation to increased physical exercise [4-8]. Such results are in line with the free radical theory of development which posits that formation of reactive species plays a very important role in development of organisms and their adaptation to environmental changes [9]. Other studies report that antioxidant supplementation may lead to increased

sport performance or shortens the process of post-exercise recovery; the effects highly desired by competitive athletes. A common feature of those studies seems to be a short and acute supplementation period, usually a few days or weeks long, which perhaps still allows for triggering the adaptation mechanisms in response to increased production of ROS. These studies are of the main interest of this review.

Glutathione – Physiological Significance and Therapeutic Potential

Glutathione (γ -L-glutamyl-L-cysteinyl-glycine, GSH) plays a very important role in physical exercise by controlling the level of oxidative stress. It is the major endogenous intracellular antioxidant and currently one of the most studied substances of this class. It is synthesized primarily in the hepatic cells and has numerous physiological functions, including protection of cells against oxidative stress, detoxication of toxins and carcinogens, posttranslational regulation of protein function, and maintenance of immune function of the immune system. In human body, GSH is

synthesized by sequential addition of the precursor amino acids, cysteine (Cys), glutamic acid (Glu) and glycine (Gly), through enzymatic catalysis by two ATP-dependent enzymes namely, glutamate cysteine ligase (GCL) and GSH synthetase (GS) [10,11]. The maintenance of tissue levels of GSH is critical for maintaining health, preventing diseases and age-related biological insults [12]. Human blood plasma contains low amounts of reduced glutathione that has been shown to increase post-exercise [13].

Almost all of blood glutathione represents blood cell content, mainly that of the erythrocytes. Oxidation of GSH to glutathione disulfide (GSSG) is a sensitive marker of the oxidative stress [14,15]. Scientific reports show that high levels of reactive oxygen species (ROS) and low GSH levels are associated with increased risk of numerous diseases. The most well-known include neurodegenerative diseases (particularly Parkinson's and Alzheimer's disease), pulmonary diseases (chronic obstructive pulmonary disease, asthma, and acute respiratory distress syndrome), cystic fibrosis, immune diseases (autoimmune disease), cardiovascular diseases (hypertension, myocardial infarction, cholesterol oxidation) as well as diseases associated with age-related oxidation (cataract, muscular degeneration, hearing impairment, and glaucoma), and cancer [16,17]. The list of conditions associated with impaired GSH homeostasis continues to grow and reflects the importance and diversity of glutathione cellular functions [17].

Thus, GSH application as a therapeutic agent is widely discussed in the literature [18-20]. Reports indicate the use of oral supplementation with GSH and its precursors for treatment of acute respiratory distress syndrome [21], in patients with cystic fibrosis [22], in autism spectrum disorders [23], for improvement of liver biochemistry (e.g. in of nonalcoholic steatohepatitis patients) [24], for significant improvement and slowing of the Parkinson's disease progress [25], as a strategy in Alzheimer's disease [26], in patients with peripheral artery disease [27], in children with chronic otitis [28], for the treatment of mercury toxicity [29], and in schizophrenia and bipolar disorder [30]. The ability to form reduced glutathione diminishes with age [31-33]. Therefore, restoration of the antioxidant potential of the body for therapeutic purposes may be required in such individuals. This could be obtained by employing various strategies such as supplementation with glutathione, its precursors, or other substances that indirectly modify GSH level.

Glutathione Supplementation and Sport Performance

The data on effects of direct GSH supplementation and sport performance is scarce. However, a recent study has showed that 2 week oral GSH supplementation (1g/day) may alleviate fatigue in healthy men during and after cycling exercise [10]. It is worth noting that the positive effects of GSH supplementation on sport performance could be observed with just minute changes in its blood levels. Data from mice suggests that this effect may result from a facilitated aerobic metabolism of lipids in skeletal muscles due to the increased mitochondria biogenesis and decreased muscle acidosis [10]. Interestingly, the observed upregulation of factors involved in mitochondria biogenesis was rather unexpected

as antioxidant supplementation (e.g. with vitamin C or E) has been shown to suppress their levels [6-8]. Thus, it is tempting to speculate that these changes manifest a GSH J-shaped activity as a signaling factor rather than an antioxidant. It is still to be determined whether it is the effect of direct GSH absorption, absorption of GSH precursors resulting from its hydrolysis, or both. Additionally, it is an interesting question as the bioavailability of ingested GSH has long been considered negligible.

However, recent studies suggest that it can be absorbed in the intact form [34,35] and the elevation of GSH body levels following its oral supplementation has been already reported [2,12,34, 36,37]. The concentration of GSH may be increased by supplementation oral or sublingual form of glutathione. Schmitt et al. demonstrated the superiority of sublingual form of GSH over oral form. Within this study 20 volunteers with metabolic syndrome received 450mg/day of both forms of GSH [37]. Other studies revealed that GSH supplementation may improve the performance in mice. Leewuenburgh et al. demonstrated improvement in endurance performance and lower muscle lipid peroxidation during prolonged exercise (mice were given 6mmol/kg body (via intraperitoneal injection) 1h before exercise [38]. Furthermore, a positive effect of GSH supplementation on swimming performance was reported by Novelli et al. [39]. Mice received GSH (by intraperitoneal injection) in doses of 500, 750 and 1000mg/kg. Increased swimming endurance (102.4%, 120.0% and 140.7%, respectively) was observed. GSH of 250mg/kg did not affect endurance when injected in a single dose but increased it by 103.7% when injected once a day for 7 days [39].

Glutathione Precursors

The body stores of GSH can be restored or increased by supplementation with GSH precursors, of which N-acetyl-L-cysteine has gained probably the most attention. It is the N-acetylated form of the amino acid L-cysteine which can be administered orally. It is a thiol donor with nonspecific antioxidant properties [19, 40]. NAC has been used in clinical practice to facilitate GSH biosynthesis, which may improve the intracellular antioxidant defense system and, possibly, decrease the damaging effects of ROS. Due to physiological decrease in GSH levels with age [32], it seems that NAC supplementation would be the most efficient in young patients, due to the age-related decrease in GSH synthesis in older individuals. The latest reports discuss an application of NAC to recovery facilitation after traumatic brain injury, cerebral ischemia, and in treatment of cerebrovascular vasospasm after subarachnoid hemorrhage [42]. Other therapeutic indications were achieved on rodent experimental models [43,44].

In the field of sport nutrition, there is a growing interest about NAC. It has been shown that NAC supplementation for seven days at 1200mg per day may improve the muscle fatigue and increase the VO₂ max in sedentary men [45]. Another study conducted by Kelly et al. showed that 1800mg of NAC ingested 45 minutes prior to exercise test reduced respiratory muscle fatigue in men during heavy exercise [46]. Moreover, NAC infusion (125mg/kg/h for 15min and then at 25mg/kg/h for 20min before and throughout the exercise) during prolonged, submaximal cycling exercise was

reported to increase its muscle content as well as skeletal muscle cysteine, cystine, and glutathione availability during the test. The NAC administration resulted also in a substantial enhancement of performance in well trained individuals [47]. Sinha-Hikim et al. demonstrated a beneficial effect of NAC supplementation (3mg/kg feed) to attenuate loss of muscle mass associated with aging in mice [48]. Another study showed that treatment with NAC represented an important factor in the defense against muscle soreness. Furthermore, it seems that NAC has some anti-inflammatory effects and acts on the downregulation of proinflammatory cytokines [49].

These performance-enhancing effects appear to be mediated by increases in circulating and myocyte levels of Cys and GSH [47,50,51]. However, the exact mechanism of NAC action is still to be elucidated and published data suggests that it may be a product of at least three different NAC activities. As NAC can be hydrolysed to Cys, which is a limiting factor of GSH synthesis, its supplementation may increase total GSH levels and GSH/GSSG ratio [52-55]. Additionally, it may reduce oxidated substances such as cystine or GSSG [50,55] or may simply modify the oxidative state of the organism by direct ROS quenching. Further research will need to determine which of these mechanisms predominate at a given condition. Nevertheless, recent studies have shown that the antioxidant activity of NAC may prolong the exercise time to fatigue, at least partially by the enhancement of Na⁺, K⁺-pump activity [51]. The pump is involved in maintenance of the cellular Na⁺/K⁺ homeostasis, disturbance of which may impair muscle force development. NAC has been shown to indirectly prevent a ROS-mediated Na⁺, K⁺-pump by improving the ROS scavenging mechanisms in the cell thereby protecting the SH groups on the transporter [51].

Additionally, NAC administration attenuates expression of mRNA coding for one of the isoforms of Na⁺/K⁺ catalytic subunit; the process known to be unregulated in response to exercise [56]. It is therefore possible that upregulation of this mechanism is suppressed due to the lowered ROS levels and the consequent reduced turnover of the transporter. Strenuous exercise is also known to induce muscle injury accompanied with an inflammatory response which may cause a neutrophile invasion into the tissue and local excessive ROS production [57]. Importantly, NAC supplementation (200mg/day) before incremental cycling test has been shown to prevent the increase in capacity of neutrophils to generate ROS in untrained healthy men [55]. In another study conducted on rowers (6g for three days before completing 6 min of maximal exercise) by Peake et al. [58] showed that NAC may cause a significant reduction of in vitro neutrophil ROS production below the pre-exercise values. Hence, NAC may prevent infliction of additional oxidative stress on the damaged tissue by ROS of neutrophile origin. Such NAC activity may be advantageous, especially in cases of prolonged exercise or during the recovery period [57].

A positive effect of supplementation by NAC (oral or intravenous) before (or during) trial on athletes performance are also described in [59-63]. Another glutathione precursor is glutamylcystin. It can be found in some of whey preparations such as Immunocal and

is more readily absorbed form of Cys. In general, whey protein is a collective term that encompasses a range of soluble protein fractions found in milk. Whey protein products are classified into several categories based on their macronutrient ratios that suit a variety of food manufacturing purposes. At the molecular level, stimulating protein synthesis and minimizing protein breakdown are the two processes essential to efficient recovery after exercise [64, 65]. The effect of Immunocal supplementation on enhancement of muscular performance was showed by Lands et al. [64]. In that study, an increased muscle performance (ability to generate more power to perform more work during a 30s of maximal effort) and increased lymphocyte glutathione concentrations of 35,5 % were observed in "Immunocal group".

Furthermore, the supplemented group also spent more time in activity, whereas there was no change in the control group. Similar results were showed by Karelis et al. Participants ingested 20g/day a cysteine-rich whey protein isolate (Immunocal→), whereas the control group received casein at the same dosing. All participants performed the same resistance training program (3 times per week). Results from this study showed a significant increase in muscle strength (~ +10 %) in subjects who were supplemented with Immunocal→ versus casein [66]. Supplementation with whey proteins is common in medicine. The results showing that oral administration of whey proteins may indeed lead to an increase in plasma glutathione levels were shown by Micke et al. in patients with advanced HIV-infection [67]. In another study by Ross et al. mice received Immunocal→ 660mg/kg/day. This supplementation significantly reduced age-associated decline in skeletal muscle functions. Furthermore, it significantly delayed the clinical onset of neuromuscular disease [68].

L-Glutamine

Athletes may increase glutathione body level by supplementation with L- glutamine (Gln), which is a naturally occurring nonessential neutral amino acid [69]. It is important in the acid base regulation, gluconeogenesis, and as a precursor of nucleotide bases and the antioxidant glutathione. Gln is the most abundant free amino acid in human muscle and plasma. Other dietary sources of Gln for athletes may include protein supplements such as whey protein and protein hydrolysates [69,70]. Various manufacturers and suppliers of Gln supplements claim that their products have features that may benefit athletes: nutritional support for the immune system and prevention of infection, improved gut barrier function and reduced risk of endotoxemia, improved intracellular fluid retention (i.e. a volumizing effect), more rapid water absorption from the gut, stimulation of muscle glycogen synthesis, muscle protein synthesis and muscle tissue growth, reduction in muscle soreness and improved muscle tissue repair, enhanced buffering capacity, and improved high intensity exercise performance [69,71].

Gln is an important fuel for some cells of the immune system and may have specific immunostimulatory effects. Furthermore, Gln have a positive effect on liver function [72-74]. Koo et al. [75] demonstrated that glutamine supplementation by rowers (6g/day) had a positive effect on the decrease in fatigue factor stimulation at

the recovery stage after maximal intensity exercise compared with supplementation with the placebo. Furthermore, enhanced immune function and the defensive inflammatory reaction were observed. Similar effects were obtained by Sasaki et al. [76]. Moreover, Gln could be the best of choice as a precursor of glutathione, because it is the main systemic transporter of nitrogen in mammals and its carbon skeleton may be used in glutathione synthesis [70,74]. It has been showed that periods of very heavy training are associated with a chronic reduction in plasma concentrations of Gln which may be partly responsible for the immunodepression effect apparent in many endurance athletes [77].

A lot of research concerning on oral supplementation with Gln and its effect on physical performance, took place in Iran [1,78,79]. Research conducted by Dabidi Roshan focused on immunological aspect. During the trial, active young males (mean 18 years old) received 0.1g/kg of Gln. Available data did not support the contention that the post-exercise change in some immune indices was caused by a decrease in plasma glutamine concentration. Glutamine supplementation attenuated the exercise-induced decline in glutamine concentration. Similar effect were obtained in Castell et al. [80] research. Glutamine supplementation at 0.1g/kg caused a significant increase in the plasma glutamine concentration within 30 min in healthy humans. Furthermore, glutamine supplementation caused that the ratio of CD4+ helper/CD8+ suppressor cells in blood samples taken 1h after a marathon and after a drink of either glutamine or placebo, was significantly lower in the placebo group than in the glutamine group.

Oral supplementation of glutamine (0.35g/kg/day) by 15 non-athlete male students for 8 weeks, caused significantly greater increases in upper and lower body strength, explosive muscular power, blood testosterone, GH and IGF-1 when compared to the placebo group. Furthermore, in the study group there was improved body composition (increased body mass, fat-free mass and reduced body fat) and vertical jump (62.31 ± 5.22 pre- and 67.11 ± 4.44 post- supplementation) [79]. Glutamine supplementation plays a key role in recovery after muscle injury. It has been shown that such supplementation (0.1g/kg of L-glutamine) caused attenuated delayed onset muscle soreness effects in "supplementation" group [1]. Oral glutamine supplementation influenced also athletes' endurance. Glutamine consumed by nine male soccer players caused great improvement in the time and distance covered. One group consumed carbohydrate with peptide glutamine, while the second group carbohydrate alone. Total distance covered in first group was 15571m and 12750m in second group. Furthermore, the first group experienced less fatigue. There are few studies that associate the effect of glutamine with the performance of athletes and thus the mechanisms involved are still unclear [81]. Studies on glutamine were also conducted in rodents. Eleven male mice administered an L-glutamine supplement through their drinking water for four weeks. The average contractile force obtained from these eleven experimental mice was higher (0.0018N/g) than in the control group (0.0008N/g). In study group the muscle weight mass (2,4g) was also increased in comparison to control group (1,66g) [82].

Alpha-Lipoic Acid

Alpha-lipoic acid (LA) is a naturally occurring dithiol compound synthesized enzymatically in the mitochondria from octanoic acid [83]. LA is also a dietary supplement that is known to strengthen the antioxidant network [84]. Like glutathione, LA is present in two states, namely, oxidated (α LA) and reduced (DHLA) [65]. LA is absorbed in the intact form from dietary sources, and it transiently accumulates in many tissues [83]. LA has already been described as a potent biological antioxidant [85] and has been demonstrated to increase muscle phosphocreatine levels and muscle total creatine concentrations [86]. Research conducted by Zembron Lacny et al. [87] showed that LA has a clear pro-glutathione effect in physically active individuals after 8-days supplementation with 600 mg LA daily doses. Administration of LA resulted in declined carbonyl groups and thiobarbituric acid-reactive substances in plasma compared with control group, which are products of lipid peroxidation and protein carbonylation. Both factors are markers of oxidative damage, which are indirectly correlated with total antioxidants status [87].

Another very important factor in the context of physical exercise is Interleukin-6 (IL-6). IL-6 is a cytokine which plays a role in muscle energy homeostasis during physical exercise. Lappalainen [88] investigated the effect of LA administration on IL-6. IL-6 is an immunomodulatory cytokine produced mainly by the cells of the immune system and is considered as a marker of inflammation. However, IL-6 is also secreted by the skeletal muscles and plasma levels of IL-6 may rise up to 100-fold after strenuous physical exercise [89]. IL-6 can improve skeletal muscle energy supply and assist in the maintenance of stable blood glucose levels by stimulating lipolysis in the adipose tissue and augmenting hepatic glycogenolysis [90,91]. Lappalainen [88] has indicated that exercise increases serum IL-6 levels irrespective of diurnal variation and showed that supplementation with LA had no beneficial effect on exercise performance and exercise-induced IL-6 response.

Some publications also presents a beneficial effect of supply LA in the haematological context. Sixteen healthy young males participated in the randomized and placebo-controlled study designed in order to show the changes in the hematological response through the combination of LA intake with running as an eccentric exercise. This study confirmed the antioxidative properties of LA, through lipid and protein protection against over-activity of reactive nitrogen/oxygen species (RN/OS) [92]. Furthermore, it is known that EPO stimulates proliferation of myoblasts in skeletal muscle and has a potential role in muscle mass maintenance [93]. Results from research indicated a possible use of LA to improve EPO production and skeletal muscle regeneration through changes in the reactive nitrogen/oxygen species (RN/OS) ratio at rest and after exercise. α -Lipoic acid enhanced EPO production both before exercise and during recovery. EPO level was especially high at 48 h after exercise [92].

It is know that, during physical exercise there are intense changes in the circulatory system and changes associated with

thermoregulation. Therefore, LA is also a research object in this context. Polyviou et al. [94] showed that a supplement containing LA provides improvements in the thermoregulatory and cardiovascular responses in training cyclists during exercise in the heat. Thus, application of LA can be useful for athletes, who are training at high temperature. Insulin and glucose play a very important role in physical exercise. There are many publications focused on supplementation with LA in order to improve insulin activity and glucose utilization in humans [95], rodents [96] and chickens [97]. Saengsirisuwan et al. [98] showed that LA supplementation improved insulin response in rats as evidenced by increased expression of insulin receptor substrate-1 (IRS-1) signaling in skeletal muscle which is a primary site for glucose uptake and storage.

Conclusion

Undeniably, supplements play key role in sport. On the other hand, there is a growing evidence suggesting that nutritional supplementation is strongly associated with doping use in elite and amateur sportsmen [99] and nutritional supplements (NS) have been promoted as safe alternatives in order to enhance performance. However, its use has also been associated with an increased risk of doping. Athletes who have used supplements, become more favorable toward doping, prior to engaging in this behavior [99]. Barkoukis et al. (2015) [99] investigated, the cognitive and behavioral components of the association between NS use and doping among adolescent sub-elite athletes. The results of the study on 650 adolescent athletes group showed that NS users who did not report doping use had significantly stronger doping intentions and more positive attitudes and favorable beliefs toward doping use, in comparison with athletes who did not use NS. Glutathione, its precursors and Sulphur compounds are considered as a potentially therapeutic wide spectrum agents in clinical practice. The need for healing injuries after intensive exercises justifies use of these compounds by athletes. Further research is being conducted on markers for detection of early signs of overload exercise athletes, such as glutamine, glutamate and glutamine/ glutamate ratio [100]. The use of performance-enhancing substances is frequent and popular in both amateur and professional sports [101]. However, it can be also done unintentionally while taking dosages of NS that are contaminated or faked with doping substances prohibited by World Anti-Doping Agency (e.g. steroids, stimulants, narcotics) [99,102-104]. Proteins, amino acids, creatine, multivitamins and mineral and herbal products are used for performance enhancement in the elite, amateur, and recreational sports across age groups [105-109]. A growing body of research implies that the NS use can serve as a "gateway" to doping [99].

Acknowledgement

Ministry of Sport and Tourism of the Republic of Poland, Grant/Award Number: 2018.0029/ 0305/UDOT/BM.

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ISSN: 2574-1241

DOI: [10.26717/BJSTR.2019.12.002293](https://doi.org/10.26717/BJSTR.2019.12.002293)

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