Exercise-induced immunodepression—plasma glutamine is not the link

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Hiscock, Natalie, and Bente Klarlund Pedersen. Exercise-induced immunodepression—plasma glutamine is not the link. J Appl Physiol 93: 813–822, 2002;10.1152/japplphysiol.00048.2002.—The amino acid glutamine is known to be important for the function of some immune cells in vitro. It has been proposed that the decrease in plasma glutamine concentration in relation to catabolic conditions, including prolonged, exhaustive exercise, results in a lack of glutamine for these cells and may be responsible for the transient immunodepression commonly observed after acute, exhaustive exercise. It has been unclear, however, whether the magnitude of the observed decrease in plasma glutamine concentration would be great enough to compromise the function of immune cells. In fact, intracellular glutamine concentration may not be compromised when plasma levels are decreased postexercise. In addition, a number of recent intervention studies with glutamine feeding demonstrate that, although the plasma concentration of glutamine is kept constant during and after acute, strenuous exercise, glutamine supplementation does not abolish the postexercise decrease in in vitro cellular immunity, including low lymphocyte number, impaired lymphocyte proliferation, impaired natural killer and lymphokine-activated killer cell activity, as well as low production rate and concentration of salivary IgA. It is concluded that, although the glutamine hypothesis may explain immunodepression related to other stressful conditions such as trauma and burn, plasma glutamine concentration is not likely to play a mechanistic role in exercise-induced immunodepression.

immune function; exercise; supplementation

IN A SERIES OF EXPERIMENTS in the 1980s, Newsholme and co-workers (1, 2) examined the role of glutamine metabolism in cultured lymphocytes. The importance of glutamine metabolism in vitro was demonstrated by the high rate of glutaminase activity in resting and stimulated lymphocytes and the inability of lymphocytes to proliferate in vitro in the absence of glutamine (2). Skeletal muscle is the major tissue involved in glutamine synthesis and is known to release glutamine into the bloodstream at a high rate. Skeletal muscle, therefore, plays a vital role in glutamine homeostasis, and, consequently, the activity of skeletal muscle may directly influence those tissues that utilize it. It was hypothesized that, during intense physical exercise, the demand on skeletal muscle and other organs for glutamine is so high that the immune system may be compromised (52, 53, 56, 57).

It is commonly believed that individuals undertaking a high-intensity, prolonged bout of exercise experience increased susceptibility to developing symptoms of upper respiratory tract illness (URTI) (18). Furthermore, epidemiological and experimental studies show that, during the incubation period of an infection, depending on the pathogen, exercise may worsen the disease outcome (28). The mechanism of this apparent susceptibility to illness is not yet understood; however, there is evidence that some aspects of in vitro cellular function may be compromised after acute exercise. Several studies have reported numerous changes in concentrations and function of blood mononuclear cells (BMNCs) as measured by in vitro immunologic meth-
GLUTAMINE METABOLISM

In 1873, glutamine was for the first time considered to be a biologically important molecule when it was found, by indirect evidence, to be a structural component of proteins (33). In 1883, free glutamine was found to be abundant in certain plants (74), and, in the 1930s, studies on the metabolism of glutamine revealed that mammalian tissues have the capacity for hydrolysis and synthesis of glutamine (41). Glutamine was classified as a nonessential amino acid based on studies showing that glutamine was not required as a dietary nutrient (50, 70). In the 1950s, Eagle and co-workers (23, 24) reported that glutamine was important for cells in vitro, and the concentration of glutamine in the circulation was shown to be more than double the concentration of any other amino acid (50).

Glutamine is a neutral glycogenic amino acid and is the most abundant amino acid in the human body. The normal plasma concentration is 500–700 μM, and in human skeletal muscle the concentration is 20 mM (64). Glutamine is available in the lumen of the intestine in the form of peptides derived from protein and can be taken up by the absorptive cells of the intestine.

However, these cells utilize glutamine at a high rate and probably utilize most of the absorbed glutamine, leaving only small amounts to enter the circulation (82).

Glutamine is utilized by many tissues within the body, including the kidneys, gut, and some cells of the cellular immune system. To accomplish the high demands for glutamine in the body, glutamine is synthesized by several organs, including skeletal muscle, kidneys, liver, lungs, and heart. Arteriovenous difference measurements indicate that skeletal muscle is the most important site for glutamine synthesis (53, 57). Skeletal muscle has high activities of branched-chain amino acid (BCAA) transaminase and glutamine synthase, which are key enzymes in the synthesis of glutamine. Glutamine is produced from glutamate and ammonia catalyzed by glutamine synthase. In the muscle, glutamate can be obtained from protein degradation or from the combination of 2-oxoglutarate (a citric cycle intermediate) and BCAA (leucine, isoleucine, and valine), catalyzed by BCAA transaminase. Furthermore, glutamate can be taken up from the circulation. The ammonia can be obtained from the free pool of ammonia or donated from the BCAAs via deamination.

ROLES OF GLUTAMINE

Glutamine plays a major part in nitrogen transport between various organs (12). The kidneys utilize glutamine to maintain acid-base homeostasis. The uptake of glutamine increases in periods of acidosis, and this increase is coupled to the increased demand by the kidneys for elimination of ammonia in the urine. The released ammonia traps protons forming ammonium ions. This loss of hydrogen ions allows for an enhanced exchange of sodium ions, and during acidosis this exchange is contributing to correct the acid-base balance. The metabolism of glutamine provides metabolic intermediates for biosynthetic pathways, such as the synthesis of purines and pyrimidines for DNA and RNA synthesis. As energy supply, glutamine has been shown to be important for tumor cells and enterocytes (39), and enterocytes and colonocytes (5). A high rate of glutamine utilization characterizes a number of different cells (tumor cells, fibroblasts, and cells of the immune system). Based on the activities of a number of key enzymes, it has been shown that there is a high capacity in lymphocytes and macrophages to utilize glutamine. Furthermore, the rate of glutamine utilization by these cells is either similar to, or greater than, that of glucose when the cells are both active and quiescent (56). Whereas mononuclear cells have a high-intracellular activity of glutaminase, they do not possess the ability to synthesize glutamine in that they do not have any activity of glutamine synthase. This has been shown by direct measurement of the enzyme (1) and by indirect measurement of the capability of lymphocytes to produce glutamine (67). The consequence of this is that lymphocytes must be supplied with glutamine in the plasma to accomplish the metabolic requirements of these cells. Release of glutamine...
from skeletal muscle is thought to be the main source to maintain the plasma glutamine concentration due to the fact that skeletal muscles contain the largest store of glutamine in the body.

Even though there is a high utilization of both glucose and glutamine, the oxidation of these compounds is only partial. The major end product of glucose metabolism is lactate, and of glutamine metabolism they are glutamate, lactate, and aspartate (56). A high rate of glutamine utilization, but only partial oxidation, characterizes several types of cells, described in tumor cells by McKeehan (49), who termed the process glutaminolysis. The high rate of glutamine utilization, but only partial oxidation, does not speak in favor of glutamine being a major supply of energy. If the role of glutaminolysis is solely to provide energy, it would be expected that the carbon skeleton would be completely oxidized by the citric acid cycle. Thus a quantitative theory of metabolic control to branched pathways has been applied to explain the high rate of glutamine utilization by lymphocytes and macrophages (19). If the flux through one branch is largely in excess of the other, then the sensitivity of the flux in the low-flux pathway to specific regulators is very high. Hence, in rapidly dividing and proliferating cells, high rates of glutaminolysis (and glycolysis) are required, not for energy or precursor provision per se, but for high sensitivity of the biosynthetic pathways involved in the use of precursors for macromolecular synthesis (e.g., the synthesis of DNA and RNA). According to this theory, the high rate of utilization provides a sensitive but stable system that allows cells to multiply very rapidly in response to a challenge, e.g., the lymphocyte proliferative response in relation to a viral infection.

**MEASUREMENT OF GLUTAMINE**

Different preparation and storage conditions of plasma samples for amino acid analysis have been compared to determine the optimal conditions concerning the stability of amino acids (79). The storage temperature was found to be the most important factor. No change was found in the concentration of glutamine in untreated rat and human plasma after storage at −70°C for 12 wk. At a storage temperature of −20°C, glutamine concentration in rat plasma decreased by 14% in untreated plasma, by 10% in plasma deproteinized with sulfosalicylic acid (SSA), and by 3% if deproteinization was followed by neutralization. In human plasma, a 4–5% reduction in glutamine concentration was found after 12 wk of storage at −20°C, but there was no statistically significant difference in the glutamine level between untreated and deproteinized plasma. As the stability of certain other amino acids is also influenced by prestorage treatment, it is recommended to deproteinize plasma with SSA before storage at −70°C as the most convenient and reliable method to determine plasma amino acid concentrations (79). In addition, several other intermediary metabolites can be determined in whole blood after single-step deproteinizing with SSA (38). Plasma samples deproteinized with SSA are suitable for analysis of amino acids by chromatography but lower the sensitivity of enzyme methodology based on reactions linked with NADH as SSA absorbs substantially at the peak absorbance of NADH (38). In another study (30a), it was found that the addition of TCA to plasma resulted in stable plasma glutamine concentrations for >1 yr when samples were stored at −70°C. Tissues may also be extracted with TCA for glutamine analysis. The glutamine-rich supernate is stable for at least 7 days without neutralization when stored at −70°C (30a). TCA does not interfere with assays based on absorbance of NADH.

Different methods have been used to measure glutamine concentrations, and HPLC is widely used in the measurement of amino acid concentrations and is a reliable method of measuring glutamine concentrations after the introduction of precolumn derivatization (32). Other techniques include the use of enzyme methodology (71) and a glutamine-dependent Escherichia coli bioassay (37). The major difference between HPLC and enzyme methodology and the bioassay is that the glutamine concentration is ~40% higher when measured with the bioassay.

**PLASMA GLUTAMINE CONCENTRATION IN RESPONSE TO EXERCISE AND OTHER STRESSES**

The plasma glutamine response to acute exercise is outlined in Table 1. The effect of acute exercise on plasma glutamine concentration appears to be largely dependent on exercise duration and intensity; however, after prolonged (>2-h duration), exhaustive exercise, there is generally a transient decrease in plasma glutamine concentration (17, 21, 59, 64–67). These levels may remain significantly lower than resting levels for 2–4 h after the cessation of exercise. However, after an ultratriathlon, plasma glutamine concentration was not different from resting levels (44). Intermittent, high-intensity, short-duration bouts may also decrease plasma glutamine concentration (37, 81) or result in no significant change (59, 75). It is interesting to note that, after repeated bouts of high-intensity exercise, plasma glutamine concentration may only significantly decrease some hours into recovery.

In contrast, a single high-intensity bout of shorter duration may increase (6, 25, 36), or not change (48, 59), plasma glutamine levels. Fewer studies have examined the plasma glutamine response to acute, eccentric exercise. Plasma glutamine concentration may significantly decrease (51), or not change (30), after high-intensity, eccentric activity.

Skeletal muscle is the major site of glutamine production (57), and an increase in the release of glutamine, resulting in a decrease in the intracellular concentration, has been shown to occur in relation to the above-mentioned stress situations. However, a decrease in plasma glutamine concentration, despite the increased release from skeletal muscle, is not well understood. The decrease in plasma glutamine concentration is without a doubt due to net overutilization,
which is probably the result either of increased amino acid extraction by the liver (for gluconeogenesis and urea formation) (6), or of an increased rate of glutamine utilization by the kidneys and cells of the immune system (53). In relation to long-term exercise and in overtrained athletes, another theory is that these conditions interfere with the rate of glutamine release from muscles and thus could be responsible for the decrease in the plasma glutamine concentration (53).

### Table 1. Changes in plasma glutamine concentration in relation to various forms of exercise

<table>
<thead>
<tr>
<th>Author and Year</th>
<th>Exercise Mode</th>
<th>n</th>
<th>Sample</th>
<th>Glutamine, µM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babij et al. (6)</td>
<td>10-min cycling at 25, 50, and 75% VO₂ max and to fatigue</td>
<td>8</td>
<td>venous</td>
<td>pre: 575</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Castell et al. (17)</td>
<td>marathon</td>
<td>12</td>
<td>plasma</td>
<td>post: 734</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Decombez et al. (21)</td>
<td>100-km run</td>
<td>11</td>
<td>serum</td>
<td>pre: 571</td>
<td>post 1 h: 421</td>
</tr>
<tr>
<td>Eriksson et al. (25)</td>
<td>3 × 15-min cycling at 35, 55, and 80% VO₂ max</td>
<td>11</td>
<td>venous</td>
<td>post: 666</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Gleeson et al. (30)</td>
<td>20 × electrically stimulated eccentric contractions at 40% VO₂ peak</td>
<td>8</td>
<td>venous</td>
<td>post 1 day: 549</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Katz et al. (36)</td>
<td>10-min cycling at 50% and fatigue at 97% VO₂ max</td>
<td>8</td>
<td>arterial</td>
<td>pre: 555</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Keast et al. (37)</td>
<td>5 × 1-min treadmill at 120% VO₂ max</td>
<td>7</td>
<td>venous</td>
<td>pre: 1,244</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lehmann et al. (44)</td>
<td>ultratriathlon</td>
<td>9</td>
<td>serum</td>
<td>pre: 500</td>
<td>NS</td>
</tr>
<tr>
<td>Maughan and Gleeson (48)</td>
<td>90-min cycling at 70% VO₂ max</td>
<td>5</td>
<td>venous</td>
<td>N/A</td>
<td>NS</td>
</tr>
<tr>
<td>Miles et al. (51)</td>
<td>maximal eccentric contractions</td>
<td>12</td>
<td>plasma</td>
<td>pre: 437</td>
<td>post 3 days: 332</td>
</tr>
<tr>
<td>Parry-Billings et al. (59)</td>
<td>overtrained athletes</td>
<td>40</td>
<td>venous</td>
<td>rest: 550</td>
<td>0.02</td>
</tr>
<tr>
<td>Rennie et al. (64)</td>
<td>225-min cycling at 50% VO₂ max</td>
<td>4</td>
<td>venous</td>
<td>pre: 557</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Rohde et al. (65)</td>
<td>marathon</td>
<td>8</td>
<td>venous</td>
<td>post 2 h: 391</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Rohde et al. (66)</td>
<td>triathlon</td>
<td>8</td>
<td>serum</td>
<td>post 1.5 h: 482</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rohde et al. (67)</td>
<td>repeated exercise (30, 45, 60 min at 75% VO₂ peak, separated by 2-h rest)</td>
<td>8</td>
<td>arterial</td>
<td>post 2 h: 318</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sewell et al. (75)</td>
<td>treadmill run to fatigue at 20 km/h</td>
<td>9</td>
<td>venous</td>
<td>post 2 h: 402</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Walsh et al. (81)</td>
<td>20 × 1-min cycling at 100% VO₂ max</td>
<td>8</td>
<td>venous</td>
<td>post 5 h: 572</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Abbreviations:** VO₂ max, maximal O₂ consumption; VO₂ peak, peak O₂ consumption; BCAA, branched-chain amino acid; pre, before exercise; post, after exercise; N/A, not applicable; NS, not significant.
In relation to other forms of stress, decreases in plasma glutamine concentration have been reported in relation to catabolic conditions such as trauma, sepsis, and burns (77). It is important to note that the largest decrease in plasma glutamine concentration is reported after major burns (>30% of total body surface), with plasma glutamine concentration declining from 490 to 200 μM (60). After acute exercise, this decrease is less pronounced (~100 μM). It is, therefore, important to examine whether these observed decreases in plasma glutamine concentration after acute exercise would actually affect the availability of glutamine to those immune cells that require it and thus result in a decrease in their function.

GLUTAMINE AND IN VITRO IMMUNITY

The initial finding of Eagle et al. (23, 24) that glutamine is an essential nutrient for cells replicating in culture has been extended to many different cell types. It is recognized that glutamine is an important tissue culture supplement, necessary for the survival and growth of a variety of mammalian cells (49, 63, 77, 86), including cells of the immune system (2, 20). Furthermore, it has been shown that other amino acids, combinations of glutamate and ammonia, or combinations of glutamate and leucine, cannot substitute for glutamine (1, 67). In humans, it has been shown that glutamine influences the in vitro proliferation of lymphocytes when stimulated with concanavalin A (ConA) (60, 67, 69), phytohemaggulutinin (67, 69), interleukin (IL)-2 (67, 69), or purified protein derivative of Mycobacterium tuberculosis (69) in a concentration-dependent manner with optimal proliferation at a glutamine concentration around the physiological level (600 μM). These studies also show that, even at lower glutamine concentrations (between 100 and 300 μM), proliferation is still augmented. The influence of glutamine on the proliferative response of lymphocytes has also been examined in rats (2, 78), and similar results have been obtained with optimal proliferation at glutamine concentrations of ~300 μM (2). Furthermore, the rate of phagocytosis by mouse peritoneal macrophages has been shown to be dependent on glutamine, with decreasing rates at glutamine concentrations <600 μM (60).

Lymphocyte proliferation is a complex reaction involving several cytokines. Calder and Newsholme (14) showed that the presence of glutamine in the medium of ConA-stimulated rat lymphocytes enhanced the production of IL-2 (measured by bioassay). Wallace and Keast (80) used a thymocyte assay to show that the secretion of IL-1 by murine macrophages in response to lipopolysaccharide stimulation was dependent on the availability of glutamine in the culture medium. Our laboratory (67) showed that IL-2 and interferon (IFN)-γ production by phytohemaggulutinin-stimulated human BMNCs, measured by ELISA kits, were enhanced by the presence of glutamine at a concentration of 600 μM, whereas the production of IL-1β, IL-6, or tumor necrosis factor-α was not influenced by glutamine. Thus it is possible that glutamine may influence the lymphocyte proliferation by inducing the production of IL-2 and IFN-γ.

The in vitro influence of glutamine on the cytotoxic activity of human lymphocytes was investigated in a study from our laboratory (69). When BMNCs were incubated for 48 h in the presence of IL-2, with or without glutamine, and tested for lymphokine-activated killer (LAK) cell activity in a ⁵¹Cr-release assay, it was shown that the presence of glutamine augmented the LAK activity with optimal lysis at a glutamine concentration of 300 μM. The increase in LAK activity was apparently not due to more LAK cells in the assay, because glutamine did not influence the percentage of CD16⁺ and CD56⁺ cells in an experiment in which BMNC were stimulated with IL-2. In contrast to the relationship between LAK cell activity and glutamine concentration, the function of NK cells was not influenced by glutamine. The supportive role of glutamine in the generation of LAK cell activity has also been found by Juretic et al. (35), who discovered that glutamine deficit affected the LAK cell activity by limiting the number of generated effector cells, whereas acquisition of broad-range killing was not affected. Fahr et al. (27) also demonstrated a relationship between glutamine and IL-2-primed LAK cells. Furthermore, in relation to a triathlon, the time course of changes in serum glutamine concentration was paralleled by changes in LAK cell activities (positive correlation between LAK cell activity and serum glutamine concentration, r = 0.39, P < 0.01) (66).

There is much evidence, therefore, that in vitro function of some immune cells is decreased when glutamine concentration is reduced below physiological levels. There are also data suggesting that a decrease in plasma glutamine concentration in vivo is associated with a decrease in immune function (66). However, it remains unclear as to whether this relationship is causal. In addition, it is unclear whether the decreases in plasma glutamine concentration after acute exercise are of such magnitude as to directly result in decreased cellular immunity in vivo.

THE INFLUENCE OF GLUTAMINE SUPPLEMENTATION ON THE IMMUNE SYSTEM

To address these questions, several studies have attempted to attenuate the exercise-induced decrease in plasma glutamine concentration by glutamine feeding. If a decrease in plasma glutamine concentration is directly associated with cellular immunodepression, provision of oral glutamine supplementation may be advantageous for these cells.

Clinical studies have examined the effect of glutamine feeding, as a part of total parenteral nutrition (TPN), on cells of the immune system and on the function of the intestine in both humans and rats. In humans, it has been shown that glutamine-enriched intravenous feeding to patients with hematologic malignancies in remission (after high-dose chemotherapy and total body irradiation) decreased the amount of
positive microbial cultures and diminished the number of clinical infections (73). Studies in rats have shown that the addition of glutamine to TPN abolished the suppressed level of biliary IgA observed in relation to standard TPN and thus may offer protection against bacterial translocation from the gut (13). Yoshida et al. (84) showed that the rate of hepatic regeneration after partial hepatectomy in rats was increased due to increased protein synthesis in the liver and increased DNA synthesis in hepatocytes, when glutamine was added to standard TPN. In septic rats, it was shown that glutamine-supplemented TPN diminished the increase in urea production, partially prevented the decrease in lymphocyte blastogenesis, and increased the phagocytic index compared with standard TPN (83). Fahr et al. (27) showed that oral glutamine supplementation of tumor-bearing rats decreased the tumor growth that was associated with an increase in IL-2-primed LAK cell activity. These studies were based on the fact that, in rats, TPN induces intestinal villus atrophy and an increased permeability of the intestine. However, in humans, Buchman et al. (11) found that the changes in the intestine resulting from TPN were substantially less significant than the changes occurring in rats, and, in another study (10), they found that TPN was not associated with intestinal immune dysfunction. A recent study by Shewchuck et al. (76) evaluated the influence of regular exercise and dietary glutamine supplementation in rats and found no effect of glutamine supplementation on immune function. Koyama et al. (40) showed that chronic exercise in rats resulted in decreased plasma glutamine concentrations paralleled by decreased ConA-stimulated T-lymphocyte proliferation. The same relationship was shown when glutamine synthesis was inhibited by injection of methionine sulfoximine, indicating an association between glutamine and T-cell proliferation.

**DOES GLUTAMINE SUPPLEMENTATION ABOLISH EXERCISE-INDUCED IMMUNODEPRESSION?**

A number of studies have also investigated whether oral glutamine supplementation to endurance athletes, to abolish postexercise decline in plasma glutamine concentration, has an effect on postexercise immunodepression. Castell et al. (18) supplemented runners participating in a marathon or ultramarathon event. The athletes received 5 g L-glutamine or placebo on a double-blind basis. The glutamine supplementation was given in two doses: one immediately after exercise and a second dose 2 h later. Plasma glutamine concentration was decreased by \( \sim 20\% \); however, the authors did not distinguish between changes in glutamine concentration in the two groups. However, other data from the same authors (17) show that plasma glutamine concentration decreases by a similar degree in both glutamine- and placebo-supplemented groups. Based on questionnaires, 80.8% of the glutamine-supplemented marathon runners in this investigation reported no infections in the 7 days postexercise compared with 48.8% in the placebo group. In contrast, Mackinnon and Hooper (46) showed no significant difference in glutamine levels between subjects who did or did not develop URTI in a study examining the effect of intensified training on swimmers. Furthermore, it was shown that the plasma glutamine concentration did not necessarily decrease during periods of intensified training. Another placebo-controlled study by Castell et al. (17) showed that glutamine supplementation to runners after a marathon race did not influence the lymphocyte distribution or the plasma concentration of IL-6, IFN-\( \gamma \), or C-reactive protein. In the latter study, glutamine was supplemented immediately after and 1 h after exercise. However, to prevent a decrease in the plasma glutamine concentration, glutamine has to be supplemented approximately every 30 min (60). In the studies outlined above, plasma glutamine concentration in both the glutamine-supplemented and the placebo group in the latter study actually declined significantly (to a similar level).

Well-trained athletes performing repeated bicycle-ergometer exercise in a blind crossover, randomized, placebo-controlled study were supplemented with glutamine (68). The athletes performed 60, 45, and 30 min of exercise at 75% of maximal oxygen consumption separated by 2-h resting periods. Glutamine (100 mg/kg body mass) was supplemented 30 min before completion of exercise, immediately postexercise, and 30 min postexercise. Arterial plasma glutamine concentration declined from 508 (preexercise) to 402 \( \mu M \) (2 h after the last exercise bout) in the placebo trial. Glutamine was maintained above preexercise level at all time points in the glutamine supplementation trial. There were no differences between the two trials in the concentration of lymphocytes, leukocyte subpopulations, lymphocyte proliferation, LAK activity, or NK activity. Thus, despite the attenuation of the exercise-induced decrease in plasma glutamine concentration, glutamine supplementation did not abolish the postexercise immunodepression characterized by a decrease in lymphocyte concentration and a decrease in the LAK cell activity. Comparable results were found in a field study (randomized, placebo controlled) (65) showing that glutamine supplementation to marathon runners did not influence the exercise-induced immunological changes. Venous plasma glutamine concentration declined from 647 \( \mu M \) prerace to 470 \( \mu M \) 120 min postrace in the placebo group, whereas plasma glutamine concentration was maintained in the glutamine supplementation group. In this study, no difference in lymphocyte proliferation was observed between the glutamine and the placebo group, whereas glutamine addition in vitro enhanced the proliferative response equally in the two groups. Similar results have been observed in a recent study comparing glutamine and placebo supplementation during, and in recovery from, 2 h of cycle ergometry at 75% peak oxygen consumption (42, 43). During recovery from exercise in the placebo group, plasma glutamine concentration was significantly decreased from 631 \( \mu M \) before exercise to 538 \( \mu M \) 2 h postexercise. However, glutamine supplementation attenuated the exercise-induced decrease in
DISCUSSION

Several studies have tried to link the changes in the immune system induced by exercise or other stress situations to changes in plasma glutamine concentration (18, 37, 53, 59, 60). The hypothesis stating that decreased plasma glutamine concentration postexercise is the main reason for the observed decrease in immune function is based on studies showing that 1) glutamine is important for cells in culture, 2) cells of the immune system have a high capacity for glutamine oxidation, and 3) glutamine addition in vitro enhances lymphocyte proliferation and LAK cell activity and increases the production of some T-cell-derived cytokines. Furthermore, studies in animals have shown a beneficial effect of glutamine addition to TPN, resulting in 1) less efflux of glutamine from skeletal muscle, 2) improved nitrogen balance, and 3) less villus atrophy in the gut.

In vitro studies examining the influence of glutamine on lymphocyte proliferation show that glutamine enhances the mitogen-stimulated response in a concentration-dependent manner, with optimal proliferation at glutamine concentrations between 100 and 600 μM (69). The in vivo decrease in plasma glutamine concentration in relation to exercise is ~100 μM, depending on the type and duration of the exercise (Table 1), and the lowest plasma glutamine concentration reported in the literature in relation to catabolic conditions is 200 μM, measured after major burns. Results from the majority of in vitro studies show that glutamine concentration has to be <100 μM to observe a decreased proliferative response (67, 69). If this is compared with the results from the in vivo exercise studies (65, 68), with relatively small decreases (10–20%) in plasma glutamine concentration, it is unlikely that glutamine supplementation and restoration of the plasma glutamine concentration would influence the proliferation response and LAK cell activity. In fact, it is unclear whether those decreases in plasma glutamine concentration observed after acute exercise actually represent a decrease in the availability of glutamine to immune cells. In a recent experiment, glutamine concentration was measured within BMNCs after an acute bout of cycle ergometry, where plasma glutamine concentration was significantly decreased from resting levels. When BMNC glutamine concentration was calculated to give glutamine concentration per 10^6 cells, it was shown that intracellular levels actually increased during recovery, when plasma levels were decreased. These data were mainly due to the fact that circulating BMNC count significantly decreased during recovery; thus, even though plasma glutamine concentration was decreased, there was no decrease, and possibly an increase, in the amount of glutamine available to the BMNCs in circulation (N. Hiscock, R. Morgan, G. Davison, J. Garcia, F. Grace, N. Boisseau, L. M. Castell, L. T. Mackinnon, B. Davies, and D. M. Bailey, unpublished observations). These data suggest that a decrease in circulating numbers of immune cells, or perhaps other factors, may be more likely to contribute to postexercise immunodepression.

Thus the fact that glutamine supplementation does not restore postexercise impairment of various immune functions is not based on a discrepancy between results as such but is explained by the fact that the decrease in the concentration of glutamine after exercise may not decrease the availability of glutamine to immune cells and, therefore, is unlikely to directly induce a decrease of immune function in vitro. However, it cannot be excluded that, in vivo, the metabolism of glutamine in the lymphocytes is influenced by exercise-induced changes in, for example, the hormonal environment.

Increased levels of plasma glutamine after short-duration exercise bouts have been shown in several studies (25, 47) and ascribed to increased release from skeletal muscle (72). In accordance with this, decreased muscle glutamine concentrations during exercise have been described (64, 72). A decrease in plasma glutamine concentration after prolonged, exhaustive exercise is also well described (Table 1). However, there is a lack of data from humans regarding postexercise muscle glutamine concentrations and exchange rates. In rats, decreases in muscle glutamine concentrations postexercise have been reported (22). It appears that, during or at the end of prolonged exhaustive exercise in humans, muscle glutamine concentration may be decreased. This indicates a glutamine release, resulting in an increase in plasma glutamine concentration. It is more difficult to explain why the muscle glutamine concentration decreases after prolonged exercise, as does the plasma concentration. Plasma glucocorticoid concentrations increase after prolonged, intensive exercise (29). This contributes to a change in glutamine metabolism by increasing glutamine synthase activity and mRNA expression, decreasing intramuscular glutamine stores, and maintaining maximal glutamine transportation, even at lowered intramuscular glutamine levels (71). However, it is possible that these changes may still
be insufficient to balance an increased rate of utilization by other organs, resulting in decreased plasma glutamine concentrations.

Newsholme and coworkers (53, 56) have proposed that the decreased plasma glutamine concentration after exercise is due to an increased utilization by some cells of the immune system. However, during acidosis in rats, the renal glutamine consumption has been shown to increase dramatically, making the kidneys the major organ of glutamine utilization. Combining this with the fact that glutamine acts as a gluconeogenic precursor makes it more possible that the “over-utilization” postexercise is due to acid-base homeostasis and gluconeogenesis and possibly, to a minor degree, to the increased utilization by cells of the immune system.

Glutamine in vitro is undoubtedly important for optimal lymphocyte proliferation, generation of LAK cell activity, and the ability of cells to produce certain cytokines. Furthermore, in rats, glutamine is important as an additive to TPN, probably to maintain the barrier of the gut. It is, however, more questionable whether the decrease in circulating glutamine concentration in relation to exercise is mechanistically related to decreased immune function. A weak point in the “glutamine hypothesis” with regard to the in vitro influence of glutamine is that, when lymphocytes are cultured in a glutamine concentration identical to the lowest plasma glutamine concentration measured post-exercise (300–400 μM), these cells will function equally well as when glutamine is added at a concentration identical to that of the resting level (600 μM). Glutamine supplementation studies from our laboratory as well as other studies have shown that maintenance of the plasma glutamine concentration does not influence the exercise-induced changes in lymphocyte proliferation, LAK cell activity, or distribution of lymphocytes. Therefore, the available data on humans do not support the contention that the postexercise decline in some immune functions is caused by a decrease in plasma glutamine concentration.

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REFERENCES


