

**Side effects of amino acid supplements**

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**Short title:**

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## **Summary**

The aim of the article is to examine side effects of increased dietary intake of amino acids, which are commonly used as a dietary supplement. In addition to toxicity, mutagenicity and carcinogenicity, attention is focused on renal and gastrointestinal tract functions, ammonia production, and consequences of a competition with other amino acids for a carrier at the cell membranes and enzymes responsible for their degradation. In alphabetic order are examined arginine,  $\beta$ -alanine, branched-chain amino acids, carnosine, citrulline, creatine, glutamine, histidine,  $\beta$ -hydroxy- $\beta$ -methylbutyrate, leucine, and tryptophan. In the article is shown that enhanced intake of most amino acid supplements may not be risk-free and can cause a number of detrimental side effects. Further research is necessary to elucidate effects of high doses and long-term consumption of amino acid supplements on immune system, brain function, muscle protein balance, synthesis of toxic metabolites, and tumour growth and examine their suitability under certain circumstances. These include elderly, childhood, pregnancy, nursing a baby, and medical condition, such as diabetes and liver disease. Studies are also needed to examine adaptive response to a long-term intake of any substance and consequences of discontinuation of supplementation.

## **Key words**

Arginine; Branched-chain amino acids; Carnosine; Citrulline; Creatine; Glutamine; Histidine; Tryptophan.

## **Introduction**

There are many people who consume chronically high amounts of amino acids as a dietary supplement. These are mainly athletes and bodybuilders who use amino acids and their derivatives to increase muscle mass and strength and delay the onset of fatigue. Humans also consume amino acids to support immune system, improve memory, ameliorate depression, prevent headaches, and to help insomniacs. Several amino acids are recommended or investigated in therapy of muscle wasting disorders, sepsis, multiple trauma, liver cirrhosis, renal insufficiency, eczema, and ageing-related disorders. However, well controlled studies on adverse effects of increased intake of specific amino acids on humans are rare. A growing problem, which this article will not address, is the frequent content of anabolic steroids in supplements that are non-standard, counterfeit and deliberately manufactured to imitate a legitimate product [1].

The aim of the present article is to examine side effects of increased dietary intake of amino acids, which are commonly used as a dietary supplement. First, I will try to outline general risks of excessive amino acid intake. Then I will examine in an alphabetic order what side effects can be induced by increased intake of a specific amino acid.

## **Side effects of increased amino acid intake**

Several studies have examined possible toxic, mutagenic, cancerogenic and teratogenic effects of high doses of specific amino acids and tried to assess the safe limits of their increased intake [2-5]. Many studies have evaluated effects on the digestive system, for example, whether or not nausea, abdominal pain, vomiting, and diarrhoea are present [3,6,7]. As the liver and the kidneys are the main organs involved in metabolism and excretion of excess substances from the body, a large part of the studies focusses on hepatic and renal functions [8-10].

However, there are a number of other side effects, which are evaluated only sporadically, although they can be harmful, especially in children, pregnancy, elderly, and illness. For example, experimental studies have clearly demonstrated that some amino acids, such as glutamine (GLN) and arginine (ARG), are essential for growth of tumour cells [4,5,11,12]. The dangerous may be increased production of ammonia in individuals with impaired hepatic or renal function, especially after consumption of high amount of amino acids with several nitrogen atoms, such as GLN, histidine (HIS), and ARG. Unfortunately, the studies examining effects of chronic amino acid intake on tumour growth and ammonia levels in humans are almost non-existent.

Furthermore, increased intake of one or more amino acids can cause imbalance in amino acid concentrations, increase concentrations of its metabolites, and affect the transport of a group of amino

acids into cells due to competition for a carrier at the cell membrane. The phenomenon of carrier competition can affect absorption of other amino acids in the gut and subsequently their appearance in the blood, transport across the blood-brain barrier, and supply for protein synthesis.

In summary, it may be assumed that chronic intake of high amounts of individual amino acid or its derivatives alters various biochemical pathways and cellular functions (Figure 1). The consequences might be detrimental effects on the course of the illness and unexpected response to various physiological and pathological conditions.

## **Side effects of increased intake of individual amino acids**

I will briefly describe the reasons for use of a specific amino acid as a dietary supplement and then possible adverse effects of its increased intake. The amino acids are listed alphabetically.

### **L-Arginine (ARG)**

ARG is a substrate for various isoforms of nitric oxide synthase (NOS) to produce nitric oxide (NO), plays a role in removing ammonia from the body through the urea cycle, serves as a substrate for synthesis of creatine and proline, and has a stimulatory role in secretion of insulin and growth hormone [6,13]. In addition, ARG decreases plasma triglycerides and exerts positive influence on immune system and collagen synthesis [5,12,14,15]. The most of endogenous ARG is synthesized from citrulline in the kidneys. The synthesis may be not sufficient in neonates, in catabolic states, and after small bowel resection [16]. Therefore, ARG is classified as a conditionally essential amino acid. Low plasma ARG concentrations are found in patients with sepsis [17].

Supplementation of ARG has been proposed to increase endothelial NO bioavailability and subsequently improve health status in elderly, treat patients with cardiovascular diseases, and improve sexual functions [18-20]. In addition, ARG is recommended to increase muscle performance in athletes and body builders, treat adiposity and metabolic syndrome, and for wound healing [21,22].

A common dosage is 6 g per day; higher doses, up to 30 g per day have also been reported [13]. It should be emphasized that the majority of orally taken ARG is degraded in enterocytes and the liver to yield ornithine, citrulline, and urea.

### ***Side effects***

There are reports of gastrointestinal distress, such as nausea and diarrhoea [6,13]. A significant increase in weight and protein content in the kidneys was observed after a two-month intake of ARG-enriched diet in the rat [14]. Overstimulation of NOS by ARG load may induce hypotension due to NO-mediated

vasodilatation, which was observed in subjects receiving a large dose of ARG intravenously [23]. Therefore, recommendations to increase ARG intake should aware its negative interaction with blood pressure medications. Unclear are the effects on tumour growth [5,6,12].

A serious problem with the use of ARG in therapy of cardiovascular disorders is that its beneficial effects disappear if it is given chronically. Benefits of ARG (3–9 g/d) in patients with peripheral artery insufficiency observed after 3 months [18] were not observed after 6 months [24]. Similarly, the short-term benefits of ARG in myocardial infarction observed after one month [19] were not observed in myocardial infarction trial with 153 patients randomly assigned to ARG (9 g/d) or placebo for 6 months. In addition, 6 patients in the ARG group died during the study [25]. The authors concluded that ARG should not be recommended following acute myocardial infarction.

It is supposed that the loss of therapeutic effects of ARG upon long-term supplementation in humans (“L-arginine tolerance”) is due to oxidative stress. It was shown that endothelial cells exposed to physiologically relevant concentrations of ARG manifest NOS down-regulation and superoxide and glucose cumulation [26]. In addition, it was shown that long-term ARG supplementation increases formation of ROS and decreases NO production in old mouse aortas and accelerates functional decline of kidney in aging. Hence, it was suggested that ARG supplementation should be avoided in elderly population [27].

Oxidative stress and ARG consumption for NO synthesis, resulting to decreased concentration of ARG in plasma, is activated during inflammatory response, especially during sepsis. The NO released by phagocytes participates in vasodilation at the site of inflammation and under conditions of simultaneously increased ROS production gives rise to cytotoxic peroxynitrite, which plays a role in potential damage to its own structures (Fig. 2). Only a few studies have evaluated effects of ARG supplementation on individuals with sepsis [17].

There have been few studies investigating the specific effects of ARG supplementation on the distribution of amino acids in plasma and tissues. In our study, in rats consuming ARG-enriched diet for 2 months increased plasma concentrations of urea, creatinine, ARG, and ornithine and decreased concentrations of most of other amino acids. ARG and ornithine increased also in muscles and kidneys. In most of examined tissues, including liver, soleus and extensor digitorum longus muscles, and kidneys, decreased methionine, phenylalanine, threonine, asparagine, glycine, serine, and taurine. An increase of lysine was observed in muscles [14].

**Resume.** Careful studies are necessary to determine under which conditions ARG supplementation is appropriate and when it is undesirable. Attention should be focused to markers of oxidative stress and blood pressure stability.

## **β-Alanine (BA)**

BA is the rate-limiting amino acid in the synthesis of carnosine ( $\beta$ -alanyl-L-histidine) a dipeptide with high buffering and antioxidant capacity present in skeletal muscle. BA is more effective at increasing carnosine content than HIS and, currently, BA supplementation is becoming a popular ergogenic strategy [28-30]. The recommended dose is 1 - 3 g/day. In some studies, more than 6 g/day was administered [31].

### *Side effects*

There is no information on toxic or carcinogenic effects. The only reported side effects are short-term paraesthesia and a slight increase in alanine aminotransferase levels [32]. BA supplementation at 6.4 g/day for 24 weeks did not significantly affect clinical markers of renal, hepatic and muscle function, nor did it result in chronic sensory side-effects [31]. However, a substantial decrease in HIS content (~30 %) in muscles and plasma after BA supplementation has been reported [29].

**Resume.** Obviously, further studies are needed to determine whether BA supplementation requires a concomitant increase in histidine (HIS) intake.

## **Branched-chain amino acids (BCAAs)**

The BCAAs (valine, leucine, and isoleucine) are essential amino acids, which serve as substrates and regulators of protein metabolism, particularly in muscles. Positive effects of BCAA on protein synthesis, especially of leucine, are realized through the mTOR signalling pathway, phosphorylation of translation initiation factors and ribosomal proteins, and stimulatory effect on insulin secretion [33,34]. The inhibitory effects on proteolysis are mediated mainly by branched-chain keto acids (BCKA;  $\alpha$ -ketoisocaproate,  $\alpha$ -keto- $\beta$ -methylvalerate, and  $\alpha$ -keto-isovalerate) and  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB).

BCAA catabolism is increased in all muscle wasting conditions, including sepsis, trauma, cancer, chronic renal failure, and liver cirrhosis. However, BCAA levels decrease markedly only in liver cirrhosis, whereas increased levels are found in diabetes. Decrease in BCAA levels in liver cirrhosis is due to their use as a donor of amino group for synthesis of glutamate, which is a direct substrate for ammonia detoxification to GLN in muscles [35]. Increase in BCAA levels in diabetes is due to their impaired catabolism related to decreased glycolysis and surplus of NADH from increased fatty acid oxidation [36].

BCAA supplementation is thought to promote anabolic pathways in athletes, mitigate cachexia in muscle wasting disorders, prevent or treat signs of hepatic encephalopathy, attenuate fatigue during exercise, promote wound healing, and stimulate insulin production [37]. A common dose of BCAA supplementation is 10 – 20 g/day, in some studies 60 g/day, resulting in 4-fold [38] and more than 10-fold [39] increase in plasma BCAA, respectively. On the basis of maintenance studies [40,41] and the

ratios of BCAAs in proteins [42], most studies involving pharmacologic BCAA administration have used mixtures containing 50 to 100% leucine more than isoleucine and valine.

### *Side effects*

There is no evidence of carcinogenesis and mutagenicity, or neurological damage as observed in maple syrup urine disease, the hereditary disorder of BCAA catabolism.

It seems that effects of exogenous BCAA are temporary limited to the early period after their administration and opposite effects are activated later. In our study, muscle protein synthesis in postprandial state was higher in rats fed by BCAA-enriched diet for two months when compared with controls. However, we failed to demonstrate positive effects of BCAA-enriched diet on muscle protein balance. Furthermore, muscle protein synthesis decreased after overnight starvation in animals fed before starvation by BCAA-enriched diet more than in controls [43].

It is well established that the rate of BCAA degradation in skeletal muscle is highly responsive to changes in dietary intake. The  $K_m$  of BCAA aminotransferases for BCAAs is two- to four-fold higher than tissue BCAA concentrations [44] and, therefore, the rate of transamination leading to the production of BCKA, glutamate, alanine, and GLN responds rapidly to changes in BCAA level (Figure 3, left side). Therefore, the effects of BCAA load may be detrimental under conditions of impaired or increased ammonia production, such as in subjects with liver disease and during heavy physical performance (Figure 3, right side). In these conditions, ammonia detoxification to GLN in muscles is activated and  $\alpha$ -ketoglutarate ( $\alpha$ -KG) synthesis from glutamate is impaired. The result is drain of  $\alpha$ -KG from the tricarboxylic acid cycle (cataplerosis) and an increased release of GLN into the blood stream to be catabolized to ammonia in visceral tissues. Hence, the positive effects of BCAAs on muscle protein balance compete with the negative effects of cataplerosis on muscles and increased ammonia levels on the brain [45-50].

Because BCAA are transported into the brain via a common transporter for large neutral amino acids, the excess of BCAA may lower brain uptake of other neutral amino acids, such as phenylalanine, tyrosine, HIS, and tryptophan (TRP), which are precursors of dopamine, norepinephrine, histamine, and serotonin. This phenomenon, which is a rationale for use of the BCAA to prevent hepatic encephalopathy and onset of fatigue during exercise, might have detrimental effect on mental functions of patients with neurological and psychiatric disorders. Due to impaired synthesis of serotonin from TRP, increased aggression can be expected in schizophrenic patients and increased quarrelsomeness in people with a tendency to irritability or aggression [51].

Some researchers suggest that increased BCAA concentrations and their metabolites are responsible for insulin resistance and might have detrimental role in pathogenesis of complications associated with diabetes [52,53].

**Resume.** Criteria should be established for the use of BCAAs in conditions where the increased ammonia production due to enhanced BCAA catabolism might be detrimental. BCAA supplementation in diabetes appears to be inappropriate.

## **Carnosine**

Carnosine ( $\beta$ -alanyl-L-histidine) is an effective buffer, antioxidant, heavy metal chelator, and anti-glycating agent in muscles [54]. Carnosine is predicted to be a more efficient proton-buffering and antioxidant compound than HIS. Hence, several intervention studies have been performed using carnosine to examine its effects on muscle performance in neurodegenerative and age-related disorders, metabolic syndrome, and inflammatory bowel disease. In most studies, daily carnosine supplementation doses ranged from 0.1 to 2 g [30,55].

### ***Side effects***

Carnosine administered orally is rapidly degraded by carnosinase to BA and HIS [56]. Therefore, the effects of carnosine supplements are induced mainly by these amino acids, which are discussed separately.

**Resume.** It should be determined whether or not carnosine supplementation has advantages over BA and HIS administration.

## **L-Citrulline (CIT)**

The most CIT provided orally or synthesized from GLN by enterocytes are taken up by the kidneys, and utilized for ARG synthesis (Fig. 4). The main possible indications for increased CIT intake are short bowel syndrome and the indications for ARG. A decrease in the blood CIT concentration in patients with small bowel resection is considered a signal for its parenteral administration [57]. The benefits of short-term therapy are reported on cardiovascular disorders, muscle wasting, intestinal resection, obesity, and insulin resistance [58,59].

### **Side effects and resume**

Because the main mediators of the effects of CIT supplementation are ARG and NO, and long-term studies of the effect of ARG supplementation indicate cardiovascular and renal risks [24,25,27], studies examining the safety of CIT supplementation are necessary.



## **Creatine**

Creatine (methylguanidoacetic acid) is the most used dietary supplement for athletes in order to increase the content of creatine phosphate, a ready source of ATP in muscles. There are different available forms, most common is creatine monohydrate. Beneficial effects of exogenous creatine are demonstrated in anaerobic disciplines (e.g. sprint). The recommended dose is 3 - 5 g/day [60].

### ***Side effects***

The most common adverse effect is transient water retention in the early stages of supplementation [61]. Some studies report muscle cramps, dehydration, gastrointestinal distress (vomiting, diarrhoea) and liver dysfunction [3,62]. Position statements of International Society of Sports Nutrition published in 2007 and 2017 are that creatine monohydrate is safe and effective ergogenic supplement [60,63]. An internationally renowned team of experts has performed an evidence-based scientific evaluation of literature and confirmed the statements [64].

However, it should be noted that there is insufficient information on the effect of creatine supplementation on the kidneys and the liver in the elderly, individuals with renal disease, and when taken at higher than recommended doses for several months [61,65]. It has been advised that high-dose (>5 g/day) creatine supplementation should not be used by individuals with pre-existing renal disease [3]. Furthermore, it has been shown that enhanced creatine consumption results in its increased conversion to sarcosine and then to cytotoxic and carcinogenic agents methylamine and formaldehyde [66]. Although excretion of methylamine and formaldehyde not reach the limit values for healthy individuals, long term studies are required to evaluate risks of enhanced production of these agents.

Because meat and/or creatine intake increases blood creatinine levels, tests that are not based on plasma levels and urinary creatinine excretion should be used to assess renal function in individuals consuming creatine.

***Resume.*** The conditions under which increased creatine intake is inappropriate should be defined.

## **Glutamine (GLN)**

GLN is proteinogenic amino acid known as an important energy fuel for rapidly growing cells (especially immune cells, enterocytes, and tumour cells), as precursor for synthesis of glucose, ammonia, and nucleic acids, and it plays a signalling role in many processes, including expression of genes. Most GLN are synthesized in muscles, in smaller amounts it is released into the blood from the brain, lungs and adipose tissue (Fig. 5).

GLN concentration in plasma and tissues decreases postoperatively, after multiple trauma, in exhaustive exercise, major burns, and during sepsis and contributes to negative protein balance,

immunosuppression and enhanced morbidity and mortality on underlying illness [67-71]. Therefore, GLN is considered as a “conditionally essential” amino acid with proposed favourable effects on immune system, gut, and protein balance [72-75]. GLN supplementation doses range from 2 g/day to 40 g/day (high-dose GLN supplementation). Due to the instability of GLN and its limited solubility, its dipeptides (glycyl-GLN or alanyl-GLN) are sometimes used instead of GLN.

### *Side effects*

GLN supplementation is without signs of toxicity or mutagenic activity [67,76-78]. However, enhanced GLN intake, especially by parenteral route, substantially increases glomerular filtration rate, renal plasma flow, and ammonia production and protein content in the kidneys [8-10,79]. Higher concentrations of serum urea nitrogen and creatinine and decrease in glomerular filtration rate were found in older volunteers who received 0.5 g GLN/kg/day than in middle-aged individuals [80]. It has been shown that chronic intake of GLN-enriched diet has negative influence on protein balance in muscles and alters amino acid concentrations in plasma and tissues in healthy rats [79,81].

Tumour tissue cells utilize GLN much faster than normal cells and die rapidly in a medium lacking GLN [4,11] and many tumours exhibit special transporters responsible for fast transport of GLN across their membranes [82]. Studies dealing with hepatoma cells demonstrated that GLN transporters referred to as ASCT2 [83] are not expressed in normal liver cells. It was found that GLN was acting as a cellular signal to maintain ASCT2 expression and that enhanced glutaminase activity mediates signalling events coupled with c-myc oncogenesis [84-86]. Hence, there is an obvious risk that increased GLN availability will promote tumour growth. On the other side, several studies have demonstrated that GLN supplementation improves the protein balance in tumour-bearing animals [87,88], the function of natural killer cells [89,90] and has no effect on tumour weight or protein and DNA synthesis [87,91,92].

Because most of the GLN administered is utilized to form ammonia, increased GLN consumption may exert adverse effects in subjects with hyperammonaemia, such as subjects with liver disease or urea cycle disorders [81]. There is strong evidence that GLN accumulation in astrocytes contributes to the cerebral oedema in acute hepatic failure and to the Alzheimer type II astrocytes in chronic hyperammonemia [93]. Impairment in the electroencephalogram and neurological deficits during oral GLN challenge in cirrhotic patients indicate that GLN intake in patients with liver disease should be restricted [94,95].

**Resume.** It is inappropriate to administer high doses of GLN in conditions not associated with GLN deficiency, especially in elderly and individuals with hepatic and renal impairment. Research is needed to elucidate whether it is safe to consume GLN supplements by patients with cancer.

### **Histidine (HIS)**

The imidazole ring in HIS structure determines its role as an important buffer, antioxidant, and chelating agent. HIS content is high in haemoproteins (haemoglobin, myoglobin, cytochromes, haem peroxidases, etc.) and epidermal barrier protein termed filaggrin [30]. Atopic dermatitis and anaemia are the first consequences of prolonged consumption of HIS-deficient diet [96].

HIS and HIS-containing dipeptides, notably carnosine, are investigated in order to increase muscle performance and delay fatigue in athletes, reduce the effect of reactive oxygen and nitrogen species in aging-related disorders, neurodegenerative diseases, metabolic syndrome, rheumatoid arthritis, and for therapy of eczema [30]. HIS supplementation doses range from 1 to 4 g/day. High amounts of HIS are in solutions employed for organ preservation before transplantation and myocardial protection in cardiac surgery [97].

### *Side effects*

There are no data on the toxic or mutagenic effects of increased HIS intake. Although HIS is a precursor of histamine, there are no data on allergies or peptic ulcer disease. Of practical significance might be anorexia, decreased folic acid levels, and increased urinary zinc losses [7,98,99]. Several articles have reported that HIS administration may increase ammonia (three nitrogen atoms are present in one HIS molecule) and affect the level of several amino acids. The most common are increased levels of alanine, glutamine, and glutamate and decreased levels of glycine and BCAA [100]. In addition, hypercholesterolemia and liver enlargement have been reported after long-term intake of HIS [101-104].

**Resume.** A long-term intake of high amounts of HIS is not suitable for people with impaired liver function.

## **$\beta$ -Hydroxy- $\beta$ -methylbutyrate (HMB)**

HMB is a metabolite of leucine with a positive effect on protein balance, repair, strength and function of skeletal muscle [105]. HMB is used as a dietary supplement mainly in athletes, studies performed with older people have demonstrated that HMB attenuates development of sarcopenia [106,107]. In a recent study was shown that HMB restores muscle protein homeostasis in liver disease [108]. HMB is commercially available in the form of calcium salts, less often as the free form. The recommended dose is 3 g/day.

### **Side effects**

Most studies report that HMB is well tolerated and has no toxic effects [2,109,110]. However, probably due to protein anabolic effect of HMB on muscles, decreased GLN level has been reported in rats at 24 hours after HMB treatment [111]. Decreased ATP concentrations and increased AMP/ATP ratios were found in the liver and muscles of HMB-treated rats with diabetes [112].

**Resume.** It should be examined whether the positive effects of HMB on protein balance in muscles are not associated with adverse effects on amino acid concentrations and ATP metabolism.

### **Leucine (LEU)**

Of all the amino acids, LEU is the most powerful anabolic agent [34,113-115]. Proponents of use of LEU alone as a dietary supplement claim that competition between LEU and other neutral amino acids for transport into muscle cells hinders the unique effects of LEU on protein synthesis and, therefore, using LEU alone is more effective than a mixture of all three BCAA (leucine, valine, and isoleucine).

The effects of a dietary supplement of LEU alone are studied mainly in athletes and old people. Several articles indicate that LEU may overcome the blunted muscle protein synthesis response to food ingestion in the elderly, treat sarcopenia, and improve glycaemic control in type 2 diabetes [114-117]. A commonly used dose is 5 g/day.

#### ***Side effects***

Most studies reporting positive effects of LEU on muscles are short-term studies, which report positive effects on protein synthesis and not on mass of muscle protein [115,18-120]. Positive influence of LEU supplementation on protein balance was not observed by most of long-term studies [121-123], and there are also reports of negative effects [43,124].

Several studies evaluating the consequences of enhanced LEU intake revealed a decrease in concentrations of valine and isoleucine in plasma [43,124,125], and even in muscles [43]. Decreased levels of valine and isoleucine are due to induction of BCKA dehydrogenase (rate limiting enzyme in BCAA catabolism) by ketoisocaproate, a product of LEU transamination, in liver, muscles, and adipose tissue [44,125-128]. A role in depletion of valine and isoleucine pools may play also their competition with LEU for transport via the L-carrier system. This already occurs in the gut. Higher intracellular concentrations of valine and isoleucine in enterocytes of jejunum of animals fed by LEU-enriched diet indicate impaired transport through basolateral membrane of enterocytes [43]. The availability of valine and isoleucine may therefore become rate limiting for protein synthesis when LEU alone is consumed.

**Resume.** There are significant limitations if LEU alone is used as a dietary supplement, although its stimulatory effect on protein synthesis in muscles has been confirmed. Due to deficiency of valine and isoleucine, administration of LEU alone could have only a transitory stimulatory effect on protein synthesis.

### **Tryptophan (TRP)**

TRP is an essential proteinogenic amino acid used to treat depression and insomnia. Its effect is induced by TRP-induced increase in synthesis of serotonin (5-hydroxytryptamine), a neurotransmitter known to regulate neuronal circuits that control sleep and mood. Next to its role as a neurotransmitter, serotonin is the precursor of melatonin, modulates gut and immune functions, and plays a role in haemostasis.

TRP supplementation has been employed as a potential treatment for depression and sleep disturbances since the early 1960s. In addition, there is considerable evidence for beneficial effects of TRP on mood and social behaviour. TRP can reduce aggression in schizophrenic patients while increasing agreeableness in people with a tendency to irritability or aggression [51].

The well-known affair of adverse effect of enhanced consumption of TRP as a supplement is the eosinophilia–myalgia syndrome reported in the 1989 [129]. Several thousand cases have occurred in users of TRP for insomnia and mood elevation, several dozen of which have resulted in deaths. The cause was traced to impurities in synthetic TRP supplements from one Japanese manufacturer. This issue will not be considered as a possible side effect of TRP supplementation.

Single doses of TRP from 1 g to 15 g were used both acutely and chronically for periods up to 2 years [130]. A commonly used dose is 3 g/day.

### *Side effects*

Although TRP has been studied for 6 decades, few side effects, which include tremor, nausea, and dizziness, have been reported (Fernstrom 2012). A more common effect of high doses of TRP, which can be expected due to the stimulating effect of TRP on serotonin synthesis, is fatigue or drowsiness.

A potentially life-threatening condition is “serotonin syndrome” (also referred to as serotonin toxicity), which includes neuromuscular abnormalities, autonomic hyperactivity, and mental state changes [131]. Serotonin syndrome is usually precipitated by the simultaneous administration of two or more drugs, which enhance serotonin availability, such as serotonin reuptake or MAO inhibitors.

In humans, intestinal cells and gut microbiota play an important role in TRP metabolism. The kynurenine-anthranilate route produces kynurenine and its derivatives (kynurenic acids, 3-hydroxykynurenine, 3-hydroxyanthranilate, and anthranilic acid). In enterochromaffin cells, hydroxylation of TRP yields 5-hydroxytryptophan, which is decarboxylated to serotonin. The degradation of side chain of TRP results in indole and a number of related substances, such as indol propionate, indol acetate, methyl indole (skatole) and indole lactate. Most of metabolites of TRP are classified as uremic toxins and act as ligands for aryl hydrocarbon receptors (AHR), which play a crucial role in immune response, epithelial renewal, barrier integrity, and metastasis of cancer cells [132]. In a recent study was shown that TRP supplementation in mice and human volunteers increases kynurenine metabolites in circulation [133].

**Resume.** Careful studies examining effects of TRP supplementation on AHR activation and concentrations of TRP metabolites should be performed. Because TRP is a precursor to serotonin, caution should be exercised when supplementing it with drugs that affect serotonin metabolism.

## **Conclusions**

Amino acids and their derivatives are commonly used dietary supplement and there are a number of studies on their beneficial effects both in healthy and sick individuals. However, data on their possible side effects, including those that may be harmful to consumers, are rare. Only sporadically are mentioned consequences of adaptive responses on long-term amino acid intake, such as altered degradation and excretion of a supplement, expression of amino acid transporters, and synthesis of toxic metabolites. Almost absent are reports on side effects of supplements based on combination of individual amino acids or combination with other substances or medications. Adverse influence on metabolism may have also the withdrawal of a supplement. Starvation for 24 hours of animals previously fed by GLN-enriched diet resulted in a significant decrease in GLN concentration in body fluids and more pronounced decrease in protein synthesis in the liver, jejunum, colon, and spleen when compared with animals fed before starvation by a basal diet [79].

In conclusion, the reports summarized in this article demonstrate that enhanced intake of most amino acid supplements can cause adverse side effects. Further research is necessary to elucidate effects of high doses and long-term consumption of amino acids on immune system, brain function, muscle protein balance, synthesis of toxic metabolites, and tumour growth and examine their suitability under certain circumstances. These include elderly, childhood, pregnancy, and nursing a baby and medical condition, such as diabetes and liver disease. Studies are also needed to examine adaptive response to a long-term intake of any substance and consequences of discontinuation of supplementation.

## **Conflict of interest**

The author declares no conflicts of interest.

## **Acknowledgements**

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## **Abbreviations**

AHR, aryl hydrocarbon receptors, ARG, arginine; BA,  $\beta$ -alanine; BCAA, branched-chain amino acids; BCKA, branched-chain keto acids; CAR, carnosine; CIT, citrulline; GLN, glutamine; HIS, histidine;

HMB,  $\beta$ -hydroxy- $\beta$ -methylbutyrate; LEU, leucine; NO, nitric oxide; NOS, nitric oxide synthase; ROS, reactive oxygen species; TRP, tryptophan.  $\alpha$ -KG,  $\alpha$ -ketoglutarate.

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### Figure Legends

**Fig. 1.** Possible side effects of increased amino acid intake.

**Fig. 2.** The role of ARG in NO and ROS formation by phagocytes during sepsis. PRR, pathogen recognition receptor; PAMP; pathogen-associated molecular pattern; PC, pentose cycle.

**Fig. 3.** Effects of BCAA load on amino acid metabolism.

On the left: BCAA administration leads to the release of GLN, alanine and BCKA from muscles. Glutamine is catabolized in visceral tissues to form ammonia. Part of the BCKA released from the muscles is used for BCAA synthesis.

On the right: Effects of hyperammonaemia. Ammonia detoxification to GLN increases flux of BCAA through BCAA aminotransferase and the drain of  $\alpha$ -KG from citric cycle. 1, BCAA aminotransferase; 2, BCKA dehydrogenase; 3, GLN synthetase; 4, alanine aminotransferase.

**Fig. 4.** CIT and ARG metabolism. 1, argininosuccinate synthetase; 2, argininosuccinate lyase; 3, nitric oxide synthase. AS, argininosuccinate.

**Fig. 5.** GLN synthesis and utilization in healthy conditions and severe illness. In a healthy individual, there is a balance between synthesis and utilization of GLN. In severe disease, GLN utilization often exceeds its synthesis.

Fig.1

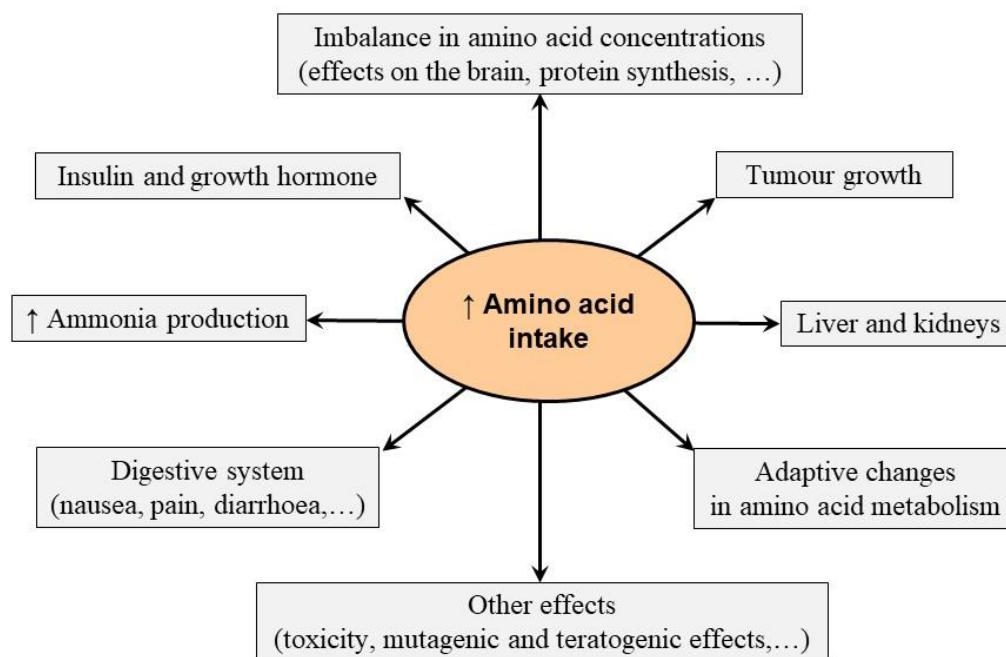


Fig.2

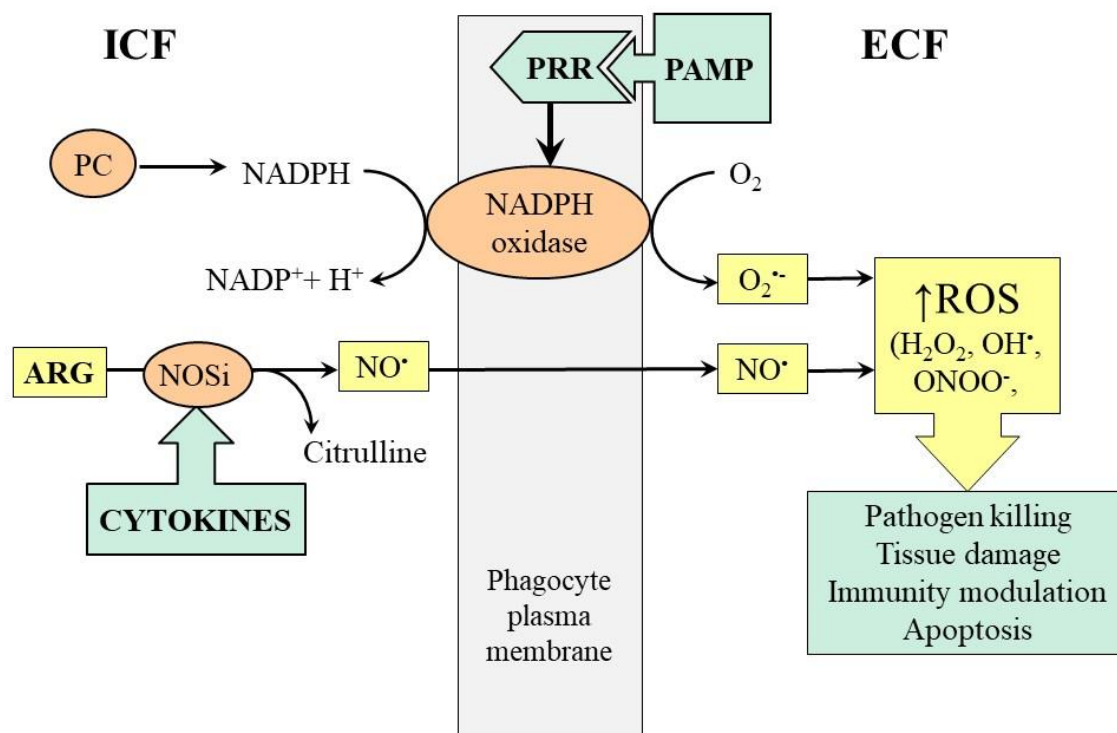




Fig.5

