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Glutamine, Exercise and Immune Function

Links and Possible Mechanisms

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Abstract

Glutamine is the most abundant free amino acid in human muscle and plasma and is utilised at high rates by rapidly dividing cells, including leucocytes, to provide energy and optimal conditions for nucleotide biosynthesis. As such, it is considered to be essential for proper immune function.

During various catabolic states including surgical trauma, infection, starvation and prolonged exercise, glutamine homeostasis is placed under stress. Falls in the plasma glutamine level (normal range 500 to 750 μmol/L after an overnight fast) have been reported following endurance events and prolonged exercise. These levels remain unchanged or temporarily elevated after short term, high intensity exercise. Plasma glutamine has also been reported to fall in patients with untreated diabetes mellitus, in diet-induced metabolic acidosis and in the recovery period following high intensity intermittent exercise. Common factors among all these stress states are rises in the plasma concentrations of cortisol and glucagon and an increased tissue requirement for glutamine for gluconeogenesis. It is suggested that increased gluconeogenesis and associated increases in hepatic, gut and renal glutamine uptake account for the depletion of plasma glutamine in catabolic stress states, including prolonged exercise.

The short term effects of exercise on the plasma glutamine level may be

cumulative, since heavy training has been shown to result in low plasma glutamine levels (<500 μmol/L) requiring long periods of recovery. Furthermore, athletes experiencing discomfort from the overtraining syndrome exhibit lower resting levels of plasma glutamine than active healthy controls. Therefore, physical activity directly affects the availability of glutamine to the leucocytes and thus may influence immune function. The utility of plasma glutamine level as a marker of overtraining has recently been highlighted, but a consensus has not yet been reached concerning the best method of determining the level.

Since injury, infection, nutritional status and acute exercise can all influence plasma glutamine level, these factors must be controlled and/or taken into consideration if plasma glutamine is to prove a useful marker of impending overtraining.

Epidemiological studies of the incidence of common infections in athletes and nonathletes have shown an effect of long term exercise on susceptibility to infection. Athletes engaged in heavy training programmes, particularly those involved in endurance events, appear to be more susceptible to infection.[1] Furthermore, the odds of getting a cold during the winter are 6 times higher in regular runners than in nonparticipants.[2] Laboratory-based experiments have implicated a suppression of immune function as being at least partly responsible for the increased incidence of infection in athletes. Although most reports of exercise-induced immune system disruption come from long distance runners, participants in many different forms of exercise appear to exhibit altered immune function, including skiers, swimmers, cyclists and ballet dancers.[3] Short term responses to prolonged bouts of strenuous exercise may also be important, since 13% of participants in the Los Angeles marathon fell sick in the week after the race, compared with 2% of control runners who did not participate.[4]

In contrast, low to moderate intensities of exercise have been shown to be of likely benefit to proper immune function compared with the sedentary state. Nehlsen-Cannarella et al.[5] estimated that five 45-minute walks per week for 15 weeks reduced the duration of infections by 50% in elderly women. Low intensity exercise enhances the lymphocyte response to mitogenic stimulation *in vitro* and increases the number of natural killer cells.[6] A biphasic leucocytosis is associated with short term continuous high intensity exercise: the number of circulating leucocytes increases during exercise and rapidly decreases during the first hour of recovery. This is followed by a second delayed leucocytosis which peaks 2 to 3 hours after stopping exercise.[7] This delayed leucocytosis is predominantly due to an elevation of circulating neutrophils.[7,8] These effects would be expected to enhance immune function, according to Nieman.[9]

With high intensity exercise the obverse is true; the deleterious effects of exercise on immune function at intensities above 70% $\dot{V}O_{2max}$ and for prolonged durations have been well documented.[1,10-14] Furthermore, the composition of different lymphocyte subpopulations changes with high intensity exercise. The ratio of CD4 (T-helper) : CD8 (T-suppressor/cytotoxic) cells was reported to decrease following a bout of maximal exercise.[15] A 40% suppression in the lymphocyte response to mitogenic stimulation *in vitro* has been reported in the first 30 minutes after a marathon race, with incomplete recovery at 3 hours and persisting hyporesponsiveness 24 hours later.[6,16]

Attenuated antibody synthesis and blood and salivary immunoglobulin concentrations[17] have been demonstrated following high intensity exercise. Bosenberg et al.[18] and Israel et al.[19] found falls in serum immunoglobulin (Ig) G after a triathlon and 10 to 28% falls in serum IgG, IgM and IgA following 45- or 75km runs. More recently, Blannin and associates[10] have shown changes in both exercise and postexercise leucocyte counts as a function of exercise intensity. Neutrophil func-

tion as measured by elastase release (degranulation) in response to stimulation by bacterial lipopolysaccharides was reduced during recovery from exercise at 70% $\dot{V}O_{2max}$.[20]

The aetiology and mechanisms of immunosuppression have remained elusive, although numerous training-related indices are commonly cited as contributing factors. These include an increased training volume, training intensity and inadequate recovery between exercise bouts.[21-23] Although chronic elevation of stress hormones (e.g. cortisol) may provide an explanation for the immunosuppression associated with heavy training loads, falls in the plasma glutamine level have been suggested as a possible mechanism for immune system disruption. Although glutamine can be synthesised in the body, and is thus not an essential dietary amino acid, it is required for a variety of immune cell functions including lymphocyte proliferation and macrophage phagocytosis.

Parry-Billings et al.[24] have postulated that regular exercise of high intensity and long duration is responsible for a sustained decrease in the plasma glutamine level and that this provides the simplest explanation for the immunosuppressive effects of exercise. Indeed, lower plasma glutamine levels have been reported in overtrained compared with healthy trained athletes.[25,26] Overtraining is defined as a stress response to excessive training characterised by poor performance, prolonged fatigue and alterations in mood state.[27] Overtraining is also commonly associated with an increased frequency of opportunistic infections. Other forms of stress including surgery, burns, sepsis and trauma have been associated with reduced plasma glutamine and increased susceptibility to infection.[27] The effects of exercise on plasma glutamine level are discussed in this review, as are the possible uses of glutamine as a marker of exercise stress and overtraining in athletes.

1. Glutamine and Immune Function

The neutral amino acid glutamine is the most abundant amino acid in human muscle and plasma. In humans, after an overnight fast the normal plasma level is between 500 and 750 μmol/L and is dependent on the net balance between the release and uptake of glutamine by organs and tissues.[28] In a recent review article, Rowbottom and associates[29] highlighted the numerous roles that glutamine may fulfil in the body. Glutamine is involved in the transfer of nitrogen between organs and the detoxification of ammonia,[30] the maintenance of the acid-base balance during acidosis,[31] acts as a nitrogen precursor for the synthesis of nucleotides,[32] regulates protein synthesis and degradation[33] and, finally, is a fuel for gut mucosal cells and cells of the immune system.[29]

Regarding this last role, it is known that glutamine is utilised at a very high rate by lymphocytes[34] and macrophages.[35] The importance of glutamine is attributed to the provision of energy through its partial oxidation in a process known as glutaminolysis, and also to its provision of carbon and nitrogen for precursors of RNA, DNA and protein synthesis.[36] In regulating the biosynthesis of pyrimidine and purine nucleotides, glutamine availability may serve to control some important aspects of immune function. Indeed, the role of glutamine as a precursor for purine and pyrimidine synthesis is essential for lymphocytes and other immune cells to replicate. It is the requirement of glutamine for both energy provision and nucleotide synthesis in immune cells that has led Parry-Billings and colleagues[24,37,38] to hypothesise that a fall in plasma glutamine level below about 600 μmol/L will have deleterious effects on immune function. The normal range in apparently healthy resting humans is 500 to 750 μmol/L but values as low as 200 μmol/L have been reported in pathological states such as those caused by burns and sepsis.[25]

The rates of proliferation of T and B lymphocytes are dependent on glutamine as are rates of protein synthesis, RNA synthesis, interleukin-2 (IL-2) production and antibody synthesis.[24,39] These events – essential to the normal effective response of the immune system to invading pathogens – appear to be sensitive to the glutamine level present in the surrounding medium.

Parry-Billings et al.[25] have shown that reductions in glutamine level (*in vitro*) below 600 μmol/L are associated with reduced RNA synthesis, IL-2 production, immunoglobulin synthesis and proliferative responses to mitogens in lymphocytes, and a decreased rate of phagocytosis in macrophages. These authors incubated blood samples with specific concentrations of glutamine in culture for between 24 and 48 hours. Both Wagenmakers[40] and Gleeson and Bishop[41] recommend caution when interpreting these findings as it is likely that glutamine concentrations will have dropped over the 24 to 48 hours required to obtain a significant lymphocyte proliferative and other responses, due to utilisation of glutamine by the lymphocytes as an energy source or in the biosynthesis of nucleotides. Glutamine concentration in culture was monitored neither during, nor at the end of, the experiments. The validity of these results is questionable as the concentrations of glutamine may have fallen below physiological levels, or to zero, an occurrence which does not happen in the body. Gleeson and Bishop[41] further add that these experiments may have effectively become a measure of glutamine utilisation.

More recent studies on the glutamine requirements of leucocytes indicate that transcription of early activation markers in lymphocytes occurs even in the absence of glutamine, but that later events (including lymphocyte proliferation and lymphokine-activated killer cell activity) depend on the provision of exogenous glutamine in a dose-dependent manner.[42,43] However, addition of glutamine *in vitro* did not normalise the attenuated lymphocyte proliferation seen during exercise for 1 hour at 75% $\dot{V}O_{2max}$.[43]

Wallace and Keast[36] have shown that a minimum glutamine level of 125 μmol/L is required for a significant increase in macrophage phagocytosis of opsonised sheep erythrocytes. A minimum of glutamine concentration of 30 μmol/L was required for the induction of significant levels of IL-1 by lipopolysaccharide-stimulated macrophages.

The provision of glutamine-supplemented total parenteral nutrition to severely ill surgical patients improves T lymphocyte mitogenic responses compared with those in control patients receiving total parenteral nutrition.[44]

These findings provide some (albeit controversial) evidence that falls in the plasma glutamine level associated with catabolic stress states such as surgery, burns, sepsis, trauma, prolonged exercise and possibly overtraining may be at least partly responsible for the associated impairment of immune function. Indeed, a decrease in the plasma glutamine level *in vivo* has been shown to result in immunosuppression in mice.[45]

Although glutamine may exert its immunological effects by a direct action on cells of the immune system, it is also possible that it may have an indirect effect by maintaining gut barrier function, or the preservation of the antioxidant glutathione.[44]

2. Glutamine Metabolism

2.1 Enzymic Control of Glutamine Production and Catabolism

Many cells known to require glutamine lack the ability to synthesise it, and must therefore obtain it from their surroundings. Glutamine synthetase catalyses the synthesis of glutamine from ammonia and glutamate, and glutaminase catalyses the hydrolysis of glutamine to glutamate and ammonia.[30,32] The direction and rate of flux through the substrate cycle is tissue-dependent, as different tissues vary considerably in the forward and reverse rates of the reaction. Therefore, the net direction of the reaction will determine whether a particular tissue is a net consumer or producer of glutamine. Those organs considered to be involved in the synthesis of glutamine include primarily skeletal muscle, the lungs, liver, brain and possibly adipose tissue.[29] The main consumers appear to be the kidneys, gut and cells of the immune system.[28-30,32] Under certain conditions (e.g. reduced carbohydrate availability) the liver may also become a net consumer of glutamine. The largest store of glutamine is found in skeletal muscle; this tissue lacks the enzyme glutaminase.

2.2 Branched Chain Amino Acids and Glutamine Synthesis

Of the total amount of amino acids metabolised following a protein containing meal, most will be metabolised by the liver. However, the branched chain amino acids (BCAA) largely escape hepatic uptake due to the fact that the liver possesses a very low BCAA aminotransferase activity. These BCAA (namely isoleucine, leucine and valine) are primarily metabolised by skeletal muscle.[32] Alanine and especially glutamine are released by human skeletal muscle in exchange for the uptake and subsequent deamination of BCAA.[46] The first step in BCAA metabolism involves the reversible transfer of the α-amino group to an oxo-acid. The α-amino group from a BCAA is donated to 2-oxoglutarate to form glutamate and branched chain oxo-acids (BCOA), catalysed by BCAA aminotransferase. Glutamate can then combine with other oxo-acids to reform amino acids; it is commonly combined with oxaloacetate to form aspartate and 2-oxoglutarate.[47]

Intramuscular glutamate is central in the transamination reactions. It can be derived from the plasma, from intramuscular protein catabolism, and from the transamination of the BCAA. Glutamate may donate its amino group to pyruvate to form alanine and regenerate 2-oxoglutarate. Alternatively, glutamate can be a source of ammonia (NH_3) via glutamate dehydrogenase where oxidative deamination forms NH_3 and 2-oxoglutarate. In the reaction catalysed by glutamine synthetase, glutamate reacts subsequently with NH_3 to give glutamine. Thus, during exercise the formation and export of alanine and glutamine from active muscle helps to prevent a large rise in free NH_3 concentration which would otherwise occur due to increased rates of NH_3 generation from the deamination of amino acids and adenosine monophosphate (AMP).[48] The latter is converted to inosine monophosphate (IMP) with the liberation of free NH_3 by the action of AMP deaminase which becomes activated in very high intensity exercise.

The carbon skeletons for the synthesis of alanine are probably derived from muscle glycogen or glucose taken up from the circulation. The carbon skeletons for the synthesis of glutamine are mostly derived from muscle glycogen or glucose and also from conversion of isoleucine and valine to tricarboxylic acid (TCA) cycle intermediates.[49] This link between carbohydrate metabolism and alanine/glutamine formation has important implications for postexercise plasma glutamine levels.

2.3 Glutamine in Skeletal Muscle

Glutamine is the most abundant free amino acid in the human body, comprising 50 to 60% of the total free amino acid pool of skeletal muscle;[46] human skeletal muscle contains about 20 mmol/kg wet weight.[50] The rate of glutamine synthesis in human skeletal muscle is higher than that for any other amino acid and in the fed state is approximately 50 mmol/h. To maintain plasma glutamine homeostasis and glutamine stores in muscle, this high synthetic rate is essential. Newsholme and Leech[30] and Parry-Billings et al.[24] speculate that skeletal muscle provides the majority of glutamine required by other tissues.

Both the synthesis and transport of glutamine are known to be influenced by the glucocorticoids. For example, muscle glutamine synthetase activity is increased following glucocorticoid treatment.[51] This elevated glutamine synthetase activity is often reported during catabolic states.[52] The glucocorticoids may also increase efflux of glutamine from skeletal muscle,[24] decrease intracellular glutamine stores and alter transport kinetics, allowing maximal glutamine efflux at lower intracellular glutamine levels. Such influences would ensure an increased release of glutamine from muscle during catabolic states.[29] During exercise, muscle glutamine level has been reported to fall by 34%.[50] A similar fall (37%) has been reported in 24-hour fasted rats after 30 minutes' swimming.[53] The same authors observed a nonsignificant fall of 14% in fed rats forced to swim for the same duration. Whether the increased efflux of glutamine during catabolic states satisfies the accompanying increased demand for glutamine by other tissues is unknown.

3. Glutamine and Exercise

It has been suggested that skeletal muscle plays an integral part in proper immune function,[24] as failure of the muscle to provide sufficient glutamine could result in an impairment of the function of the immune system. As muscular activity may sometimes influence the rate of release of glutamine, exercise could directly influence the immune system. The important role of skeletal muscle in glutamine metabolism and immune function has prompted investigation into the effects of muscular work on plasma glutamine levels both during and after exercise.

3.1 Plasma and Tissue Glutamine Changes During Acute Exercise and Recovery from Exercise

The effects of exercise on glutamine metabolism are not well established. A lack of consensus exists in the literature concerning the effects of exercise on the plasma glutamine level. Reasons for equivocal data include the intensity and duration of exercise used, the nutritional status of the individuals involved, and differences in measurement technique, blood sampling times and sample storage.

A number of studies have shown an increase in plasma glutamine level following brief (<1 hour) high intensity exercise in humans[54-57] (table I). For example, Babij et al.[54] observed increases from 575 μmol/L at rest to 734 μmol/L during exercise at 100% $\dot{V}O_{2max}$, and Eriksson et al.[55] found plasma glutamine level increased from 538 to 666 μmol/L during 45 minutes of incremental exercise at 80% $\dot{V}O_{2max}$. These findings were supported by Katz et al.,[60] who reported elevation of plasma glutamine from 555 to 699 μmol/L following 4 minutes of exercise at 100% $\dot{V}O_{2max}$. Following treadmill running to exhaustion at 20 km/h, a significant increase in plasma glutamine level from 662 to 757 μmol/L was reported by Sewell et al.[58] Hood and Terjung[67] claim such increases in plasma glutamine level during exercise suggest that glutamate acts as a sink for NH_3 in the formation of glutamine during enhanced NH_3 production in exercise. During brief fatiguing high intensity exercise it is likely that most of the increased ammonia production arises from increased breakdown of adenine nucleotides.[48,58] Haemoconcentration is also an important factor in the rise of plasma glutamine level during high intensity exercise.[58]

In contrast to high intensity exercise, there is a consistent body of evidence that plasma glutamine level falls substantially during and/or after very prolonged exercise (table I). Rennie et al.[50] monitored plasma glutamine for 4.5 hours following 3.75 hours of cycling at 50% $\dot{V}O_{2max}$. A fall from 557 μmol/L at rest to 470 μmol/L immediately after exercise was reported. After 2 hours' recovery, plasma glutamine level had fallen to a nadir of 391 μmol/L. After 4.5 hours of recovery the figure was 482 μmol/L. Parry-Billings et al.[25] reported significant falls in plasma glutamine level following a marathon race from 592 μmol/L (pre-race) to 495 μmol/L (post-race) in 24 club standard athletes. However, both a 30km treadmill run and a cycle ride to exhaustion at 73% $\dot{V}O_{2max}$ had no effect on plasma glutamine level during exercise. Following a triathlon in which 8 volunteers swam 2.5km, cycled 81km and ran 19km, mean serum glutamine level declined from 468 μmol/L (prerace) to 318 μmol/L at 2 hours after the race.[66]

The majority of research supports a postexercise fall in plasma glutamine following very long duration protocols.[26,45,58,66,67] In a recent experiment conducted in our laboratory, continuous cycling at 55% $\dot{V}O_{2max}$ for 3 hours in 18 healthy men led to a 23% fall in plasma glutamine 1 hour after exercise (580 μmol/L pre-exercise compared with 447 μmol/L after 1 hour's recovery). However, continuous cycling to exhaustion at 80% $\dot{V}O_{2max}$ (which occurred within 1 hour) in the same group of volunteers did not alter the plasma glutamine level compared with the pre-exercise value (fig. 1).[59]

It is important to note that the plasma glutamine level at any one time reflects a net balance between release and utilisation of glutamine by various organs and body tissues. Therefore, glutamine levels

Table I. Plasma glutamine concentration following exercise in humans

Reference	Study participants	Exercise intensity/duration	Methodology	Change in plasma glutamine level[a] (%)	
				immediately after exercise	during recovery
Continuous or intermittent high intensity exercise					
Parry-Billings et al.[25]	10 RA	10 × 6 sec treadmill sprint	Enzymatic (A)	↑11	
Sewell et al.[58]	9 RA (2 F)	60 sec treadmill running at 20 km/h	Enzymatic (G)	↑5	Return to baseline at 5 min after
		20 km/h treadmill run to exhaustion		↑14	
Robson et al.[59]	18 T M	Cycling to exhaustion at 80% $\dot{V}O_{2max}$	Enzymatic (G)	↑3	↑2 at 5h
Katz et al. [60]	8 RA M	Cycling to exhaustion at 100% $\dot{V}O_{2max}$	HPLC	↑26	↑13 at 10 min
van Hall et al.[61]	8 T M	3 min cycling at 50% W_{max} and 6 min at 80% W_{max} alternated to exhaustion	Enzymatic (–)	↓9	↓16 at 2h
van der Schoor et al.[62]	8 T M	2 min cycling at 90% W_{max} alternated with 2 min at 50% W_{max} until exhaustion	Enzymatic (–)	↓20	↓24 at 2h
Keast et al.[63]	7 T M	15 × 1 min treadmill exercise at 90% $\dot{V}O_{2max}$	Bioassay	↓44	
		15 × 1 min treadmill exercise at 120% $\dot{V}O_{2max}$		↓55	
Walsh et al.[64]	8 T M	20 × 1 min cycling at 100% $\dot{V}O_{2max}$	Enzymatic (G)	↓2	↓16 at 5h
Prolonged light-moderate intensity exercise					
Parry-Billings et al.[25]	22 T (20 M)	Marathon ≈150 min	Enzymatic (A)	↓16	
	12 T M	30km self-paced treadmill run		↑8	
	4 T M	Cycling to exhaustion at 73% $\dot{V}O_{2max}$		↑8	
Rennie et al.[50]	4 RA M	3.75h cycling at 50% $\dot{V}O_{2max}$	HPLC	↓16	↓30 at 2h
Babij et al.[54]	8 RA M	10 min incremental cycling at 25, 50 and 75% $\dot{V}O_{2max}$ and to exhaustion	Column chromatography	↑28	Returned to baseline at 10 min
Eriksson et al.[55]	11 RA M	45 min incremental cycling to 75% $\dot{V}O_{2max}$	HPLC	↑24	↑12 at 1h
Maughan & Gleeson[56]	5 RA M	90 min cycling at 70% $\dot{V}O_{2max}$	Enzymatic (G)	↑3	
Robson et al.[59]	18 T M	Cycling at 55% $\dot{V}O_{2max}$ for 180 min	Enzymatic (G)	↓11	↓23 at 1h
Decombaz et al.[65]	8 T M	100km run	Column chromatography	↓16	↓7 at 24h
Rohde et al.[66]	8 T M	2500m swim, 81km cycle, 19km run	HPLC	↓20	↓32 at 2h

a Percentage difference from pre-exercise plasma glutamine level (not corrected for plasma volume changes).

A = asparaginase used to determine plasma glutamine; **F** = female; **G** = glutaminase; **HPLC** = high performance liquid chromatography; **M** = male; **RA** = recreationally active but not specifically endurance trained; **T** = trained; $\mathbf{W_{max}}$ = maximal work rate attained during an incremental exercise test; ↑ = increased; ↓ = decreased.

in other tissues and rates of uptake and release must also be considered during and following exercise to fully understand exercise-induced changes in the plasma concentration. Sahlin et al.[57] have shown a 2-fold increase in the release of glutamine from leg muscles during cycling at 75% $\dot{V}O_{2max}$. Earlier data from Rennie et al.[50] support the claim that muscle glutamine release is accelerated during prolonged exercise, where muscle glutamine level fell from 21.6 to 14.3 mmol/kg wet weight following 3.75 hours of cycle exercise at 50% $\dot{V}O_{2max}$. Two other studies have reported that human muscle glutamine levels fall during exercise,[28,68] but there are also reports of an increase[69] or no change.[55] At the onset of exercise a substantial proportion of the intramuscular glutamate pool is transaminated to yield 2-oxoglutarate. Other amino acids (e.g. isoleucine, valine, aspartate) are

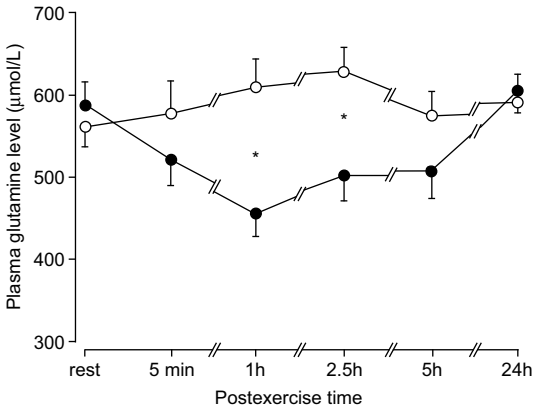

Fig. 1. Changes in plasma glutamine concentration in 18 healthy male volunteers after 3 hours' cycling at 55% $\dot{V}O_{2max}$ (closed symbols) and after exercise to exhaustion at 80% $\dot{V}O_{2max}$ (open symbols). The mean (± SEM) endurance time at 80% $\dot{V}O_{2max}$ was 38 ± 9 minutes. * $p < 0.05$ between exercise intensities.[59]

also transaminated to increase the intracellular concentration of TCA cycle intermediates and carboxylation of pyruvate may also play an important role. This is required to maintain a high rate of flux through this important pathway of energy metabolism.

A 43% fall in liver glutamine level during exercise was reported by Dohm et al.[68] when the concentrations of all other amino acids were elevated. After prolonged swimming in trained rats, falls in the muscle glutamine level from 4.15 to 3.13 mmol/kg wet weight were observed together with a fall in liver glutamine from 7.05 to 2.61 mmol/kg wet weight.[53]

Keast and colleagues[63] have investigated the effect of interval exercise on the plasma glutamine level. 20 bouts of treadmill running for 1 minute (90 to 120% $\dot{V}O_{2max}$), each separated by 2 minutes' recovery, led to a drastic reduction in plasma glutamine level at 5 minutes *post* exercise. From a mean fasted plasma glutamine level of 1244 μmol/L at rest, mean levels of 702 μmol/L and 560 μmol/L were recorded after the exercise protocol at 90 and 120% $\dot{V}O_{2max}$, respectively.[63] The very high plasma glutamine values reported by these authors raise a question over the validity of the method used to measure glutamine level. In a similar experimental protocol performed in our laboratory, 20 bouts of cycle exercise for 1 minute at 100% $\dot{V}O_{2max}$ separated by 2 minutes' recovery at 30% $\dot{V}O_{2max}$ did not affect plasma glutamine level at 5 minutes *post* exercise (681 μmol/L before compared with 663 μmol/L after exercise). However, a fall in plasma glutamine to 572 μmol/L was reported 5 hours after exercise.[64]

3.2 Why Does Plasma Glutamine Level Fall After Very Prolonged Exercise?

The fall in plasma glutamine level observed following very prolonged exercise may result from an increased demand and uptake of glutamine by tissues of the body that require it. Alternatively, it could be caused by a decreased production and/or altered transport kinetics of this amino acid, resulting in diminished release of glutamine by muscle. It also follows that the fall in plasma glutamine could be due to a combination of increased uptake of glutamine and decreased production/altered transport kinetics.

In rat muscle, glutamine is transported by a stereospecific saturable mechanism (system N^m) that is faster than any other amino acid transport mechanism. The transport kinetics have been studied by Rennie et al.[70] and revealed a maximum velocity (V_{max}) of 333 μmol/kg/min for alanine uptake and 1156 μmol/kg/min for glutamine uptake. System N^m has by far the highest capacity for glutamine transport of any of the muscle amino acid transport systems, and is sodium (Na^+) dependent.[71] Glutamine transport depends upon the net electrochemical potentials of the amino acid plus Na^+. Such is this system that, whenever intracellular Na^+ concentration rises, glutamine efflux from muscle increases. This Na^+ dependence of glutamine transport could have implications for glutamine turnover and whole-body nitrogen metabolism.[70] Intracellular Na^+ concentrations in muscle have been shown to increase during injury, sepsis and infection. Furthermore, intracellular Na^+ concentrations may be elevated by the action of corticosteroids. Over several hours, this could produce a substantial fall in the muscle glutamine level.

Prolonged exercise is known to cause an elevation in plasma cortisol concentration which stimulates not only protein catabolism and glutamine release but also hepatic, gastrointestinal and renal gluconeogenesis.[72,73] The latter mechanism may explain the observed fall in plasma glutamine during and after very prolonged moderate intensity exercise. As liver glycogen becomes depleted and blood glucose concentration starts to fall, an increased rate of gluconeogenesis in the liver – primarily from blood-borne precursors (namely glutamine, alanine and glycerol) – could place a significant drain on plasma glutamine availability. Recent work by Nurjhan et al.[74] indicates that in humans glutamine is a major gluconeogenic precursor and that overall, carbon transfer to glucose from glutamine is similar to that from alanine. During prolonged exercise, plasma levels of glucagon, growth hormone and cortisol rise.[75] Glucagon[76] and cortisol[77] increase uptake of glutamine (and other amino acids) by the liver, allowing increased utilisation of glutamine for gluconeogenesis and acute-phase protein synthesis. Growth hormone stimulates glutamine uptake by the gut and kidneys[78] and in surgical patients this effect occurs despite reduced muscle and whole-body glutamine release.

Both starvation and endotoxaemia have been shown to increase hepatic uptake of glutamine in rats.[79] Similar changes in plasma hormones seem to occur after starvation, surgical trauma and prolonged exercise and all of these states of catabolic stress are characterised by plasma glutamine depletion, immunosuppression and increased gluconeogenesis.[12,14,24,32,42,72,74,80]

Wagenmakers et al.[80] have suggested an alternative explanation for falls in plasma glutamine levels, whereby glutamine synthesis is depressed. Early in exercise the BCAA aminotransferase reaction is counteracted by anaplerotic conversion of pyruvate (derived from glycolysis) into TCA cycle intermediates. However, when the glycogen stores are depleted the rate of glutamine synthesis will decrease while the rate of draining of this amino acid will increase, due to gradual activation of the BCAA transferase complex. Subsequently, the concentration of TCA intermediates will fall, a proposed mechanism of fatigue, possibly reducing flux through the TCA cycle.[81] The fall in TCA intermediates may lead to a reduction in the glutamine synthesis rate and an increased production of ammonia.

This is supported by Broberg and Sahlin[82] who have shown that muscle NH_3 efflux was increased during a prolonged exercise bout when exercise was begun in a glycogen-depleted state. The administration of a carbohydrate drink during exercise reduced plasma NH_3 levels compared with placebo.[80]

It was hypothesised that a reduction in glycogen or blood glucose could therefore be responsible for the fall in plasma glutamine during recovery. Subsequently, van Hall et al.[61] investigated the effect of consumption of a carbohydrate drink during exercise on postexercise plasma glutamine level. Volunteers performed repeated bouts of 3 minutes' cycling at 50% peak power output (PPO) followed by 6 minutes at 80% PPO until exhaustion. An 8.3% w/v carbohydrate solution (2 ml/kg bodyweight) was administered at 15-minute intervals. Although plasma glutamine fell by 21% in the second hour of recovery, carbohydrate ingestion did not prevent this fall. Thus, it was concluded that carbohydrate ingestion during exercise cannot prevent the fall in plasma glutamine during recovery. However, Gleeson and Bishop[41] have shown that consuming a diet low in carbohydrate for 3 days leads to larger falls in plasma glutamine levels during recovery from 60 minutes' cycling at 70% $\dot{V}O_{2max}$ compared with the same exercise after a normal diet.

Studies in rats also indicate that pre-exercise carbohydrate availability is an important factor, since 24-hour fasted animals exhibited a much greater fall in plasma glutamine after 30 minutes' swimming compared with the same exercise performed in the fed state.[53] Apparently, patients with untreated type 1 (insulin-dependent) diabetes mellitus exhibit abnormally low plasma glutamine levels,[83] but to our knowledge there are no reports

on the effects of exercise on plasma glutamine level in patients with diabetes.

Mackinnon and Hooper[27] have hypothesised that increased glutamine uptake by activated immune cells may be responsible for exercise-induced falls in the plasma concentration of this amino acid. By monitoring the numbers of lymphocytes displaying activation markers, Fry et al.[84] have shown increased activation of immune cells during 10 days of intensive running training. This period of training was associated with a reduction in plasma glutamine level.[63] Thus, activated immune cells, increased gluconeogenesis, altered transport kinetics and depressed glutamine synthesis could all explain lowered plasma glutamine following exercise.

The postexercise falls in plasma glutamine observed following endurance and interval-type exercise may also be due to an increased uptake of glutamine by the kidneys in an attempt to buffer metabolic acidosis. Acidosis during and after exercise can arise from increased lactic acid production (though this is usually short-lived and rapidly cleared from the circulation) and from a more gradual but sustained accumulation of other organic acids including free fatty acids, acetoacetate and 3-hydroxybutyrate. Ammonia production in the kidneys and its secretion into the distal tubules together with excretion of excess protons in the urine protects against acidosis. By the action of glutaminase, glutamine is hydrolysed to glutamate in the distal tubules, producing NH_3 which can be combined with a hydrogen ion to form ammonium ions.

In conditions of metabolic acidosis the renal uptake of glutamine has been shown to increase to provide for ammoniagenesis.[31] Hems[85] and Newsholme et al.[86] agree that during acidosis the kidney is the major organ of glutamine utilisation. During chronic acidosis there is an adaptive increase in kidney glutaminase activity and a 4-fold rise in urinary ammonia excretion.[87] Greenhaff et al.[88] reported that diet-induced metabolic acidosis with a high-protein (24%), high-fat (72%) diet for 4 days led to an ≈25% reduction in both plasma and muscle glutamine levels. These authors proposed that muscle glutamine release may have increased along with renal uptake in an attempt to maintain acid-base balance.

In conclusion, the raised plasma glutamine level during exercise[57] and immediately following acute high intensity exercise[25,58,60] may be explained by haemoconcentration, increased ammoniagenesis from adenine nucleotide breakdown and the effects of cortisol on muscle Na^+-dependent glutamine transport. However, during recovery from prolonged exercise and exercise of an intermittent nature, the observed falls in plasma glutamine level suggest an increased uptake by other tissues (e.g. liver, kidneys or leucocytes) that is outstripping the rate of release of glutamine from muscle at this time. Rowbottom and colleagues[29] have suggested that, of the potential explanations for the postexercise fall in plasma glutamine, increased renal uptake of glutamine to maintain acid-base balance is the most likely. This is based on their findings that exercise intensity is positively and linearly related to the magnitude of the postexercise fall in plasma glutamine level.[63] However, other authors have not been able to confirm this, which may be due to differences in the methods used to measure the level. We suggest that a common mechanism may be responsible for depletion of plasma glutamine after prolonged exercise, starvation and physical trauma: namely increased hepatic and gastrointestinal uptake of glutamine for gluconeogenesis at a time when muscle release of glutamine remains constant or falls.

4. Glutamine Measurement

Glutamine is unstable in acid media; therefore, the usual storage of blood or plasma for metabolite analysis as perchloric acid supernatants cannot be applied. Keast et al.[63] have described a bioassay using a strain of *Escherichia coli* that is dependent on glutamine for replication, but which appears to give glutamine values that are double the plasma levels found by standard enzymatic spectrophotometric techniques. A bioassay method may not be valid for studies investigating changes in plasma glutamine level during and after exercise as other

factors may change in the blood plasma during exercise that could influence the growth of bacteria in culture.[41]

Plasma glutamine can be determined by measurement of the free ammonia (or glutamate) concentration in plasma before and after treatment of the plasma with the enzyme glutaminase (which breaks down glutamine into glutamate and ammonia).[64,89] Another enzymatic spectrophotometric method has been described using asparaginase.[38] High performance liquid chromatography (HPLC) has also been used to determine plasma glutamine,[50,54] giving values similar to those using the above enzymatic spectrophotometric procedures. However, using HPLC, Mackinnon and Hooper[27] reported resting plasma glutamine levels in well trained and overtrained athletes which were twice those reported elsewhere.[24-26,37,38,50,56,59,64,65,88] The validity of using HPLC to determine plasma glutamine levels is questionable as the stability of the amino acid in acidic or alkaline media, and the low sensitivity of automated amino acid analysers in detecting changes in plasma glutamine levels,[83] both remain concerns.

5. Overtraining, Infection and the Role of Glutamine

The plasma concentration of glutamine has been reported to be lower in overtrained athletes than in well trained athletes and sedentary individuals. To date, there are only 4 studies that have reported this finding. Parry-Billings et al.[25] have reported values of 503 μmol/L for plasma glutamine in overtrained athletes compared with 550 μmol/L for healthy control athletes (9% difference). Rowbottom et al.[90] have observed a lower mean plasma glutamine level in a group of 10 athletes classified as overtrained (703 μmol/L) compared with sedentary (1030 μmol/L) and athletic age-matched controls (1179 μmol/L) using their bioassay technique for the estimation of plasma glutamine level.

Mackinnon and Hooper[27] recorded 23% lower plasma glutamine levels after 2 weeks of intensified training in elite swimmers. A recent study[26] involved a screening programme of elite athletes before and after the 1992 Olympic Games. Three groups of athletes were studied, based on varying levels of training fatigue. Group A had no lasting fatigue, group B had heavy fatigue at night during a period of heavy training but recovered overnight to continue training, and group C had chronic fatigue and had been unable to train normally for several weeks. Group A exhibited normal amino acid patterns with a mean plasma glutamine level of 554 μmol/L. In comparison, both group B and group C showed markedly lower plasma glutamine levels (group B: 356 μmol/L, and group C: 383 μmol/L). Following the Olympic competition during lighter training there were no changes in plasma glutamine for group A. However, of the 12 athletes in group B all but 2 showed increases in plasma glutamine level to above 450 μmol/L. In group C, 53% of athletes still had a plasma glutamine level below 450 μmol/L.

That overtrained athletes and those experiencing chronic fatigue may be in a constant 'glutamine debt' has important ramifications for (i) those cells and tissues that require glutamine, and (ii) those cells and tissues that do not have the capability to produce this amino acid. Both the lymphocytes and monocytes (macrophages) of the immune system are such cells. It has therefore been hypothesised that a sustained low plasma glutamine level may have deleterious consequences for immune function and render the individual susceptible to invading pathogens.[25] However, to date, there has been no direct evidence linking low plasma glutamine levels with impaired immune function and increased susceptibility to infection in humans.

Reports of lower plasma glutamine levels in athletes with upper respiratory tract infections (URTI) have been made by a number of authors.[26,91,92] In particular, Kingsbury et al.[26] have shown that in athletes presenting with infection, 73% had a plasma glutamine level below 450 μmol/L. Conversely, Mackinnon and Hooper[27] found no relationship between low plasma glutamine level and the occurrence of URTI in trained swimmers. Surprisingly, URTI was more common among well

trained swimmers (with 23% higher plasma glutamine) than overtrained swimmers.

6. Oral Glutamine Supplementation and Infection

Although there is no direct evidence implicating low plasma glutamine (either after exercise or in the overtraining syndrome) with impaired immune function, epidemiological data showing low plasma glutamine and an increased occurrence of URTI in some groups of highly trained athletes has raised interest in glutamine supplementation. van der Schoor et al.[62] have shown that a drink containing 0.3 g/kg bodymass protein hydrolysate given at exhaustion following a prolonged cycling protocol can prevent the postexercise fall in plasma glutamine. Kingsbury et al.[26] also show that 3 weeks of additional dietary protein intake (≈20 g/day) increased plasma glutamine levels in 9 of 10 athletes exhibiting initial plasma glutamine levels below 500 μmol/L. Of these 10 athletes, all had presented signs of infection, although the authors gave no indication of the effect of dietary protein supplementation on recovery from infection.

However, the subject of protein supplementation has provided equivocal data regarding plasma glutamine levels. To elucidate, Greenhaff et al.[88] have shown that a high protein diet (24% protein, 72% fat, 3% carbohydrate) consumed for 4 days leads to a lowering (≈25%) of plasma glutamine level. These authors suggested that this was probably due to increased renal uptake of glutamine to re-establish normal acid-base balance. It would appear wise to increase dietary protein and to maintain a high intake of carbohydrate in those athletes exhibiting low plasma glutamine levels. The protein requirements of athletes training for strength or endurance are increased considerably compared with the sedentary population.[93] An adequate level of daily dietary protein intake in athletes has been estimated to be 1.2 to 1.8 g/kg body mass, compared with 0.8 g/kg for untrained individuals. Analysis of the diets that athletes habitually consume suggests that a substantial proportion of them are not eating enough protein.[93] Severe protein malnutrition has long been associated with a depressed immune system and an increased susceptibility to opportunistic infections.[94]

Shewchuk et al.[95] found that rats fed a high protein diet (2% w/w L-glutamine) for 1 week showed no change in plasma glutamine level or natural killer cytolytic activity. However, Moriguchi et al.[96] have shown maintained proliferation of mitogen-stimulated blood lymphocytes following exercise in rats receiving a 3-week glutamine-supplemented diet. The control group showed decreased proliferation of blood lymphocytes following mitogen stimulation after exercise, and a lower uptake of glutamine by lymphocytes.

Castell et al.[91] have to date provided the best evidence for a prophylactic effect of oral glutamine supplementation on the occurrence of infection. Ultra-marathon and marathon runners participating in races were given either a placebo drink (malto-dextrin) or a glutamine solution (glutamine 5g in water 330ml) immediately after and 2 hours after the race. Athletes were given questionnaires to self-report the occurrence of symptoms of infection for 7 days after the race. In those receiving the glutamine supplement (n = 72), 81% experienced no infection in that period. In those athletes receiving the placebo preparation (n = 79), only 49% experienced no infection in the same period. Although in both groups the level of infection increased following the race, it was concluded that the provision of 2 glutamine drinks in the first 2 hours decreased the incidence of infection in the week after the event.

7. Conclusions

Several authors have suggested that plasma glutamine level may provide a useful biochemical marker of overtraining.[25,26,29,63,67,90] However, since the plasma glutamine level is influenced by short term exercise, nutritional status, diet, infection and physical trauma, it is important that researchers be aware of these factors and take them into account if they propose to include plasma glutamine measurement as part of the battery of tests

designed to monitor athletes for signs of impending overtraining.

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