BJSM reviews: A to Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance—Part 18

P Newsholme,[1] M Krause,[2] E A Newsholme,[3]
S J Stear,[4] L M Burke,[5] L M Castell[6]

[1]UCD School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland
[2]Department of Science, Institute of Technology Tallaght, Dublin, Ireland
[3]Merton College, Oxford, UK
[4]Performance Influencers, London, UK
[5]Australian Institute of Sport, Canberra, Australia
[6]University of Oxford, Green Templeton College, Oxford, UK

Correspondence to
L M Castell, University of Oxford, Green Templeton College, Oxford OX2 6HG, UK; lindy.castell@gtc.ox.ac.uk

LMB, LMC and SJS edited this series.

Accepted 7 January 2011

INTRODUCTORY REMARKS

Part 18 extends the general amino acids overview in Part 2, by reviewing specifically glutamine and glutamate, and the tripeptide antioxidant, glutathione.

Glutamine supplementation has been well studied in both clinical and exercise situations, particularly in terms of its effects on immune function. Sports drinks containing glutamine as a free amino acid or part of a dipeptide are widely available but the low levels recommended are unlikely to help improve immune or muscle function.

The first product of glutamine metabolism, catalysed by the enzyme glutaminase, is the excitatory neurotransmitter glutamate. The latter has sometimes been used for supplementation. This may seem rather surprising, since its appearance in plasma at a high concentration correlates neurotoxicity and sometimes with clinical problems.

Glutathione, for which glutamine is a precursor via glutamate, is a powerful antioxidant and, in its reduced form, is a good marker of antioxidant capacity, while an increase in its oxidised form is a good marker of oxidative stress.

GLUTATHIONE AND GLUTAMATE

P Newsholme and M Krause

Glutathione (γ-glutamyl-cysteinyl-glycine; GSH) is the predominant low molecular weight thiol (0.5–10 mmol/l) in mammalian cells. Most GSH (85–90%) is cytosolic, with the remainder located in organelles (including mitochondria, nuclear matrix and peroxisomes).[1] This tripeptide is a key antioxidant within cells, critical to regulating the reactive oxygen species (ROS) concentration.[2] Reduced glutathione (GSH) may be used to remove damaging ROS such as H_2O_2 and convert it to harmless H_2O, generating oxidised glutathione (GSSG) via glutathione peroxidase (figure 1). Disulphide formation and gluathionylation are reversible forms of protein covalent modification dependent on glutathione and can provide mechanisms for regulation of metabolic, signalling and transcriptional processes,[3] including skeletal muscle adaptation to exercise and training.[4] The cellular redox state is crucial for molecular signalling, and glutathione is a key regulator/sensor for redox status; thus strategies aiming at increasing GSH synthesis should be beneficial to exercise performance.

Exercise, free radical production and dissipation

Exercise stimulates ROS and reactive nitrogen species (RNS) production, dependent on exercise type, duration and intensity, culminating in changes in skeletal muscle redox state.[5] ROS/RNS production and various antioxidant roles are summarised in figure 1. Excessive ROS and RNS production is associated with deleterious effects in many diseases including diabetes.[6 7] Antioxidant supplementation strategies have been assessed for their ability to decrease ROS levels and the deleterious effects of oxidative/nitrosative damage.[8] While excessive ROS and RNS can exert harmful effects within skeletal muscle during exercise, lower levels are crucial for adaptation of metabolic and signalling pathways in response to exercise. For example, redox changes are essential for the production and release of myokines such as interleukin 6 (IL-6), which, in part, optimises fuel provision for sustained activity.[9] Although antioxidant supplementation may at first be considered as beneficial, the consequent reduction of ROS/RNS could have negative effects. Muscle redox state may be best improved by providing skeletal muscle cells with key natural precursors for GSH synthesis and allowing the cells to synthesise what they actually require. Exercise-induced free radical production in skeletal muscle is not detrimental to human health; thus endogenous antioxidants may be sufficient to protect against exercise-induced oxidative damage.

Regulation of glutathione synthesis

The synthesis of GSH from glutamate, cysteine and glycine is catalysed sequentially by two key cytosolic enzymes, γ-glutamylcysteine synthetase (GCS) and GSH synthetase. The availability of these amino acids is essential for GSH synthesis (figure 2). Supplementation with cysteine precursors, such as N-acetylcysteine, increases glutathione levels.[10] However, de novo GSH synthesis depends on glutamate, which is a constituent of the GSH molecule. It also acts as an amino acid donor in serine synthesis, which can subsequently be converted to glycine. GSH is a non-allosteric feedback inhibitor of GCS but competes with glutamate; thus high intracellular glutamate concentrations will enhance GSH synthesis.[11]

In conclusion, amino acid supplementation that increases intracellular glutamate and cysteine could improve muscle GSH synthesis. Future

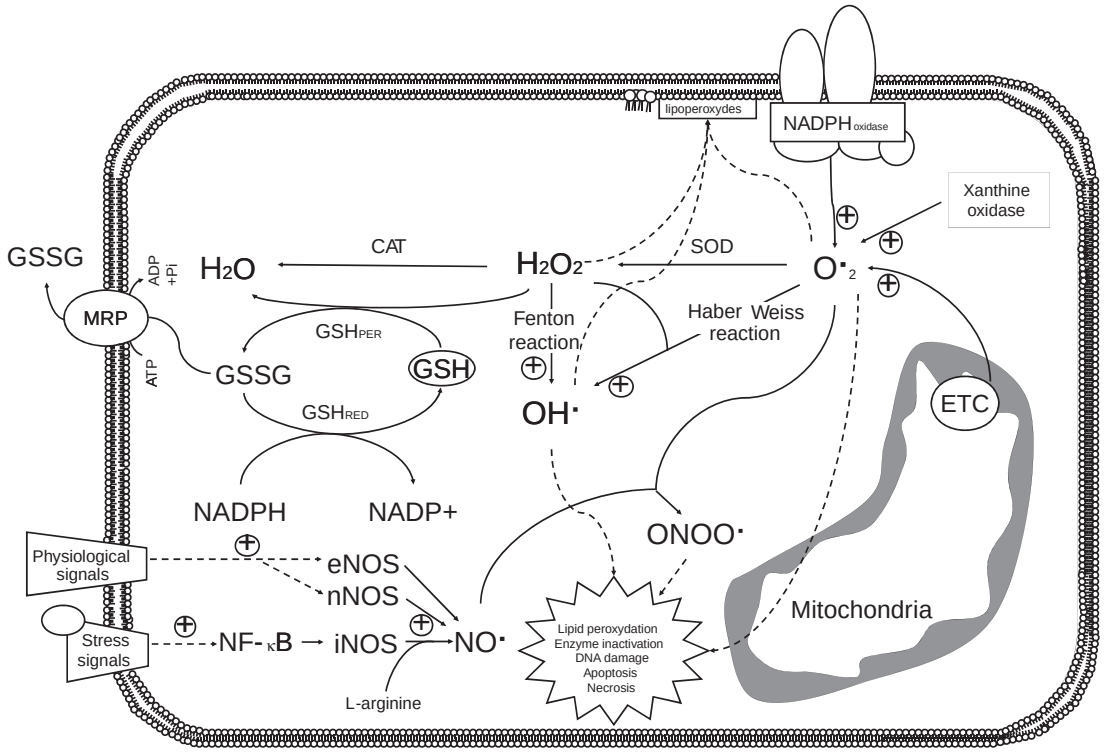

Figure 1 Reactive oxygen species (ROS)/reactive nitrogen species (RNS) synthesis and the role of endogenous antioxidants. Cells need antioxidant systems to neutralise ROS and RNS. Superoxide (O_2^-) is enzymatically converted into H_2O_2 by a manganese superoxide dismutase (Mn-SOD) within mitochondria. H_2O_2 can then be rapidly removed by the mitochondrial enzyme glutathione peroxidase (GPX). Glutathione is a tripeptide (γ-glutamyl-cysteinyl-glycine) composed of glutamate, cysteine and glycine, with the amino group of cysteine joined in peptide linkage to the γ-carboxyl group of glutamate. A further antioxidant enzyme CAT (catalase) is the major H_2O_2-detoxifying enzyme found exclusively in peroxisomes. In addition to the classic antioxidant enzymes, multidrug-resistance proteins (MRPs), such as the MRP pump (a transmembrane protein that acts by exporting intracellular glutathione disulfide, reducing accumulation and redox imbalance), are also important.[2]

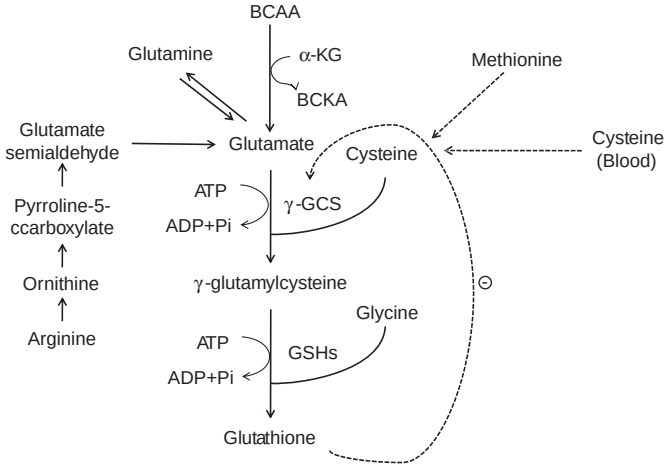

Figure 2 Glutathione synthesis and the possible amino acid candidates which may increase intracellular glutamate and glutathione (see main text for further explanations). α-KG, α-ketoglutarate; BCAA, branched chain amino acids; BCKA, branched chain keto acids; GCS, γ-glutamylcysteine synthetase; GSH, γ-glutamyl-cysteinyl-glycine.

studies need to determine which amino acids can increase intracellular glutamate and GSH synthesis in skeletal muscle without the risk of blunting essential redox changes required for exercise adaptation. Potential amino acid candidates are summarised in figure 2 and include branched chain amino acids (BCAA), arginine and glutamine.

GLUTAMINE

L M Castell, P Newsholme and E A Newsholme

Glutamine, the most abundant amino acid in the body, has recently become regarded as conditionally essential rather than non-essential. Glutamine is synthesised, stored and released predominantly by skeletal muscle: it is taken up by intestinal cells, such as enterocytes and colonocytes, by the liver and kidney and by some key immune cells. In clinical studies, the plasma concentration of glutamine (p[Gln]) is decreased in trauma and starvation: glutamine provision has been reported to have a beneficial effect on gut function, morbidity and mortality, and immune cell function. Clinical evidence suggests that glutamine provision helps recovery from surgery and maintains muscle protein mass.

The normal resting, fasting p[Gln] is 500–700 μmol/l and is often higher in athletes: the muscle concentration can reach 20 mM (60% of the intramuscular pool). During short-term strenuous exercise, p[Gln] is usually markedly increased, probably due to the release of glutamine into the circulation from skeletal muscle. However, p[Gln] is often substantially reduced by prolonged, exhaustive exercise: this decrease often occurs concomitantly with relatively transient immunodepression. Decreased p[Gln] may contribute to overtraining.[12] Glutamine supplementation after exercise reduced the self-reported incidence of illness in endurance athletes.[13] However, when glutamine was given to athletes to combat exercise-induced depletion of circulating glutamine, no effects were observed on the immune parameters studied, apart from reduced neutrocytosis and increased circulating IL-6. It remains to be

determined which other aspects of exercise-induced immunodepression might be altered by glutamine supplementation. Although the main focus of the series and this article is the ergogenic effects of supplementation, immunodepression must also be taken into account, since its elimination will allow more effective training and thus better performance.

After endurance exercise, muscle glycogen repletion is an important factor in recovery and subsequent performance. Post-exercise intake of carbohydrate provides a substrate for glycogen synthesis and also stimulates insulin secretion, which subsequently activates glucose transport and the glycogen synthase enzyme in muscle. Varnier *et al*[14] and Bowtell *et al*[15] suggested that glutamine supplementation might also promote glycogen synthesis—perhaps an indirect effect via promotion of insulin secretion? However, Marwood and Bowtell[16] found no effect of glutamine supplementation (0.125g/kg) on performance in high-intensity exercise after glycogen depletion.

Glutamine supplementation (l-alanyl-l-glutamine dipeptide, at 0.05 and 0.2 g/kg) led to a significant ergogenic benefit by increasing time to exhaustion during a mild hydration stress.[17] This ergogenic effect was thought likely to be mediated by an enhanced fluid and electrolyte uptake.

There is evidence of a role for glutamine, versus alanine, in protecting footballers against an exercise-induced increase in blood ammonia,[18] which would have an impact on fatigue. Earlier, the same group also observed that glutamine+carbohydrate reduced blood ammonia accumulation in endurance athletes.

A mixture containing vitamins and minerals, and 12 amino acids, including glutamine, was provided to improve training efficiency in athletes.[19] However, it cannot be deduced whether any one amino acid had a more specific effect than another. When BCAAs (precursors for glutamine) were given, although p[Gln] was increased, and muscle recovery was helped, the supplementation did not actually enhance athletic performance.[20]

Welbourne[21] found that acute glutamine administration (16–36 mg/kg) increased both plasma bicarbonate and growth hormone amino acid supplementation. However, glutamine administration (0.03 g/kg 90 min pre-exercise) did not improve maximum effort on a bicycle ergometer.[22] By contrast, Lehmkuhl *et al*[23] observed an enhanced initial rate of power production during cycle ergometer bouts (4 g glutamine/day), combined with creatine monohydrate, but no significant difference was observed in the combined supplement group compared with creatine monohydrate alone.

Overall, there is no consensus or unifying concept to explain the efficacy of exogenous provision of glutamine alone on performance in athletes, although in combination with carbohydrate or other amino acids, significant improvements have been reported.

CONCLUDING COMMENTS

Although there is some evidence that glutamine is effective in decreasing the self-reported incidence of upper respiratory tract illness, it has been difficult to obtain evidence of an effect on any specific aspect of the immune system. There is no doubt that it is important for the athlete to combat immunodepression, and glutamine would be particularly advantageous if it could be proved useful in this way, since it is not a banned substance. Its effects on performance per se are not convincing and, although space precludes citing every study, it is clear that more studies are needed to back up the small amount of evidence already reported.

Because of the importance of glutathione as a key regulator or sensor for redox status, increasing GSH synthesis may be beneficial to exercise performance. Thus, amino acid supplementation that increases intracellular glutamate and cysteine might improve muscle GSH synthesis.

Competing interests None.

Provenance and peer review Commissioned; not externally peer reviewed.

REFERENCES

1. **Wu G,** Fang YZ, Yang S, *et al.* Glutathione metabolism and its implications for health. *J Nutr* 2004;**134**:489–92.
2. **Krause MS,** Oliveira LP Jr, Silveira EM, *et al.* MRP1/GS-X pump ATPase expression: is this the explanation for the cytoprotection of the heart against oxidative stress-induced redox imbalance in comparison to skeletal muscle cells? *Cell Biochem Funct* 2007;**25**:23–32.
3. **Ghezzi P.** Oxidoreduction of protein thiols in redox regulation. *Biochem Soc Trans* 2005;**33**:1378–81.
4. **Ji LL.** Modulation of skeletal muscle antioxidant defense by exercise: role of redox signaling. *Free Radic Biol Med* 2008;**44**:142–52.
5. **Powers SK,** Lennon SL. Analysis of cellular responses to free radicals: focus on exercise and skeletal muscle. *Proc Nutr Soc* 1999;**58**:1025–33.
6. **Newsholme P,** Homem De Bittencourt PI, O' Hagan C, *et al.* Exercise and possible molecular mechanisms of protection from vascular disease and diabetes: the central role of ROS and nitric oxide. *Clin Sci* 2010;**118**:341–9.
7. **Newsholme P,** Haber EP, Hirabara SM, *et al.* Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. *J Physiol (Lond)* 2007;**583**:9–24.
8. **Stear SJ,** Burke LM, Castell LM. BJSM reviews: A–Z of nutritional supplements: dietary supplements, sports nutrition and ergogenic aids for health and performance Part 3. *Br J Sports Med* 2009;**43**:890–2.
9. **Pedersen BK.** IL-6 signalling in exercise and disease. *Biochem Soc Trans* 2007;**35**:1295–7.
10. **Townsend DM,** Tew KD, Tapiero H. The importance of glutathione in human disease. *Biomed Pharmacother* 2003;**57**:145–55.
11. **Griffith OW.** Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Radic Biol Med* 1999;**27**:922–35.
12. **Parry-Billings M,** Budgett R, Koutedakis Y, *et al.* Plasma amino acid concentrations in the overtraining syndrome: possible effects on the immune system. *Med Sci Sports Exerc* 1992;**24**:1353–8.
13. **Castell LM,** Poortmans JR, Newsholme EA. Does glutamine have a role in reducing infections in athletes? *Eur J Appl Physiol Occup Physiol* 1996;**73**:488–90.
14. **Varnier M,** Leese GP, Thompson J, *et al.* Stimulatory effect of glutamine on glycogen accumulation in human skeletal muscle. *Am J Physiol* 1995;**269**:E309–15.
15. **Bowtell JL,** Gelly K, Jackman ML, *et al.* Effect of oral glutamine on whole body carbohydrate storage during recovery from exhaustive exercise. *J Appl Physiol* 1999;**86**:1770–7.
16. **Marwood S,** Bowtell J. No effect of glutamine supplementation and hyperoxia on oxidative metabolism and performance during high-intensity exercise. *J Sports Sci* 2008;**26**:1081–90.
17. **Hoffman JR,** Ratamess NA, Kang J, *et al.* (2010) Examination of the efficacy of acute l-alanyl-l-glutamine ingestion during hydration stress in endurance exercise. *J Int Soc Sports Nutr* 2010;**7**:8.
18. **Bassini-Cameron A,** Monteiro A, Gomes A, *et al.* Glutamine protects against increases in blood ammonia in football players in an exercise intensity-dependent way. *Br J Sports Med* 2008;**42**:260–6.
19. **Ohtani M,** Sugita M, Maruyama K. Amino acid mixture improves training efficiency in athletes. *J Nutr* 2006;**136**:538S–43S.
20. **Negro M,** Giardina S, Marzani B, *et al.* Branched-chain amino acid supplementation does not enhance athletic performance but affects muscle recovery and the immune system. *J Sports Med Phys Fitness* 2008;**48**:347–51.
21. **Welbourne TC.** Increased plasma bicarbonate and growth hormone after an oral glutamine load. *Am J Clin Nutr* 1995;**61**:1058–61.
22. **Haub MD,** Potteiger JA, Nau KL, *et al.* Acute l-glutamine ingestion does not improve maximal effort exercise. *J Sports Med Phys Fitness* 1998;**38**:240–4.
23. **Lehmkuhl M,** Malone M, Justice B, *et al.* The effects of 8 weeks of creatine monohydrate and glutamine supplementation on body composition and performance measures. *J Strength Cond Res* 2003;**17**:425–38.

BJSM reviews: A to Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance ––Part 18

P Newsholme, M Krause, E A Newsholme, et al.

Br J Sports Med 2011 45: 230-232
doi: 10.1136/bjsm.2010.080978