ELSEVIER

Participation of blood cells in the changes of blood amino acid concentrations during maximal exercise

A. Aguiló, E. Castaño, P. Tauler, M.P. Guix, N. Serra, and A. Pons

Bioquímica i Biologia Molecular, Departament de Biologia Fonamental i Ciències de la Salut, Universitat de les Illes Balears, and Servicio de Análisis Clínicos, Laboratorio del Carmen, Hospital Son Dureta, Balears, Spain

We determined the participation of the cellular compartment in the changes of plasma amino acid concentrations during maximal exercise test on a cycle ergometer. Following an overnight fast, male athletes were submitted to a maximal exercise test until fatigue (for 25 min approximately) to determine maximal oxygen uptake. The amino acid concentrations in total blood, plasma, and blood cells were determined before and after the maximal exercise test. Most essential amino acids were decreased significantly in the total blood concentration as a result of the maximal exercise test. However, the concentrations of most nonessential amino acids tended to be significantly increased. Amino acid concentration was increased most in plasma. Concentrations of blood cell alanine and proline were significantly increased by 26% and 15%, respectively, after the maximal exercise test. No significant differences in blood cell concentrations of other amino acids induced by the exercise test were found, although the amount of tryptophan in blood cells was increased after exhaustive exercise. (J. Nutr. Biochem. 11:81–86, 2000)

Keywords: amino acid; tryptophan; glutamine; exercise; erythrocyte; fatigue

Introduction

It has been suggested that changes in plasma amino acid concentration could play a role in central fatigue by increasing the rate of synthesis and hence the level of the neurotransmitter 5-hydroxytryptamine in some parts of the brain.[1] The entry of tryptophan, the plasma precursor of 5-hydroxytryptamine, into the brain is influenced by the plasma level of free tryptophan (that not bound to albumin) and, due to competition for entry into brain, by the plasma level of branched chain amino acids (BCCA).[2] Experimental evidence exists that the supplementation of BCAA in the drink of athletes during competition produces an improvement in their performance[3] and in the perception of mental effort,[4] although a balanced review of the literature does not confirm any important ergogenic effect of BCAA intake. However, a possible explanation for central fatigue in prolonged exercise related to amino acid metabolism is that the plasma level of BCAA falls and that of fatty acid increases: The latter increases the free tryptophan level so that the plasma concentration ratio (free tryptophan/BCAA) increases, thus leading to higher levels of tryptophan and therefore of 5-hydroxytryptamine in the brain.[5]

Blood amino acids are distributed between the plasma and cellular compartments, and the importance of erythrocytes in amino acid transport has been noted in several classical studies[6–8] as well as in more recent studies.[9–14] Amino acid distribution between plasma and blood cells is subject to important changes induced by different nutritional and physiologic conditions, such as starvation and obesity, and is also determined by gender and genetic and hormonal factors.[10,13–16] Generally, blood amino acid changes during exercise are described only in the plasma fraction and after prolonged and intense exercise protocols.[1,3,17] However, the participation of the erythrocytes and

Address correspondence to Dr. A. Pons, Bioquímica i Biologia Molecular, Departament de Biologia Fonamental i Ciències de la Salut, Universitat de les Illes Balears, Cra Valldemossa km 7.5, 07071-Palma de Mallorca, Balears, Spain.
Received June 17, 1999; accepted October 25, 1999.

655 Avenue of the Americas, New York, NY 10010
0955-2863/00/$–see front matter
PII S0955-2863(99)00073-X

the changes during intense short exercise protocols are not known.

The goal of this study was to determine the participation of the cellular compartment in the changes of plasma amino acid concentrations during intense maximal exercise of well-trained athletes. The general trend is that plasma presents the main changes of amino acid concentration during maximal exercise, with few changes in the concentration in cell amino acids, but in the case of tryptophan and alanine the participation of blood cells in the total blood amino acids transport is evidenced by the blood cells' retention of tryptophan in the bloodstream during intense maximal exercise.

Materials and methods

Subjects

Seven well-trained male athletes volunteered to participate in this study. They all trained 14 ± 1 h each week. Their mean (±SEM) age was 29.6 ± 2.4 yr, height 181 ± 5.2 cm, weight 76.7 ± 4.4 kg, body mass index 23.3 ± 0.5 kg/cm^2, and maximal oxygen uptake (VO_2 max) 65.1 ± 2.2 mL/kg/min.

Subjects were informed as to the purpose of the study and the possible risks involved before giving their oral consent to participate. The study was approved by the Ethical Committee at the Son Dureta University Hospital.

Experimental procedure

All exercise tests were performed on an electromagnetic cycle ergometer (Ergometrics 900, MedGraphics™, St. Paul, MN USA) equipped with a counter to measure the exact number of revolutions. The subjects exercised at a pedaling rate of 60 rpm. The exercise test was a maximal test, which ended when the subjects manifested their subjective fatigue status and when increased work did not increase or decrease oxygen consumption; this value was the VO_2 max. The duration of the test was 25.3 ± 0.9 min and the athletes explained their lack of leg muscular fatigue. Subjects warmed up on the cycle ergometer for 3 minutes at 30 watts prior to starting the test. The test started at 50 watts and the subjects' work rate was increased by 30 watts every 3 minutes until fatigue. Expired oxygen was continuously monitored with a Cardiopulmonary Exercise System CPX (MedGraphics™, St. Paul, MN USA) and VO_2 was determined automatically. Subjects reported to the laboratory in the morning after a 12-hour overnight fast during which they were able to drink mineral water ad libitum but not in the laboratory before the exercise test. During the exercise test they did not drink anything.

Blood samples were collected from the arm vein 5 minutes before exercise and 5 minutes after exercise in suitable vacutainers for biochemical and hematologic clinical parameters. The blood vacutainers, which were transported to other laboratories to analyze serum parameters and blood cell characters, were maintained at 4°C. The clinical analysis of these blood vacutainers were carried out the same morning. Sample preparations for amino acid analysis was carried out immediately after sampling.

Sample preparation and blood analyses

The levels of amino acids were determined in hemolyzed total blood and in plasma following the procedure described previously.[13] Plasma was obtained by centrifuging the blood (1,000 × g for 30 min) at 4°C. The plasma was diluted with an equal volume of working internal standard solution (L-methionine sulfone 0.4 mM). Hemolysis of blood was achieved by diluting the blood (1:1) with a solution of internal standard as the case of plasma. The two fractions of blood were deproteinized with cold acetone (1:1.5, v/v)[18] and the protein-free supernatant fraction was used for individual amino acid measurements.

The amino acids were assessed by high performance liquid chromatography (HPLC), using the PICO.TAG™ method[19] developed by Waters Associates (Waters Chromatography Division, Milford, MA USA) by derivatization of amino acids using phenylisothiocyanate. L-methionine sulfone 0.4 mM was used as the internal standard. Sample amino acid levels were calculated from the peak area using the Maxima 820 program (Waters).

Hematologic parameters were assessed by Technicon H*2 (Bayer, Pittsburgh, PA USA) and serum parameters using a computer-controlled random access clinical chemistry analyzer such as Technicon (Bayer) DAX-78 (Toshiba, Tokyo, Japan). The accuracy of the latter has recently been published.[20]

Calculations and statistics

The blood cell amino acid concentrations (*C*) were calculated by subtracting the measured plasma concentration (*P*) from the measured total blood concentration (*B*) with a correction for hematocrit (*H*), taking into account that 10% of plasma was retained in the blood cell precipitate,[21] according to the following formula:

$$C = (B - (1 - H)P)/H$$

This formula was applied to each individual value of plasma, whole blood, and hematocrit of the same individual, before or after exercise, to obtain the individual values of amino acid concentrations in blood cells. This modus operandi was followed in all calculations made in this study.

Plasma volume (*PV*) loss (in percentage) during exercise was calculated from the hematocrit values before (*Hb*) and after (*Ha*) exercise, according to the following formula:

$$PV = 100 - ((100 - Ha)/(100 - Hb) \times)Hb/Ha))$$

This formula was developed from the presumption that cellular volume was maintained during exercise.

Plasma amino acid loss (*PAL*) was calculated from the difference (*D*) between plasma amino acid concentration (expressed per milliliter of blood) before (*Pb*) and after (*Pa*) exercise, but the latter was calculated from the real plasma concentration (*RPa*) after exercise, taking into account the loss of plasma volume (*PV*; expressed per milliliter). This difference was expressed as a percentage when divided by the blood concentration before exercise (*Bb*). The formulas applied were:

$$PAL = 100 \times D/Bb$$

$$D = (Pb - Pa)(1 - Hb)$$

$$Pa = RPa \times (1 - PV)$$

The meaning of the term PAL is to specify the quantity of amino acid loss from the plasma per each 100 units of amino acid present in blood during exercise.

The statistical analyses were made using the program StatView SE+Graphics™ (Abacus Concepts, Inc., Berkeley, CA USA). After calculating each parameter for each subject and situation, we compared the values after versus before exercise using the paired Student's *t*-test. The statistical comparison of the ratios between the values after and before exercise with respect to serum protein concentration or hematocrit were also made using the paired Student's *t*-test, taking into account the respective ratios for each individual subject.

Table 1 Hematological and metabolic parameters before and after acute intense exercise

	Before	After	Ratio
Blood cell parameters			
Hematocrit %	45.4 ± 1.1	48.8 ± 1.3*	1.07 ± 0.004
Erythrocyte number ($\times 10^{-6}$)	5.03 ± 0.10	5.37 ± 0.12*	1.07 ± 0.004
MCV (fL)	90.1 ± 0.7	90.8 ± 0.9	1.01 ± 0.004
MCH (pg/cell)	29.4 ± 0.2	29.5 ± 0.2	1.00 ± 0.005
Leukocyte number ($\times 10^{-3}$)	6.27 ± 0.69	8.55 ± 0.88*	1.37 ± 0.05†
Platelet number ($\times 10^{-3}$)	218 ± 18	263 ± 17*	1.22 ± 0.03†
Serum parameters			
Glucose (mg/100 ml)	85.7 ± 2.4	119 ± 6*	1.39 ± 0.05†
Urea (mg/100 ml)	29.9 ± 1.8	30.0 ± 1.7	1.01 ± 0.01†
Protein (g/100 ml)	6.95 ± 0.08	7.67 ± 0.10*	1.10 ± 0.01

The results are the mean ± SEM of 7 male athletes.
*$P < 0.05$. Paired Student's *t*-test, comparing before and after situation.
†$P < 0.05$. Paired Student's *t*-test comparing blood cell ratios *vs* hematocrit or serum parameters ratio *vs* serum protein ratio.
MCH–mean corpuscular hemoglobin.
MCV–mean corpuscular volume.

Results

Changes in the concentration of blood metabolites and in hematologic parameters were examined before and after maximal exercise of seven well-trained male athletes. Hematocrit and blood cell number, both leukocytes, platelets, and erythrocytes, increased significantly ($P < 0.05$ by paired Student's *t*-test) after intense acute exercise until extenuation (*Table 1*). However, specific characteristics of erythrocytes such as the mean corpuscular volume (MCV) or the mean corpuscular hemoglobin concentration (MCHC) were unchanged by this exercise protocol. The loss in plasma volume calculated using hematocrit was 12.7 ± 0.8% of plasma; the change in serum protein concentration was similar to that of hematocrit, as indicated by the lack of statistical differences between the serum protein concentration after/before ratio and the respective ratio for the hematocrit. The increases of leukocytes and platelets were higher than those of erythrocytes and hematocrit, in accordance with the significantly higher ratio presented by the former. A net increase of leukocytes and platelets, which was not attributed to the hemoconcentration observed with this exercise protocol, was produced.

Maximal exercise until fatigue induced significant alterations in the concentration of blood glucose and serum proteins, whereas the urea concentration remained unchanged (*Table 1*). The increase observed in glucose was higher than that of hematocrit; thus, a net appearance of glucose was evident in the blood at the end of the exercise, even after calculating these concentrations by taking into account the plasma volume loss. Urea concentration was not modified by exercise, but by taking into account the plasma volume loss, there was actually a net disappearance of seric urea during exercise.

Individual amino acid concentration changed in total blood as a result of intense acute exercise (*Table 2*). In general, the differences in blood amino acid concentration were very low between before and after exercise, but the statistical paired Student's *t*-test was able to detect the changes because the individual response was very homogeneous. The concentrations of most essential amino acids decreased significantly in total blood as a result of exercise. However, the concentrations of most nonessential amino acids tended to increase significantly. Thus, exercise produced a significant decrease in the total blood concentration of Trp, Phe, Val, Ile, Thr, Lys, Ser, and Hyp. The concentrations in total blood of Glu, Ala, Gly, and Arg significantly increased by exercise. The total blood concentrations of Tyr, Leu, Gln, Asp, Asn, Hys, Met, and Pro remained unchanged by exercise.

To differentiate the effects of plasma volume loss on total blood amino acid concentration from other effects, we statistically compared the ratio between amino acid concentration after/before exercise versus the same ratio for the hematocrit. Most amino acids presented an after/before ratio that was lower than that of the hematocrit. Only for Ala, Pro, and Arg was this ratio higher that of hematocrit. No significant differences were found for the cases of Glu, Asp, and Met.

On the whole, the changes described in blood amino acids can be attributed to plasma amino acids. Intense acute

Table 2 Changes in blood amino acid concentration before and after intense acute exercise

Amino acid	Before exercise (μM)	After exercise (μM)	Ratio A/B
Tryptophan	34.9 ± 1.7	30.0 ± 1.3*	0.863 ± 0.021†
Phenylalanine	60.6 ± 1.6	54.1 ± 2.3*	0.893 ± 0.031†
Tyrosine	61.4 ± 3.7	57.6 ± 4.6	0.933 ± 0.028†
Valine	254 ± 47	233 ± 47*	0.906 ± 0.015†
Leucine	89.3 ± 12.9	86.5 ± 13.4	0.964 ± 0.019†
Isoleucine	72.7 ± 10.3	66.3 ± 11.3*	0.896 ± 0.023†
Glutamic acid	122 ± 10	135 ± 10*	1.113 ± 0.031
Glutamine	716 ± 29	708 ± 33	0.987 ± 0.009†
Aspartic acid	62.7 ± 15.4	56.9 ± 11.6	0.990 ± 0.083
Asparagine	92.0 ± 5.6	90.8 ± 5.6	0.987 ± 0.009†
Alanine	333 ± 20	434 ± 26*	1.274 ± 0.038†
Serine	121 ± 7	112 ± 7*	0.926 ± 0.021†
Threonine	125 ± 15	106 ± 15*	0.837 ± 0.021†
Glycine	300 ± 13	305 ± 14	1.017 ± 0.004†
Histidine	62.4 ± 2.2	58.4 ± 3.7	0.932 ± 0.038†
Arginine	18.2 ± 2.6	21.5 ± 2.9*	1.209 ± 0.073†
Lysine	139 ± 17	129 ± 18*	0.921 ± 0.030†
Methionine	25.5 ± 1.5	26.7 ± 1.7	1.057 ± 0.065
Proline	208 ± 17	214 ± 20	1.023 ± 0.020†
Hydroxyproline	11.5 ± 1.3	11.0 ± 1.3*	0.956 ± 0.014†
Total	2914 ± 127	2930 ± 154	1.00 ± 0.01†
Essential	801 ± 71	731 ± 78*	0.91 ± 0.02†
Nonessential	2113 ± 84	2199 ± 100*	1.07 ± 0.004†
Trp/BCAA	0.094 ± 0.011	0.088 ± 0.010*	

For definition of parameters see Materials and methods.
The results are the mean ± SEM of 7 male athletes and are expressed as μmoles/L.
*$P < 0.05$. Paired Student's *t*-test, comparing before and after situation.
#$P < 0.05$. Paired Student's *t*-test comparing all ratios with respect to the hematocrit ratio (1.074 ± 0.004).
BCAA— branched chain amino acids.

Table 3 Amino acid concentration in plasma and blood cells before and after intense acute exercise

Amino acid	Plasma			Blood cells	
	Before	After	% Loss	Before	After
Tryptophan	62.2 ± 4.1	49.3 ± 2.8*	32.7 ± 5.9†	UD	5.42 ± 1.74
Phenylalanine	72.6 ± 6.3	56.5 ± 3.0*	22.6 ± 5.3†	43.7 ± 7.9	51.3 ± 3.3
Tyrosine	61.4 ± 3.9	57.1 ± 34.7	11.7 ± 2.9†	61.1 ± 5.2	58.1 ± 4.6
Valine	308 ± 62	270 ± 58*	18.0 ± 3.5†	178 ± 32	185 ± 35
Leucine	102 ± 15	95.1 ± 16*	13.9 ± 2.5†	70.7 ± 12	75.7 ± 11
Isoleucine	86.9 ± 13.0	76.7 ± 13.2*	17.8 ± 3.1†	52.0 ± 9.4	52.8 ± 9.7
Glutamic acid	41.0 ± 3.9	53.1 ± 4.0*	−3.23 ± 2.86	238 ± 16	239 ± 18
Glutamine	838 ± 40	797 ± 43	11.9 ± 2.6†	536 ± 38	595 ± 38
Aspartic acid	5.85 ± 0.44	6.15 ± 1.00	1.07 ± 0.70	147 ± 39	123 ± 26
Asparagine	67.8 ± 5.4	58.7 ± 4.2*	10.4 ± 1.5†	127 ± 6	132 ± 8
Alanine	349 ± 24	492 ± 34*	−14.1 ± 2.3†	294 ± 19	372 ± 23*
Serine	113 ± 5	98.4 ± 6.4*	13.9 ± 3.2†	132 ± 14	129 ± 8
Threonine	145 ± 19	119 ± 19*	20.7 ± 3.6†	94.9 ± 13	89.3 ± 14
Glycine	242 ± 16	227 ± 18	8.87 ± 1.89	383 ± 16	405 ± 12
Histidine	68.3 ± 3.6	63.3 ± 3.8	12.5 ± 3.7†	53.5 ± 3.5	51.7 ± 4.2
Arginine	52.3 ± 5.3	59.1 ± 7.1*	1.79 ± 10.3	UD	UD
Lysine	148 ± 21	126 ± 25*	17.5 ± 1.8†	128 ± 21	136 ± 20
Methionine	28.0 ± 1.6	25.9 ± 1.8	12.5 ± 2.9†	22.1 ± 2.3	27.7 ± 3.0
Proline	235 ± 18	229 ± 24	11.0 ± 3.1†	169 ± 19	194 ± 15*
Hydroxyproline	12.8 ± 1.4	11.8 ± 1.3	13.3 ± 4.8†	9.39 ± 2.0	9.81 ± 1.8
Total	3054 ± 149	2952 ± 202	10.1 ± 2.8†	2704 ± 162	2908 ± 156
Essential	952 ± 98	818 ± 101*	18.5 ± 3.0†	584 ± 59	624 ± 62
Nonessential	2102 ± 93	2133 ± 131	6.93 ± 2.68†	2120 ± 113	2283 ± 114
Trp/BCAA	0.139 ± 0.016	0.126 ± 0.01*		UD	0.019 ± 0.008

Results are the mean ± SEM of 7 male athletes and are expressed as μmoles/L.
*$P < 0.05$, before versus after by paired Student's *t*-test.
†$P < 0.05$, % loss significantly different to 0.
UD–undetected. BCCA–branched chain amino acids.

exercise produced a loss of most amino acids from plasma (*Table 3*). We defined the plasma amino acid loss as the percentage of loss or gain (negative values) of plasma amino acids during the exercise with respect to the amino acid concentration present in total blood before exercise. The difference between the plasma amino acid concentration after and before exercise was achieved by correcting the plasma amino acid concentration after exercise by taking into account the plasma volume loss. During intense exercise to extenuation only Glu, Asp, and Arg maintained an unalterated plasma content. Only Ala was incorporated to plasma during exercise. The other amino acids were lost from plasma during exercise in a greater proportion in the case of essential amino acids compared with that of nonessential amino acids Trp being the amino acid that had the greatest loss from plasma.

Most amino acids did not change their blood cell concentrations after acute intense exercise. Only Ala and Pro increased their concentration significantly in blood cells by exercise. It is interesting to note that Trp was incorporated into blood cells during exercise. Before exercise, the Trp concentration was undetected in blood cells, but after exercise Trp was present in blood cells. This mechanism allowed approximately 22% of plasma Trp loss into bloodstream to be retained in the cellular compartment. On the other hand, Arg was undetected both before and after exercise in the blood cell compartment.

Discussion

Maximal exercise until fatigue on a cycle ergometer has been shown to be an appropriate protocol to study blood amino acid metabolism. Large changes in human skeletal muscle amino acid concentrations and NH_3 production have been described after similar, or even shorter, maximal exercise until fatigue protocols.[22]

Maximal exercise on the cycle ergometer employed in this study produced an increase of hematocrit and number of erythrocytes mostly as a result of loss of plasma volume, because the erythrocyte parameters MCV and MCH were maintained after exercise. The decrease in plasma volume during exercise is higher than values previously described[23,24] during similar types of exercise, probably attributable to the lack of drinking water in our experimental design. The loss of plasma volume would produce an increase in serum levels of metabolites and plasma amino acids; however, we found a slight decrease or no increase in the concentration of most amino acids, indicating an important amino acid output from blood to tissues during exercise. Plasma amino acid uptake is mediated by cellular carriers. Their function during short-term exhaustive exercise could adjust the concentration of most amino acids to a slightly decreased value after exercise; however, we can expect the anionic amino acid transport systems to decrease because Glu and Asp plasma concentration increase after exercise in the same order as the hemoconcentration.

Measures of nitrogen balance indicate that the current protein recommended daily allowance is inadequate for the protein demands of athletes in training,[25] although protein requirements of athletes may be easily attained by a normal increase in food intake. The components of protein metabolism that are altered by intense physical activity and training are not well understood. This study shows that the total blood concentration of some essential amino acids are higher in athletes than in a healthy human population[13] and are used during intense exercise.

Amino acid tissue utilization in most cases is regulated by tissue uptake.[26] Thus, the plasma amino acid output observed during maximal exercise can be related to a possible amino acid utilization for energy purposes during exercise, mainly by the muscle. In fact, during maximal exercise, muscle alanine increases,[22] probably as a result of utilization of amino acids and glucose or glycogen, and the rate of alanine release from muscle also increases.[8,27] The muscle alanine produced is driven to liver for glucose synthesis de novo. During maximal exercise until fatigue, both alanine and glucose present high levels in blood, probably indicating an important operation of glucose-alanine cycle. The elevated concentration of glucose in blood could facilitate its uptake by muscle, which in turn could use it to obtain energy to maintain the intense work. This availability of glucose at the end of testing could indicate a more general fatigue than a local muscle fatigue, as indicated by the participants in the test. It is noticeable that alanine is transported both in plasma and in blood cells (*Table 3*). In fact, after exercise only 69% of blood alanine surplus is transported in plasma whereas the rest is in the blood cell compartment. The alanine transport in blood cells allows a decrease in the plasma alanine concentration and would then facilitate the alanine output from muscle. We have obtained in vivo evidence for the role of erythrocytes in the interorgan transport of proline from muscle in fed and starved rats.[28] Starvation increases the participation of blood cells in the proline transport from muscle to splachnic bed of rats.[28] The existence of an intercellular proline cycle between erythrocytes and hepatocytes that brings oxidizing equivalents to the red blood cells has been proposed.[29] The increase of approximately 15% in blood cell proline concentration after exercise is in accordance with the operation of a muscle-hepatocyte proline cycle during exercise.

The greatest amount of amino acid extraction during exercise occurs in plasma, whereas blood cells maintain practically the same amino acid concentration, participating to a minor extent in buffering blood amino acid changes during exercise. Blood cell amino acid concentrations are maintained practically unaltered by exercise, but in the same period the number of leukocytes and platelets is increased, indicating that their contribution to blood cell amino acid concentration is practically negligible; thus, this blood cell amino acid pool could be mainly attributed to the erythrocyte fraction. Although the participation of erythrocytes in buffering amino acid output from the bloodstream during exercise is in general limited, blood cells buffer the output from the bloodstream of tryptophan, an amino acid that participates in the mechanism of central fatigue.[15] Trp uptake by tissues such as the brain competes with other large neutral chain amino acids such as BCAA; however, the increased concentration of Trp in blood cells is not accompanied by a comparable increase in BCAA, indicating a specific uptake of Trp by erythrocytes. The tryptophan uptake by blood cells could delay the appearance of central fatigue. Several factors are known to alter the amino acid distribution between plasma and blood cells.[10,12–15,30] We have pointed out the existence of an amino acid pool that is transported in the bloodstream adsorbed onto the erythrocyte membranes in addition to those that are dissolved in the plasma and the intracellular compartment.[9,11] This amino acid pool, as part of the cellular compartment, also undergoes deep changes in differing physiologic and nutritional conditions.[11–13] The human erythrocyte membrane capabilities of absorbing Trp are not known and would need further investigation because this could be a system to modify the availability of blood Trp to peripheral tissues such as the brain.

Acknowledgments

This work has been supported by grants PB92-0748 from the Dirección General de Investigación Científica y Técnica (DGICYT) of the Spanish government and from the Conselleria d'Educació, Cultura i Esports of the Balear goverment. We are grateful to Ms. E. Fuentespina and Ms. M. Parera for excellent analytical assistance.

References

1 Newsholme, E.A. and Blomstrand, E. (1996). The plasma level of some amino acids and physical and mental fatigue. *Experientia* **52,** 413–415
2 Fernstrom, J.D. (1990). Aromatic amino acids and monoamine synthesis in the CNS: Influence of diet. *J. Nutr. Biochem.* **10,** 508–517
3 Blomstrand, E., Hassmén, B., Ekblom, B., and Newsholme, E.A. (1991). Administration of branched-chain amino acids during sustained exercise–effects on performance and on plasma concentration of some amino acids. *Eur. J. Appl. Physiol.* **63,** 83–88
4 Newsholme, E.A., Blomstrand, E., Hassmén, P., and Ekblom, B. (1991). Physical and mental fatigue: Do changes in plasma amino acids play a role? *Biochem. Soc. Trans.* **19,** 358–362
5 Newsholme, E.A. and Blomstrand, E. (1995). Tryptophan, 5-hydroxytryptamine and a possible explanation for central fatigue. *Adv. Exp. Med. Biol.* **384,** 315–320
6 Elwyn, D.H., Launder, W.J., Parikh, H.C., and Wise E.M. Jr. (1972). Roles of plasma erythrocytes in interorgan transport of amino acid in dogs. *Am. J. Physiol.* **222,** 1333–1342
7 Felig, P. (1975). Amino acid metabolism in man. *Annu. Rev. Biochem.* **44,** 933–952
8 Felig, P., Wahren, J., and Raf, L. (1973). Evidence of interorgan amino acid transport by blood cells in humans. *Proc. Natl. Acad. Sci. USA* **70,** 1775–1779
9 Picó, C., Pons, A., and Palou, A. (1991). A significant pool of amino acids is adsorbed on blood cell membranes. *Biosci. Rep.* **11,** 223–230
10 Picó, C., Pons, A., and Palou, A. (1992). Effect of diet-induced obesity on kinetic parameters of amino acid uptake by rat erythrocytes. *Biochem. Int.* **28,** 509–517
11 Picó, C., Pons, A., and Palou, A. (1995). Decrease of the pool of amino acids adsorbed on blood cell membranes caused by starvation in rats. *Life Sci.* **7,** 675–683
12 Proenza, A.M., Palou, A., and Roca, P. (1994). Amino acid distribution in human blood. A significant pool of amino acids adsorbed onto blood cell membranes. *Biochem. Mol. Biol. Int.* **34,** 971–982
13 Proenza, A.M., Roca, P., Virgili, J., Remesar, X., Alemany, M., and Palou, A. (1995). Estrogen effects on blood amino acid compartmentation. *Life Sci.* **57,** 1589–1597
14 Serra, F., Picó, C., Johnston, J., Carnie, J., and Palou, A. (1993).

Opposite response to starvation of Trp/LNAA in lean and obese Zucker rats. *Biochem. Mol. Biol. Int.* **29,** 483–491
15 Gianotti, M., Roca, P., and Palou, A. (1990). The effects of cafeteria diet induced obesity on rat blood amino acid compartmentation. *Arch. Int. Physiol. Biochim.* **98,** 155–161
16 Serra, F., Johnston, J., Carnie, J., and Palou, A. (1991). Altered blood amino acid distribution in genetically obese mice. *Biochim. Biophys. Acta* **1097,** 289–292
17 Van Hall, G., Saris, W.H., and Wagenmakers, A.J. (1998). Effect of carbohydrate supplementation on plasma glutamine during prolonged exercise and recovery. *Int. J. Sports Med.* **19(2),** 82–86
18 Arola, L.L., Herrera, E., and Alemany, M. (1977). A new method for deproteinization of small samples of blood plasma for amino acid determination. *Anal. Biochem.* **82,** 236–239
19 Bidlingmeyer, B.A., Cohen, S.A., and Tarvin, T.L. (1984). Rapid analysis of amino acids using the column derivatization. *J. Chromatogr.* **336,** 93–104
20 Parera, M., Guix, P., Pérez, G., Quetglas, P., Fuentespina, E., and Llompart, I. (1996). Estudio del error aleatorio en distintos parámetros de rutina bioquímica en el autoanalizador DAX-78. *Quim. Clin.* **15,** 232–240
21 Ferrando, A., Bobadilla, I.G., Bobadilla, M.S., Palou, A., and Alemany, M. (1981). Comparative estimation of haematocrit and trapped plasma in the packed cell volume in man, rabbit and chicken blood. *Comp. Biochem. Physiol.* **70A,** 611–613
22 Katz, A., Broberg, S., Shalin, K., and Wahren, J. (1986). Muscle ammonia and amino acid metabolism during dynamic exercise in man. *Clin. Physiol.* **6,** 365–379
23 Blomstrand, E., Andersson, S., Hassmén, P., Ekblom, B., and Newsholme, E.A. (1995). Effect of branched-chain amino acid and carbohydrate supplementation on the exercise-induced change in plasma and muscle concentration of amino acids in human subjects. *Acta Physiol. Scand.* **153,** 87–96
24 Montain, S.J. and Coyle, E.F. (1992). Fluid ingestion during exercise increases skin blood flow independent of increases in blood volume. *J. Appl. Physiol.* **83,** 903–910
25 Lemon, P.W.R. (1991). Protein and amino acid needs of the strength athlete. *Int. J. Sport Nutr.* **1,** 127–145
26 Christensen, H.N. (1990). Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol. Rev.* **70,** 43–77
27 Ahlborg, G., Felig, P., and Hagenfeldt, L. (1974). Substrate turnover during prolonged exercise in man. *J. Clin. Invest.* **53,** 1080–1090
28 Pericás, J., Picó, C., Matamala, J.C., Palou, A., and Pons, A. (1992). Metabolic utilization of muscular L-proline in 24-hr starved rats. *Int. J. Biochem.* **11,** 1725–1730
29 Phang, J.M., Yeh, G.C., and Hagedorn, C.H. (1980). The intercellular proline cycle. *Life Sci.* **28,** 53–58
30 Picó, C., Pons, A., and Palou, A. (1992). Regulation of rat erythrocyte L-glutamine, L-glutamate and L-lysine uptake by short term starvation. *Int. J. Biochem.* **24,** 1731–1735