Amino acid levels following beef protein and amino acid supplement in male subjects

Anthony M Uhe MSc, Kerin O'Dea PhD and Greg R Collier PhD

School of Nutrition and Health, Deakin University, Geelong, Australia

In the present study the plasma amino acid response of six lean subjects to a protein meal and a commercial amino acid supplement were compared. The amino acid supplement studied was formulated and marketed to be taken after exercise and at other times with the aim of increasing protein synthesis and/or decreasing protein degradation and to lower the ratio of tryptophan to the other large neutral amino acids (LNAA); tyrosine, valine, leucine, isoleucine, phenylalanine and methionine (trp/LNAA), to reduce fatigue. The amino acid supplement administered at the dose recommended by the manufacturer (4 g) was able to bring about a rapid but short-lived (15-30 min) increase in plasma amino acid concentrations and to produce a similarly brief decrease in the trp/LNAA and tyr/LNAA ratios and therefore achieved these aims with respect to amino acid levels even if only briefly. The changes in trp/LNAA and tyr/LNAA ratios after the supplement were of the same order as those produced after the much larger (50 g) protein meal but of shorter duration. However the relatively small insulin response after the amino acid supplement points to a lower level of amino acid uptake by muscle and other tissues for protein synthesis compared to that produced by the beef meal.

Key words: Amino acids, beef, supplements, insulin, LNAA (large neutral amino acids)

Introduction

In view of the current popularity of amino acid supplements, particularly with athletes, we compared a commercially available amino acid supplement with a muscle protein source, lean beef to determine the relative effectiveness of these two sources of amino acids in modulating the plasma amino acid profile. Amino acid supplements are marketed with many claims including enhancement of sporting performance, increasing protein synthesis, decreasing protein degradation, accelerating tissue repair and altering neurotransmitter levels affecting fatigue and mood. It has even been proposed that effects such as weight loss and increased fatty acid oxidation can be produced by amino acid supplements[1-5]. There is a large range of different amino acid supplements available (from single amino acids to complex mixtures), but a paucity of information in the literature on their effects, benefits and hazards in humans. Most research on the administration of amino acids to humans has been in the area of parenteral and enteral nutrition and not in supplementation of the diets of healthy, active individuals or elite athletes.

One aspect of amino acid supplementation potentially of interest, is whether they effect the levels of the precursors of neurotransmitters. Monoamine neurotransmitters such as serotonin (5-hydroxytryptamine) and the catecholamines (noradrenaline and dopamine) have been implicated in the regulation of fatigue, food intake, mood, pain sensitivity and sedative effects[1,4,6,7]. The level of serotonin and catecholamines are influenced by the level of their precursors tryptophan (trp) and tyrosine (tyr) in the brain which in turn depend on the competition between these amino acids and the other large neutral amino acids (LNAA, tyrosine, valine, leucine, isoleucine, phenylalanine and methionine[6] for transport across the blood brain barrier.

Amino acid supplements have the potential to alter or modulate all of these effects. However, the plasma amino acid profile following amino acid supplements has not been investigated. In the current study we compared the amino acid profile of the test meal with the plasma amino acid profiles, glucose and insulin levels in six lean male subjects in response to a meal of lean beef containing 50g of protein and 4g of the amino acid supplement.

Experimental

Subjects

Six lean male subjects were recruited: age 19.5 ± 1.2 years (mean ± SEM), (range 17 - 25), and mean body mass index BMI 21.7 ± 0.7 kg/m^2 (range 19.4 - 23.4). Only male subjects were included in this study because of reports of changes in trp/LNAA ratios during the menstrual cycle[7]. Ethics approval was obtained from the Human Ethics Committee at Deakin University and all subjects gave their informed consent.

Protocol

Subjects consumed two "meals" in random order after an overnight fast: 230g of grilled lean topside steak, equivalent to 50g of protein and 6g of fat, and 4g of the amino acid supplement "Kuan the Creative" (Musashi Pty Ltd Mulgrave Victoria Australia). The dose was that recommended by the manufacturer: a 4g dose twice a day or immediately after sport/exercise. Both the amino acid supplement and the beef meal were taken with a glass of water (200ml). Subjects were requested to chew their beef

Correspondence address: Dr Greg R Collier, School of Nutrition and Public Health, Deakin University,
Geelong VIC 3217 Australia
Tel: +61 (3) 5227 1323; Fax: +61 (3) 5227 2170
Email: beacon@deakin.edu.au

meal thoroughly and complete the meal within 15 minutes, the amino acid supplement was consumed in the form of a powder. To control activity the subjects remained seated during the course of the study.

Blood samples were collected from a soft indwelling catheter inserted into an antecubital vein 15 minutes before and immediately before commencing the meal and then at 15, 30, 45, 60, 90, 120, 150 and 180 minutes after the commencement of eating. Blood samples were collected into 5 ml fluoride heparin tubes, placed on ice and the plasma separated by centrifugation within 30 minutes of the last sample. Samples were then stored frozen either at -20°C for glucose and insulin or –70°C for amino acid measurements.

Analysis

Amino acids were measured by an automated HPLC method with ophthaldialdehyde pre-column derivatisation and separation on a reversed phase column. Samples were deproteinised with acetonitrile prior to analysis and homoserine was used as the internal standard, the coefficient of variation for the assay was 5.2% for plasma samples[8]. The amino acid supplement (300mg) was mixed with water and acidified with HCl to ensure that the amino acids had dissolved and made up to a total volume of 100ml, a sample of this solution was then analysed as for the plasma samples. The amino acid composition of the beef was determined by the same method after lipid extraction and hydrolysis with HCl and 2-mercaptoethanol[9]. The nonprotein amino acid content of the beef protein was also measured in tissue homogenate to assess the contribution of nonprotein amino acids to dietary levels. A sample of beef was homogenised with a Ystral x10/20 tissue Homogeniser (Ystral GMBH Ballrechten-Dottingen) and 100µg sample of homogenate was analysed after deproteinisation as per the amino acid analysis method. The amino acid composition reported are the sum of the acid hydrolysis and tissue homogenate and are in the form of the free base. Glucose was measured in plasma by glucose oxidase assay (Peridochrom Glucose Boehringer Mannheim GMBH Diagnostica) on a centrifugal analyser (Centrifichem 550 Baker Instruments). Insulin was measured by radioimmunoassay, double antibody solid phase technique (Phadeseph Insulin RIA, Pharmacia Diagnostics AB Uppsala, Sweden). The fat content of the beef was determined by CEM rapid automated moisture and fat analyser (CEM Corp Indiana Trail NC, USA)[10] and was 1.6% wet weight.

Statistical analysis

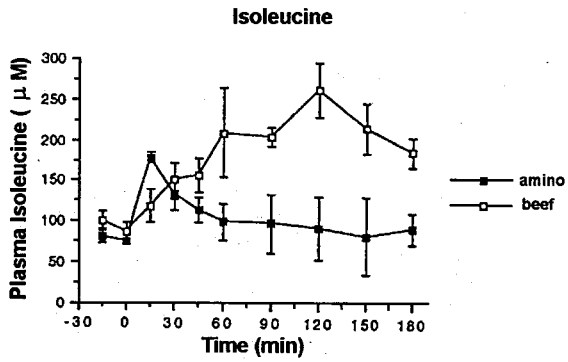
Analysis of variance (two-way ANOVA with. randomised block design) was used to determine the significance of any differences between the treatment groups and individuals with respect to glucose, insulin, amino acid profiles, trp/LNAA ratio and tyr/LNAA ratio profiles over time. Computer packages (Open Access 3 SPI) and Minitab were used for statistical analysis. Regression analysis was also performed where appropriate. P values < 0.05 were considered significant, all values are expressed as mean ± SEM.

Results

Amino acid composition of meals

Amino acid composition of beef from protein hydrolysis and homogenate was also analysed to determine the level of both protein and non-protein amino acids. The composition of the amino acid mixture was also analysed (Table 1). As would be expected the amino acid content of the beef meal was greater than the amino acid supplement. The amino acid supplement did not contain all amino acids found in protein.

Figure 1. Plasma isoleucine levels in response to a meal of 50g of protein in the form of lean beef topside (□) and 4g of the Musashi amino acid supplement (■) in 6 lean male subjects.



Plasma Amino Acids

Amino acid profiles were measured for 20 plasma amino acids. The profiles for the two meals were different with isoleucine being a representative of amino acids present in the amino acid supplement (Figure 1). Amino acid levels took significantly longer to reach peak levels after the beef meal (111 ± 8 minutes) than after the amino acid supplement (19 ± 2 minutes) ($p < 0.01$), this effect was consistent across all the amino acids measured.

Table 1. Amino acid content of each meal (g).

Amino acid (in the form of free base)	Beef 50g protein	Amino acid supplement 4g supplement
Aspartic acid (asp) & Asparagine (asn)	7.9	n
Glutamic acid (glu) & Glutamine (gln)	10.2	n
Serine (ser)	4.2	n
Histidine (his)	1.1	0.26
Glycine (gly)	4 3	0.33
Threonine (thr)	4.0	0.25
Arginine (arg)	3.6	0.13
Taurine (tau)	0.23	n
Alanine (ala)	4.6	n
Tyrosine (tyr)	1.9	0.13
Tryptophan (trp)	0.07	n
Methionine (met)	0.75	0.04
Valine (val)	3.4	0.25
Phenylalaninie (phe)	1.7	0.28
Isoleucine (ile)	3.0	0.5
Leucine (leu)	3.9	0.9
Lysine (lys)	3.4	0.19

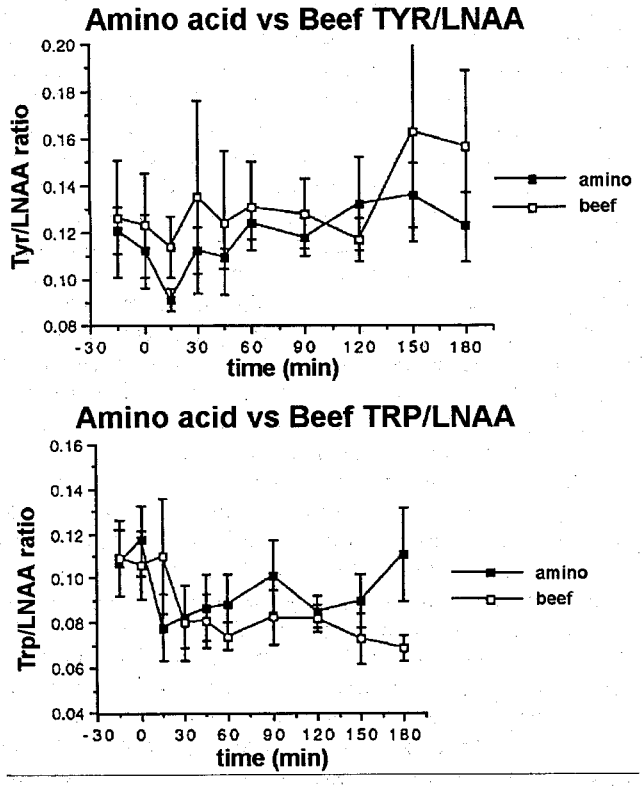
n - not present in supplement.

Tyr/LNAA and trp/LNAA ratios

The tyr/LNAA ratios reached a minimum at 15 minutes after both meals. The tyr/LNAA ratio being significantly lower at 15 minutes after the amino acid supplements ($p < 0.05$) than the beef meal (Figure 2a). Trp/LNAA ratio declined significantly from baseline after both the meals ($p < 0.05$) with the minimum being reached earlier after the

amino acid meal than the beef meal (Figure 2b). However the ratio returned to normal for the amino acid supplement while the ratio after the beef meal was still depressed after 180 minutes although the differences between the two treatments with respect to the trp/LNAA ratio failed to reach the level of significance.

Figure 2. Plasma tyr/LNAA (a) and trp/LNAA ratio (b) in response to a meal of 50 g of protein in the form of lean beef topside (□) and 4g of the Musashi amino acid supplement (■) in 6 lean male subjects.



Insulin and Glucose

Insulin levels increased significantly more after the beef meal compared with the amino acid supplement (Figure 3a) ($p < 0.05$). The insulin values reached a peak at 15 and 60 minutes for the amino acid supplement and beef respectively. Correspondingly, glucose levels declined significantly after both meals with minimum values at 45 and 90 minutes for amino acid supplement and beef respectively (Figure 3b) ($p < 0.05$ and $p < 0.01$ respectively).

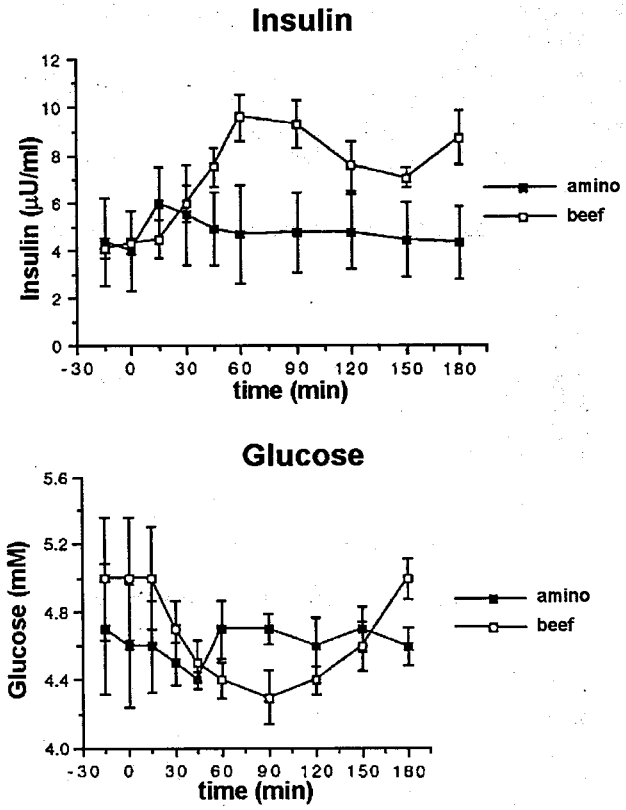
Correlation between dietary and plasma amino acid levels

The amino acid composition of meals was plotted against the increase in plasma amino acid levels expressed as total incremental area (the incremental area being the area above baseline under the curve of the amino acid response) (Figure 4). Linear regression showed good correlation with essential amino acids except for lysine after the beef meal (4a) and good correlations with all amino acids after the amino acid supplement (4b) ($r = 0.927$ and 0.930 for the beef and amino acid supplement respectively).

Discussion

The beef meal was larger in volume and contained more amino acids, in the form of protein (50g) than the supplement which contained free amino acids (4g). However the aim of this study was to give the supplement at the recommended dose and to compare this with the response to a standard protein meal. This particular amino acid supplement is formulated (according to the manufacturer) to be taken after exercise and at other times with the aim of increasing protein synthesis and/or decreasing protein degradation ("allowing a man to rebuild his body to its ideal state" according to the advertising material) and to lower the trp/LNAA ratio, purportedly to reduce fatigue. From our observations the amino acid supplement was able to bring about a rapid but short-lived increase in plasma amino acid concentrations and to produce a decrease in the trp/LNAA and tyr/LNAA ratios.

Figure 3. Plasma insulin (a) and plasma glucose (b) in response to a meal of 50 g of protein in the form of lean beef topside (□) and 4g of the Musashi amino acid supplement (■) in 6 lean male subjects.

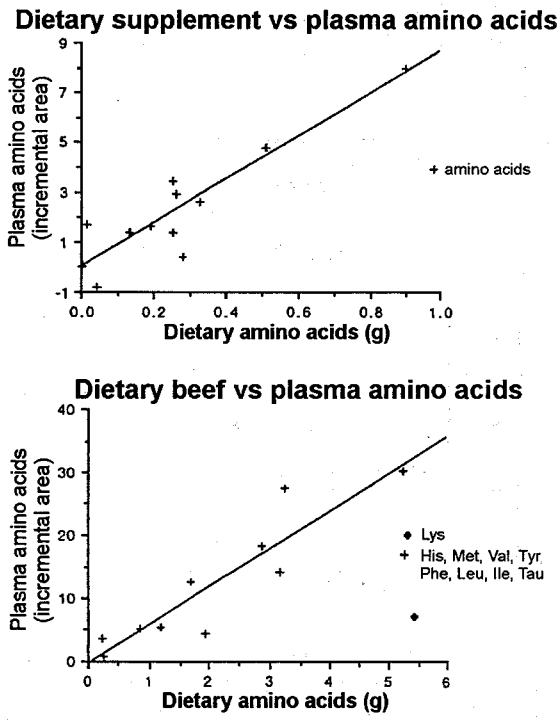


The amino acid supplement resulted in a rapid increase (15 minutes) and subsequent decline in plasma amino acids indicating that the free amino acids found in the amino acid supplement are absorbed quickly into the bloodstream and cleared quickly (within 60 minutes of ingestion). However after the beef meal plasma amino acids did not peak until much later (120-150 minutes) reflecting the time that would be needed to digest the protein in the beef and absorb the resulting amino acids and peptides. Plasma levels of most amino acids were elevated significantly above baseline from 30 minutes after ingestion of the beef meal and were maintained for at least three hours compared to the more transient increase after the amino acid supplement.

The tyr/LNAA ratio reached a minimum at 15 minutes after both meals with the decline being significantly greater after the amino acid supplements than the beef meal. Trp/LNAA ratio declined significantly after both the meals with the decline after the amino acid meal being faster than

that for the beef meal. However, the ratio returned to normal levels while the ratio after the beef meal was still depressed after 180 minutes. The differences between the trp/LNAA ratio for the beef and the amino acid supplement were not statistically significant. The decline in the trp/LNAA and tyr/LNAA ratio is due to relatively lower levels of trp and tyr in muscle protein and absence of trp and low levels of tyr in the amino acid supplement compared to the levels of the other LNAA (Table 1). It is notable that a small amount of amino acid supplement (4g) was able to produce effects (at least briefly) on these amino acid ratios of the same order or greater than a large protein meal (50g), but of much shorter duration. The physiological significance of these changes in plasma amino acids is unknown. The putative effects of these changes on mood and fatigue were not measured in this study.

Figure 4. Correlation between dietary amino acid levels (g) and plasma amino acid levels (incremental area) in response to meal of 50g of protein in the form of (a) lean beef topside (+) (Amino acids: His, Met, Val, Leu, Ile, Tyr, Tau, Phe), r = 0.914, (◆) Lys and (b) 4 g of the Musashi amino acid supplement (+) (Amino acids: His, Gly, Hr, Rag, Tyr, Met, Val, Phe, Ile, Leu, Lys), r = 0.930 in six lean male subjects.



Several recent studies have looked at the effect of exercise on amino acid concentration and metabolism[1,11-13]. Amino acid supplements have the potential to alter or modulate metabolism after exercise, by changes in trp/LNAA and tyr/LNAA ratios and in the levels of other important amino acids such as gluconeogenic precursors and branched chain amino acids. However, more work is needed on the short term nature of the changes in amino acid levels compared with the longer term changes seen after the beef meal before the potential effects flowing from them can be characterised.

The insulin level increased significantly more after the beef meal than the amino acid supplement. However, the increase, while large in percentage terms, was only small compared with that after glucose or a mixed meal. The increased insulin response to the beef meal is attributable to the greater quantity of amino acids released after hydrolysis compared with the supplement (50g of protein vs 4g free amino acids) via amino acid stimulation of insulin release[14-16]. Insulin levels peaked at 15 minutes for the amino acid supplement in agreement with the peak amino acid levels. However the insulin peaked earlier than the amino acid levels after the beef meal (60 vs 120 minutes). The greater insulin response to the beef meal compared with the amino acid supplement would be expected to coincide with increased amino acid uptake into muscle and other tissues and protein synthesis. This would suggest that the anabolic effects of the beef meal would be greater than that produced by the amino acid supplement. Glucose levels declined by a small but significant amount after both meals with minimum values at 45 and 90 minutes for amino acid supplement and beef respectively probably reflecting the earlier insulin response to the amino acid supplement compared to the beef meal (15 vs 60 minutes). These data are similar to the results reported by Schmid et al[16] after a tenderloin pork meal and intravenous amino acid infusions.

There was good correlation between plasma amino acid and the amino acids found in the supplement; the correlation was similarly strong for all essential amino acids (EAA) except for lysine. Relative plasma concentrations of lysine increased proportionately less than the other EAA. Of the non-essential amino acids only taurine increased to the same extent as the EAA. Plasma alanine, arginine, serine and glycine increased to a lesser extent than the EAA. Aspartate/asparagine and glutamate/ glutamine showed very little increase in plasma levels compared to dietary levels probably due to splanchnic and hepatic uptake[3]. This is in accordance with the findings of other workers who have found similar correlations between dietary and plasma amino acid in both humans[15,17,18] and the rat[19]. It would be possible on the basis of this sort of correlation to predict with some degree of accuracy the likely plasma response to amino acid supplements and to a lesser extent dietary protein in the absence of carbohydrate induced insulin effects.

Conclusion

The particular amino acid supplement studied is formulated to be taken after exercise and at other times with the aim of increasing protein synthesis and or decreasing protein degradation and to lower the trp/LNAA ratio. From our observations the amino acid supplement was able to bring about rapid but short-lived increase in plasma amino acid concentrations and to produce a decrease in the trp/LNAA and tyr/LNAA ratios. However the physiological significance of these changes in plasma amino acids is unknown. The relatively small insulin response after the amino acid supplement points to a lower level of amino acid uptake by muscle and other tissues for protein synthesis compared to that produced by the beef meal.

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男性補充牛肉蛋白質和氨基酸後的血漿氨基酸水平

摘要

作者選擇了 6 位 17～25 歲的瘦男子（BMI 19.4～23.4）爲對象，比較了補充蛋白質膳食和市售氨基酸後對他們血漿氨基酸水平的影響。氨基酸補充研究的目的是：在運動後和其它時間，能否增加蛋白質的合成和／或減少蛋白質的分解，能否降低色氨酸與其它大量中性氨酸(LNAA)，如酪氨酸、纈氨酸、亮氨酸、異亮氨酸、苯丙氨酸和蛋氨酸的比值（$\frac{\text{trp}}{\text{LNAA}}$），能否減少疲勞。氨基酸每次補充量爲 4 克是按照製造商的意見，他們認爲可迅速並短暫增加血漿氨基酸濃度，並同時可短暫降低 $\frac{\text{色氨酸}}{\text{LNAA}}$ 和 $\frac{\text{酪氨酸}}{\text{LNAA}}$ 的比值。雖然補充氨基酸後，胰島素的反應較補充牛肉膳食爲低，這指出了補充氨基酸後肌肉和其它組織對氨基酸的攝取和蛋白質的合成較低。但是在補充氨基酸後，$\frac{\text{色氨酸}}{\text{LNAA}}$ 和 $\frac{\text{酪氨酸}}{\text{LNAA}}$ 比值的變化與補充牛肉蛋白質膳（50 克）的次序相同，僅時間較短而已。

References

1. Blomstrand E, Celsing F, Newsholme EA. Changes in plasma concentrations of aromatic and branched-chain amino acids during sustained exercise in man and their possible role in fatigue. Acta Phisiol Scand 1988; 133:115-121.
2. Cerretelli P, Marconi C. L-carnitine supplementation in humans. The effects on physical performance. Int J Sports Med 1990; 11:1-12.
3. Christensen HN. Role of amino acid transport and countertransport in nutrition and metabolism. Physiol Rev 1990; 70:43-71.
4. Lopez-Ibor JJ. The involvement of Serotonin in Psychiatric disorders and behaviour. Brit J Psych 1988; 153:26-39.
5. May ME, Buse MG. Effects of branch-chain amino acids on protein turnover. Diabetes/Metabolism Rev 1989; 5:227-245.
6. Anderson GH. Metabolic regulation of food intake. In: Shils ME, Young VR, eds. Modern Health and Disease 7th edition. Philadelphia: Lea and Febiger 1989, 557-569.
7. Anderson GH, Li ETS. Protein and amino acids in the regulation of quantitative and qualitative aspects of food intake. Int J Obesity 1987, 11(suppl 3):97-101.
8. Uhe AM, Collier GR, McLennan EA, Tucker DJ, O'Dea K. The quantitation of tryptophan and other plasma amino acids by automated precolumn derivatization high performance liquid chromatography: Improved sample preparation. J Chromatogr 1991; 564:81-91.
9. Ng LT, Pascaud A, Pascaud M. Hydrochloric acid hydrolysis of proteins and determination of tryptophan by reversed-phase high performance liquid chromatography. Anal Biochem 1987; 167:47-52.
10. Mann N, Sinclair A, Watson M, O'Dea K, Evaluation of rapid fat determination in meats using the CEM automated analyser. Food Australia 1991; 43:67-69.
11. Conlay LA, Wurtman RJ, Lopz G-Coviella I, Blusztajn JK, Vacanti CA, Logue M, During M, Caballero B, Maher TJ, Evoiuk G. Effects of running the Boston marathon on concentrations of large neutral amino acids. J Neural Transm 1989; 76:65-71.
12. Devlin JT, Brodsky I, Scrimgeour A, Fuller S, Bier M. Amino acid metabolism after intense exercise. Am J Phisiol 1990; 258:E249-E255.
13. Einsparh KJ, Tharp G. Influence of endurance training on plasma amino acid concentrations in humans at rest and after intense exercise. Int J Sports Med 1989; 10:233-236.
14. Beylot M, Chambrier C, Moneger A, Cohen R. Effect of small variations in insulin and glucagon levels on plasma amino acids concentrations. Diabete & Metabolisme 1989; 15:38-44.
15. Nuttall FQ. Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. Diabetes Care 1984; 7:465-470.
16. Schmid R, Schusdziarra V, Schulte-Frohlinde E, Maier V, Classen M. Role of amino acids in stimulation of postprandial insulin, glucagon and pancreatic polypeptide in humans. Pancreas 1989; 4:305-314.
17. Ashley DV, Barclay DV, Chauffard FA, Moennoz D, Leathwood PD. Plasma amino acid response in humans of differing nutritional composition. Am J Clin Nutr 1982; 36:143-153.
18. Fernstrom JD, Wurtman RJ, Hammarstrom-Wiklund B, Rand WM, Munro N, Davidson CS. Diurnal variations in plasma concentrations of tryptophan, tyrosine, and other neutral amino acids: effect of dietary protein intake. Am J Clin Nutr 1979; 32:1912-1922.
19. Johnson D, Anderson GH. Prediction of plasma amino acid concentration from diet amino acid concentration. Am J Physiol 1982; 243:R99-R103.