Targeted Amino Acid Supplementation: A Novel Strategy for Raising Glutathione and Optimizing Health

Over the past decade, there has been a groundswell of scientific and clinical interest in glutathione, a powerful endogenous antioxidant found in nearly every tissue of the body. Biochemists and cell biologists around the globe have documented the vital roles played by this unique tripeptide in maintaining cellular and systemic homeostasis. At the same time, clinical researchers have identified glutathione deficiency in association with a number of common health challenges.

Given that glutathione is found in nearly all tissues of the body, it has become a subject of great interest to functional medicine practitioners seeking ways to influence metabolic function, reduce oxidative stress, improve hepatic detoxification, balance immune system function, and affect multiple organ systems simultaneously.

Glutathione (Fig. 1) even reached popular consciousness, when Dr. Mehmet Oz referred to it as “the superhero of antioxidants” on his popular TV show.

Though glutathione is produced endoenously, its synthesis tends to diminish with age (Samiec PS, et al. Free Radic Biol Med. 1998; 24: 699-704). This fact has led many researchers to look for ways to increase circulating glutathione levels via supplementation. But this quest has been challenging.

Like other antioxidants such as vitamin E, polyphenols, and carotenoids, glutathione can be obtained to some extent from the diet. Foods rich in glutathione include whey protein, avocados, asparagus and parsley (Fig. 2). However, most people—especially those who are glutathione-deficient—will not be able to obtain sufficient quantities of this antioxidant from food sources alone.

Taken orally, glutathione itself has low bioavailability, in part because it is hydrolyzed by intestinal and hepatic gamma-glutamyltransferase. That, along with the compound’s somewhat offensive sulfurous odor, has rendered oral glutathione supplementation of limited practical value. Intravenous infusion of glutathione is effective in raising blood and tissue levels, but this is impractical for routine use.

Some investigators have advocated the use of N-acetyl cysteine (NAC) as a method for increasing glutathione levels. As is the case with glutathione itself, oral NAC supplementation gives only modest results at best. Intravenous NAC will increase glutathione production, but is not practical (Eur J Respir Dis. 1987; 70: 73-7; Biopharm Drug Dispos. 1987; 8: 377-86).

Several years ago, Japanese investigators developed a new way of raising endogenous glutathione production through supplementation with a precise combination of cystine and theanine. These two naturally occurring amino acids provide two of the three key building blocks that serve as substrates for the endogenous synthesis of glutathione.

By providing essential precursors that enable the body to increase synthesis of its own glutathione, this approach circumvents the enzymatic degradation that has hindered the efficacy of supplementation with glutathione itself (Fig. 3 & 4).

What is Glutathione?

Glutathione is a tripeptide comprised of the amino acids glutamate, cysteine and glycine. It also contains a sulfur molecule, which plays a role in its unique anti-oxidant properties.

Glutathione is produced and used by nearly every cell in the body, and plays a fundamental role in protecting cell membranes from the ravages of free radicals generated in the normal course of mitochondrial oxygen metabolism. It is important to understand that any condition that increases oxidative stress will increase the demand for glutathione.

The Dangers of Deficiency

In 2007, a research team in Spain showed that activity of glutathione peroxidase—the primary antioxidant pathway in endothelial cells—correlates inversely with heart health. Their study of 137 participants showed that two-year event free survival was significantly lower in people with elevated glutathione peroxidase, suggestive of high oxidative stress (Garcia-Pinilla JM, et al. Texas Heart Inst J. 2008; 35(3): 262-267).

The authors concluded that while some clinical studies of interventions with antioxidants have failed to show improvement, it should be borne in mind that the inclusion criteria did not take into consideration the baseline oxidative or antioxidant status.

Glutathione is very important in hepatic detoxification pathways. It is capable of binding metals and exogenous toxins, and transforming them into water-soluble compounds that can be excreted in bile or urine. Because it is the primary organ of detoxification, the liver harbors the body’s largest stores of glutathione. Like oxidative stress, unusually heavy toxic loads will increase the demand on the liver to make new glutathione. Deficiency results when tissues cannot keep pace with the demand.

Fig. 1 Molecular structure of Glutathione, an important antioxidant compound

| Vitamin C | broccoli, papaya, red bell peppers, oranges, strawberries, kiwi |
| Vitamin E | nuts, sesame seeds, vegetable oils |
| Polyphenols | berries, grapes, cocoa, red wine, green/black tea |
| Carotenoids | pumpkin, carrots, sweet potatoes, cantaloupe |
| Selenium | beef poultry, tuna, brown rice, sunflower seeds |
| Zinc | red meat, sesame seeds, pumpkin seeds, nuts, spinach |

GLUTATHIONE: whey protein, asparagus, avocado, parsley

Fig. 2 Key antioxidants and their food sources

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Glutathione is very important in hepatic detoxification pathways. It is capable of binding metals and exogenous toxins, and transforming them into water-soluble compounds that can be excreted in bile or urine. Because it is the primary organ of detoxification, the liver harbors the body’s largest stores of glutathione. Like oxidative stress, unusually heavy toxic loads will increase the demand on the liver to make new glutathione. Deficiency results when tissues cannot keep pace with the demand.
A number of studies point to an association between glutathione levels and neuroognitive function. One important aspect of this line of research is that it suggests that there can be localized deficiencies of glutathione in the substantia nigra in the brain and also in muscle, though blood levels may be normal. These declines are related in part to changes in the levels of an antioxidant enzyme, glutathione peroxidase, and may contribute to neurodegeneration.

Because glutathione is found in nearly all of the body's tissues, sufficient levels are necessary to ensure optimal organ function across many physiological systems. Deficiency raises multiple health concerns (Mao Y, et al 2016; Neurobiol Dis 2018; 113: 35-42).

There are a number of causes of glutathione deficiency, including depression, stress, and chronic disease. These deficiencies have been linked to a number of health problems, including increased risk of age-related diseases, decreased immune function, and decreased cognitive function.

Mediating Exercise-Induced Damage

Intense endurance exercise produces marked changes in physiology that affect multiple organ systems. These changes can be highly detrimental over the long term (Lakie Smith L, Sports Med 2003; 33 (5): 347-354). Not only can they diminish athletic performance, they can also result in alterations of immune system function (Macklinn LT, Immunol Cell Biol 2000; 78(5): 502-509). Similarly, Kukulski et al. (Sci Sports 2007; 23 (suppl 1): 593–599). Some researchers have described this condition as "overtraining syndrome." Dr. Shigekazu Kusuda and colleagues at the University of Hawaii at Manoa showed that exercise can improve immune system function in elderly, healthy endurance athletes under intense training conditions.

The investigators randomized 16 male long-distance runners to two groups: one received the Aji-CAT® formulation (700 mg cysteine, 280 mg theanine), while the other received a placebo. The groups were matched for physical fitness levels and the duration of the training program.

The participants were housed in a dorm during the training period, and their meals and training schedule were identical. Prior to the training camp, the runners averaged 19.9 km/day, and during the camp they logged an average of 28.6 km/day. Inversely, there was a marked improvement in their endurance, with group 1 running an average of 11.6 km per day. The group 2 runners showed a similar increase in endurance, running an average of 11.6 km per day.

Both groups showed a significant increase in their physical performance, with a marked improvement in their endurance. The group 1 runners showed a marked increase in their endurance, running an average of 11.6 km per day. The group 2 runners showed a marked decrease in their endurance, running an average of 11.6 km per day.

As expected, neutrophil counts rose in both groups after the first day of training, but the increase was smaller in the Aji-CAT® group (mean increase of 200% vs. 163%). Interestingly, there was no meaningful difference between the two groups in terms of the neutrophil surge on the final day of training (149.5% vs. 154.9%). Lymphocyte counts decreased markedly in both groups, which is also an expected effect of intense exercise. Those taking Aji-CAT® did show post-exertion lymphocyte drops compared with their baseline levels, but they had consistently higher lymphocyte counts than the runners in the placebo group.

"In general, WBC count and neutrophil count are known to increase after intense exercise, while lymphocyte count is known to decrease," Dr. Murakami notes. "Neutrophils reduce lymphocyte counts which, in turn, causes a reduction in immunological function."

Cysteine and theanine appear to attenuate the negative impact of intense exercise on immunological function. The two amino acids, "may suppress oxygen reactive species (ROS) production from neutrophils, accumulation of lactate, and excessive damage through imbalanced immune response," the authors write. An experiment with athletes performing intense exercise with the breakdown of skeletal muscle. Dr. Murakami's work builds on an earlier study by Dr. Shigeki Kusuda and colleagues, who showed that Aji-CAT® supplementation could attenuate the suppression of natural killer (NK) cell activity that typically follows intense resistance exercise. They showed this in a cohort of 15 young healthy men involved in three-weekly resistance training (3x per week) for at least 6 months (Kawada et al. J Strength Cond Res. 2018; 32(3): 846-51).

A big question—one worthy of future research—is whether supplementation in the absence of chronic exercise would have any effect on athletic performance itself.

These studies cited above were not designed to address this question, but Dr. Kusuda—who was a co-author on the Murakami paper—pointed out that the reduction of inflammation seen in the previous studies also reduces the hyper-catabolism of skeletal muscle that often follows intense exercise.

"Cysteine-theanine supplementation could be helpful for recovery after exercise. It is not yet known whether the supplement has any impact on athletic performance itself." Dr. Kusuda notes.

"Immunonutrition" in Other Settings

Researchers in Japan are studying the effects of supplementation with cysteine and theanine in a variety of individuals and contexts. Among these, athletes and the elderly are the regimens in elderly nursing home residents.

Given the age-associated decline in tissue glutathione production and the fact that many elderly people do not eat well for impaired nutritional status, the use of cysteine and theanine supplementation could help improve these symptoms. In particular, the combination of cysteine and theanine has been shown to improve cognitive function in elderly individuals, and supplementation with these nutrients may help to improve memory and mental performance in older adults.

"In conclusion, the combination of cysteine and theanine supplementation could be a promising approach for improving cognitive function and reducing inflammation in elderly individuals." Dr. Kusuda notes.

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"In conclusion, the combination of cysteine and theanine supplementation could be a promising approach for improving cognitive function and reducing inflammation in elderly individuals." Dr. Kusuda notes.
A number of studies point to an association between glutathione levels and neuropsychiatric function. One important aspect of this line of research is that it suggests there can be localized deficiencies of glutathione in the substantia nigra in the brain and also in muscle, though blood levels may be normal. "This is the golden rule," Dr. Perry, "TMU, Mt. Neurosciencesellites. 1982; 120(3): 333(3): 305-10. Martin HL, Teissmann P. FASEB J. 2009, 23(10) 2012; 13(1): 333-42. However, glutathione is found in nearly all of the body's tissues. Sufficient levels are necessary to ensure optimal organ function across many physiological systems. Deficiency raises multiple health concerns (Mak P, et al. J Nut Trout (2012): 901-6). Any type of extreme physiological stress can lead to glutathione deficiency, including intense exercise (athletic performance, excessive physical work, etc.) exposure to environmental toxicants, and other extreme stressors. Ageing itself is associated with diminished glutathione levels, and elderly people with poor nutritional status are particularly at risk.

Restoring Glutathione: A Novel Approach

The good news is that the right building blocks, in the right proportions, the body will readily manufacture ample quantities of glutathione and quickly restore optimal anti-oxidative function. This is the rationale behind the new approach pioneered by researchers at the University of Co., which uses a 100-year-old Japanese company that specializes as the world's leader in amino acid based ingredients for pharmaceutical nutritional and nutraceutical applications.

Supplementation with a fixed combination of cystine and theanine in a ratio of 2.5 to 1—known commercially as "CAZT™"—has been shown to increase the endogenous production of glutathione, notes Dr. Shigekazu Kurihara, PhD, a senior researcher at Ajinomoto. Cystine consists of two molecules of cysteine (Cysteine (3)) held by a disulfide bond, while theanine is comprised of glutamate and ethylamine. Cystine and theanine are two of the three components of glutathione, the other being glycine. By providing a fixed ratio of 700 mg cystine and 280 mg theanine, the AJ-CAZT™ formula provides two of the essential building blocks of glutathione. One daily stack pack of the AJ-CAZT™ formulation provides the amount of cysteine equivalent to 5 whole eggs, and the amount of theanine equivalent to 28 cups of green tea.

It is interesting to note that glycine—the third key amino acid in glutathione—is not needed in supplemental form because there is typically a vast amount of glycine in our diets and it is produced endogenously. It is found abundantly in many foods throughout the world, and it is also a common food additive. Most people will obtain sufficient levels of glycine from their diets to meet the demands of glutathione production, given adequate amounts of cysteine and glutamine, explained Dr. Kurihara. "On the other hand, cysteine is the rate-limiting factor in cellular glutathione synthesis. This amino acid is relatively rare in our foodstuffs. Glutathione helps in the cellular synthesis of glutathione, but bear in mind that glutamine is one of the body's preferred amino acids from a nutritional point of view, so ingested glutamate tends to be rapidly absorbed and utilized. We thought that theanine, which can be metabolized to glutamate, could be a useful and efficient donor of glutamate."

There has also been published literature to show that the serum level of glutamate is increased after oral administration of theanine (Scheidlin, et al. J Nut 2012; 142(12): 2091-6)

Following a decade of promising animal studies, researchers in Japan have been testing the efficacy of the AJ-CAZT™ combination as an oral supplement in human subjects experiencing a wide variety of real-world physiological stresses. Among other important findings, these studies have shown improvements in immune function (Fig. 5A) and in mediating inflammatory response (Fig. 3B).

Mediating Exercise-Induced Damage

Intense endurance exercise produces marked changes in physiology that affect multiple organ systems. As we age, these changes can be highly detrimental over the long term (Lakier Smith L. Sports Med. 2003; 33 (5): 347-354). Not only can they diminish athletic performance, they can also result in alteration of immune system function (McKenna NN, Immunol Cell Biol. 2000; 78(5): 502-509). Nemiro M, Eklblom B.J. Sports Sci. 2007; 23 (suppl 1): 593-102). Some researchers have described this condition as "overtraining syndrome."

Dr. Shigekazu Kurihara, PhD, a senior researcher at Ajinomoto, notes that cysteine-theanine combination has been shown to increase immune system defenses in older adults. The investigators randomized 16 male long-distance runners to two groups: one received the AJ-CAZT™ formulation (700 mg cystine, 280 mg theanine), while the other received a visually identical placebo for 7 days prior, and throughout the duration of a 9-day intensive training camp program.

The participants were housed in a dorm during the training period, and their meals and training schedule were more or less the same. Prior to the training camp, the runners averaged 19.9 km/day, and during the camp they logged an average of 28.6 km/day. Investigators collected blood samples at the initial and final days of the camp, before and after morning interval workouts (1,000 m buns t x 15 times).

"It has been reported that athletes often experience overtraining syndromes where they are unable to recover physically after a certain period of intense strenuous exercise."

The associated acute and chronic immune system function may increase the susceptibility to prolonged period of fatigue and reduced physical performance, the authors note. The study participants took the assigned supplements after dinner every day. They were prohibited from using green tea (which contains theanine), or any other supplements containing amino acids, proteins, or creatine for 5 days prior to and throughout the study.

Athletes in the placebo group showed a surge in white blood cell counts after interval training on the first and last days of the training camp; no such increase was seen in those taking AJ-CAZT™ (Murakami S, et al. J Int Soc Sports Nutr. 2010; 7: 23).

As expected, neutrophil counts rose in both groups after the first day of training, but the increase was smaller in the AJ-CAZT™ group (mean increase of 20% vs 163%). Interestingly, there was no meaningful difference between the two groups in terms of the neutrophil surge on the final day of training (149.5% vs 145.3%). Lymphocyte counts decreased markedly in both groups, which is also an expected effect of intensive exercise. Those taking AJ-CAZT™ did show post-exercise lymphocyte drops compared with their baseline normal (Perry "et al. Neurosciences. 1982; 120(3): 333(3): 305-10.

"In general, WBC count and neutrophil count are known to increase after intense exercise, while lymphocyte count is known to decrease," Dr. Murakami notes. Neutrophils reduce lymphocyte counts which, in turn, causes a reduction in immunological function.

Cystine and theanine appear to attenuate the negative impact of intense exercise on immunological function. The two amino acids, "may suppress reactive oxygen species (ROS) production from neutrophil, accumulate in the lungs due to inflammation, suppressing further accumulation of neutrophils," the authors write. AJ-CAZT™ might also help athletes during periods of intense training by suppressing the breakdown of skeletal muscle.

Dr. Murakami's work builds on an earlier study by Dr. Shigeo Kawada and colleagues, who showed that AJ-CAZT™ supplementation could attenuate the suppression of natural killer (NK) cell activity—activity that typically follows intense resistance exercise. They showed this in a cohort of 15 young healthy men involved in chronic three-weekly resistance training (free weights) for at least 6 months (Kawada S, et al. J Strength Cond Res. 2010; 24(3): 846-51).

"A big question—one worthy of future research—is whether supplementation with amino acid and theanine would have any effect on athletic performance itself."

The studies cited above were not designed to address this question, but Dr. Kurihara—who was a co-author on the Murakami paper—pointed out that the reduction of inflammation seen in the previous studies also reduces the hyper-catabolism of skeletal muscle that often follows extreme exercise.

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"Immunonutrition" in Other Settings

Researchers in Japan are studying the effects of supplementation with cystine and theanine in a variety of individuals and contexts. Among these projects is a study of the regimen in elderly nursing home residents. Given the age-associated decline in tissue glutathione production and the fact that many elderly people do not eat well for have impaired metabolic function, the efficacies of the above results were confirmed in elderly nursing home residents. The response to cystine-theanine products in elderly nursing home residents was found to be positive.

In contrast, the elderly residents who did not receive cystine-theanine products showed a decrease in their immune function, as well as an increase in their metabolic function. The results suggest that cystine-theanine products may be beneficial in elderly nursing home residents.
digestion, it is likely that many such people are highly deficient in this important antioxidant. The deficiency predisposes elders to suboptimal immune function (Miyagawa K, et al. Geriatr Gerontol Int (2008); 8 (4):243–50).

In Japan, and also in many European countries, the concept of "immunonutrition"—the use of carefully targeted nutraceutical interventions to optimize immune system function—is quite advanced, says Dr. Kurihara. Use of a fixed-dose cystine-theanine combination like Aji-C&T™ to increase glutathione production is one example of this approach.

In all studies so far, Aji-C&T™ has been very well tolerated, with few adverse effects. In a 2008 early safety study involving 40 healthy men and women, daily doses of 2,100 mg cystine and 840 mg theanine showed no adverse effects, and no adverse changes in clinical laboratory parameters after four weeks (Kurihara S, et al. Seikatsu Eisen. 2008; 52(4):229–236). These doses are three times higher than what is delivered by the commercial aminoDefense™ product.

All of the Japanese study protocols advise users of Aji-C&T™ to take the supplement with a meal. The reason for this, says Dr. Kurihara, is that glycine is, like glutamate, one of the body’s preferred nutrients.

"Glycine taken in the absence of foods would most likely be shunted into "nutritive" pathways, rather than being used to increase glutathione production. If supplemental glycine is taken with food, there is more likely to be an overall surplus of glycine, providing enough to satisfy nutritional needs as well as enough to raise glutathione production."

"Strictly speaking, there could be subtle differences of physiological impact in terms of when amino acids are taken during the day," says Dr. Kurihara. "However, the most important thing for us is to take cystine-theanine combination every day in order to keep the glutathione level high, no matter when it is taken."

He noted that both cystine and theanine are absorbed mainly in the small intestine. This raises the question of how absorption—and subsequent glutathione production—would be affected by digestive system disorders or pharmaceuticals that affect digestion. This question has not yet been studied scientifically.

Ajinomoto is committed to a strong and long-term program of research on cystine-theanine supplementation. Investigators are also planning to study the direct impact on blood glutathione levels in humans soon. Dr. Kurihara says he is particularly interested in applications having to do with attenuating the side effects of surgical interventions. "We feel there are a lot of possibilities for cystine-theanine supplementation."

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