Amino Acids (2004) 26: 71–76
DOI 10.1007/s00726-003-0008-x

Amino Acids
Printed in Austria

Plasma amino acids and sports injuries

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Received April 15, 2002
Accepted February 10, 2003
Published online June 6, 2003;

Summary. The aim of this study was to explore the relationship between changes in plasma amino acids and the incidence of sports injuries during a soccer season. Fourteen plasma amino acids were assayed at monthly intervals in 12 young soccer players during a whole soccer season. Based on the number and severity of injuries the soccer players were divided into an injury-prone and a non-injury-prone group. The mean plasma level of the amino acid glycine was significantly lower ($P = 0.009$) in the injury-prone group than the other group, while the mean plasma levels of tyrosine, tryptophan and lysine were higher in the injury-prone group during this period ($P < 0.05$). However there were no significant differences in the calculated plasma tryptophan and tyrosine/large neutral amino acids ratios. Significant linear time trends were observed for taurine, ornithine, lysine and the tryptophan/large neutral amino acids ratio.

These results indicate that the plasma concentrations of glycine and to a lesser extent those of tyrosine, tryptophan and lysine may be promising peripheral markers for injury-proneness in young soccer players. Whether a role for glycine substitution will be indicative to reduce the occurrence of sports injuries will need to be investigated in future studies.

Keywords: Amino acids – Glycine – Tryptophan – Tyrosine – Injury-prone – Plasma

Introduction

There are differences in the occurrence of sports injuries in soccer players of a Dutch soccer team. Based on data of a relationship between a cerebral dysfunction of the monoaminergic neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) and aggressive behaviour, inwardly or outwardly directed (Van Praag et al., 1986; Mann, 1995), we were interested to know whether 5-HT was also involved in the occurrence of sports injuries. Animal studies by Bailey et al. (1992) showed that drugs which enhanced brain 5-HT reduced treadmill run time in rats, while the opposite effect emerged with 5-HT antagonists (Bailey et al., 1993a and b). The synthesis and metabolism of brain 5-HT increases in response to exercise (Barchas and Freedman, 1963; Blomstrand et al., 1989; Chaoulouff, 1989).

However in humans it is difficult to investigate directly central 5-HT function. Since serotonergic transmission depends, in part, both on its release from nerve terminals and on its synthesis from the precursor tryptophan, in these present study we investigated whether changes in the level of brain tryptophan are associated with the occurrence of sports injuries. As tryptophan cannot be directly measured in the brain, we assayed plasma concentrations of tryptophan and of other large neutral amino acids (LNAA): tyrosine, phenylalanine, valine, isoleucine and leucine. These five LNAA share the same uptake system as tryptophan, which means that competition exists between these LNAA for entry in the brain (Fernström and Wurtman, 1972) such that changes in the ratio of the plasma concentration of tryptophan to the sum of the other LNAA (tryptophan-ratio) might indicate changes in brain tryptophan levels which in turn reflects 5-HT release (Møller et al., 1996). It may be argued that it is actually the free tryptophan level which will traverse the blood-brain barrier, such that its measurement may be a more pertinent measure than total levels of this amino acid. Nevertheless, in many studies the ratio of the plasma level of total tryptophan to the summed concentrations of the other LNAA is considered as a good peripheral index of tryptophan availability to the brain (DeMyer et al., 1981; Curzon and Sarna, 1984).

It is also hypothesized that enhancement of transmission in dopaminergic and noradrenergic systems is related to aggressivity, which in turn may result in enhanced incidence of injuries (Mann, 1995). Central 5-HT levels are dependent on the concentration of tryptophan in brain, while the cerebral levels of the neurotransmitters dopamine and noradrenaline are dependent on their precursor amino acid tyrosine. Therefore, in order to obtain some peripheral indication on the functioning of the latter neurotransmitters, we also calculated, analogous to the determination of the tryptophan-ratio, the so-called tyrosine-ratio.

The objective of this study was to explore the relationship between changes in plasma amino acid concentrations, with particular emphasis on the tryptophan- and tyrosine-ratios, and their relationship with the incidence of sports injuries.

Materials and methods

Subjects and procedures

Twelve male soccer players (age between 14 and 17 yr) from the Philips Sport Vereniging (PSV, a Dutch soccer team in the premier league), who were trained for future professional soccer activities, agreed to participate in this study. Subjects were asked to keep a daily diary of the type, circumstance and severity of possible injuries during a whole soccer season. The latter comprised a period of 10 months (November until next August) in which 8 blood samples were taken on a monthly basis, except for the months of June and July. Blood sampling was performed just before the Monday training at 6:00 p.m. and approx. 5 hrs after the regular lunch, which consisted of 3–5 slices of bread containing cheese, meat or jam. The latter precautions make an effect on the plasma amino acids unlikely. Appropriate ethical safeguards and protocols have been followed.

Since no suitable instrument to assess propensity to injury was available during our experiments, we decided to compose a registration protocol ourselves in order to record systematically the injuries of the soccer players. These players were given a diary format in which they had to record both the amount of training hours during weekdays and the very real time in the field during the weekends. The injuries including the moment and kind of occurrence were recorded. Every event which occurred after direct (e.g. kicking against limbs) or indirect contact (e.g. strain one's ankle) with another player, which resulted in (partly) stopping of either the current or next training session or match was considered to be an injury. In addition, the players were asked to record their daily food intake at breakfast, lunch and dinner. The diary was checked every two weeks by the first author. A crosscheck for the injuries was conducted by means of the logbook of the medical staff of PSV. If the player had to stop the exercise or to quit the game, the injury was scored positively. Every day immediately after an injury, which caused the player to miss training or needed adapted training, was scored positively as well. On basis of these data two experts (MB and HJD) categorized independently whether the soccer player was injury-prone or not injury-prone. In case of discrepancy, the two experts judged in consensus.

Biochemical analysis

EDTA blood samples (7 ml) were collected, cooled on ice and transported to the laboratory within 2 hrs. The plasma was isolated by centrifugation at 2650 g_{max} for 20 min at 20°C and this specimen was stored at -70°C until amino acid analysis.

Fourteen amino acids were assayed by high-performance liquid chromatography (HPLC) using automated pre-column derivatization with *o*-phthaldialdehyde (Fekkes et al., 1995). The intra-assay coefficient of variation of all amino acids measured was below 4%, while the inter-assay variation was also below 4% except for taurine (4.4%) and methionine (4.6%). In addition to the assay of total amino acids, we calculated the tryptophan- and tyrosine-ratio. The tryptophan-ratio was calculated by dividing the total plasma tryptophan level by the sum of the other LNAA, which compete for the transport of tryptophan through the blood-brain barrier. The tyrosine-ratio was calculated analogously by substituting tryptophan for tyrosine.

Statistical analyses

The data of 10 out of 12 soccer players were used for calculations. One player was lost to follow up after the first blood sample and another player was left out because of acute hepatitis with consequent disturbances of the amino acid concentrations. Data are presented as mean ± standard deviation, unless otherwise stated. Level of significance was set at 0.05 (two-sided).

The relationships between the sixteen biochemical variables and the physical injury were explored by the Student *t*-test for independent variables and by the method of random regression modeling (RRM) after logarithmical transformation of the data. RRM can flexibly cope with different error covariance structures, time-varying and time-constant covariables, (individual-specific) unequal time intervals between the measurements. This approach allows individual-specific deviations from the average trend. In addition, it can handle missing data under realistic assumptions. The type of error covariance structure was 'unstructured', that is to say that the (co)variances of the repeated measurements were allowed to be different (Molenberghs and Verbeke, 1997). The analyses were conducted with the computer package SAS version 6.12 (Statistical Analysis Systems, 1991).

Results

Based on the injuries recorded by the soccer players and scored by the experts, it was decided that 4 of the 10 remaining soccer players were injury-prone. Table 1 shows the mean plasma amino acid levels and the tryptophan- and tyrosine ratios in both the injury-prone and noninjury-prone group during the whole soccer season. In view of legibility the values of all 8 time points first were averaged for each individual before the mean values of the parameters were calculated. Significant higher mean concentrations of tyrosine, tryptophan and lysine were observed in the injury-prone group compared to the noninjury-prone group ($P = 0.03$). However, no differences were found for the tryptophan- and tyrosine-ratios. Furthermore, the mean plasma level of glycine in the injury-prone soccer players was significantly lower than in the noninjury-prone group ($P < 0.01$). It has to be noted that the latter statistical calculations have some limitations, i.e. the time points were put together before the data were analyzed, which makes the analysis less appropriate. Therefore, we reanalyzed the data using the method of random regression modeling. With this method all time points can be taken into consideration without making any restrictions, and not only potential differences in plasma amino acid levels between injury-prone and noninjury-prone players during

Table 1. Mean plasma amino acid levels and the tryptophan- and tyrosine ratios in injury-prone and noninjury-prone soccer players for the period of November 1993 to August 1994 including

Amino acid	Plasma concentration (μmol $\cdot$ l^{-1})	
	Injury-prone group ($n = 4$)	Noninjury-prone group ($n = 6$)
Serine	108.6 ± 11.9	115.2 ± 24.1
Glycine	216.9 ± 21.2	257.2 ± 49.1*
Arginine	61.0 ± 19.7	57.5 ± 16.0
Alanine	472.8 ± 66.0	485.4 ± 99.3
Taurine	38.8 ± 7.2	38.6 ± 8.4
Tyrosine	75.2 ± 15.2	66.8 ± 15.0*
Valine	260.0 ± 40.9	247.7 ± 54.2
Methionine	27.7 ± 4.9	26.6 ± 6.6
Tryptophan	52.7 ± 6.7	48.7 ± 6.9*
Phenylalanine	69.3 ± 8.1	65.3 ± 13.4
Isoleucine	88.2 ± 15.6	83.3 ± 20.5
Leucine	159.2 ± 24.3	149.1 ± 34.5
Ornithine	99.6 ± 28.6	107.6 ± 25.6
Lysine	169.3 ± 37.2	148.7 ± 35.8*
Tryptophan-ratio	8.2 ± 1.0	8.2 ± 1.4
Tyrosine-ratio	12.0 ± 2.1	11.3 ± 1.4

Data are presented as mean ± SD. For each individual, plasma amino acid levels measured during the period of November 1993 to August 1994 including (8 time points) were first summed and averaged before the mean values for both groups were calculated. Statistical testing was done using Student *t*-test for independent variables. *$p < 0.05$ as compared with injury-prone soccer players

the whole soccer season can be determined, but also the existence of a possible time trend can be analyzed. Table 2 shows the results of this analysis. It was found that the plasma levels of glycine in the injury-prone soccer players were significantly lower ($P = 0.009$) compared to the noninjury-prone group. Also relevant are the higher levels of tyrosine ($P = 0.046$), tryptophan ($P = 0.046$) and lysine ($P = 0.043$) in the plasma of the injury-prone versus noninjury-prone soccer players. On the contrary, no differences were found for the tryptophan- and tyrosine-ratios.

In addition, it can be seen (Table 2) that taurine and the tryptophan-ratio showed a significant negative linear time trend ($P = 0.000$ and 0.024, respectively), while this trend was positive for ornithine and lysine ($P = 0.015$ and 0.022, respectively). Although, a positive trend was also found for alanine, valine, tyrosine and the tyrosine-ratio, no significance was reached. For none of the parameters was a significant effect modification found, which means that there were no differences in time trend between both groups (data not shown). Figures 1 and 2 show the course of the mean plasma amino acid levels and their ratios in the injury-prone and noninjury-prone groups, which differ with regard to linear time trend or injury-proneness.

Table 2. Linear time trends of and differences in plasma amino acid levels in injury-prone ($n = 4$) and noninjury-prone soccer players ($n = 6$), as assessed by the method of random regression modeling

Amino acid	Linear time trend		Injury vs. noninjury	
	b-value	*p*-value	*b*-value	*p*-value
Serine	0.015 ± 0.012	0.206	− 0.015 ± 0.104	0.882
Glycine	− 0.006 ± 0.009	0.526	**− 0.219 ± 0.080**	**0.009**
Arginine	− 0.029 ± 0.018	0.110	0.084 ± 0.157	0.593
Alanine	0.021 ± 0.011	0.053	− 0.064 ± 0.096	0.510
Taurine	**− 0.045 ± 0.011**	**0.000**	− 0.009 ± 0.074	0.903
Tyrosine	0.025 ± 0.013	0.068	**0.217 ± 0.106**	**0.046**
Valine	0.020 ± 0.011	0.066	0.151 ± 0.103	0.150
Methionine	0.019 ± 0.015	0.215	0.169 ± 0.099	0.095
Tryptophan	− 0.005 ± 0.009	0.570	**0.143 ± 0.062**	**0.024**
Phenylalanine	0.014 ± 0.009	0.145	0.125 ± 0.094	0.188
Isoleucine	− 0.006 ± 0.014	0.669	0.129 ± 0.115	0.267
Leucine	0.011 ± 0.013	0.369	0.138 ± 0.103	0.190
Ornithine	**0.033 ± 0.013**	**0.015**	− 0.052 ± 0.156	0.742
Lysine	**0.032 ± 0.014**	**0.022**	**0.227 ± 0.110**	**0.043**
Tryptophan-ratio	**− 0.019 ± 0.008**	**0.024**	0.002 ± 0.091	0.980
Tyrosine-ratio	0.014 ± 0.007	0.054	0.083 ± 0.081	0.310

Data are presented as regression coefficients (*b*) with their respective standard errors (SE) and *p*-values. The *b*-values of the linear time trend indicate the linear slope across time and the *b*-values of injury vs. noninjury signify the difference between the two groups (a positive value implies that the level of the injury-prone players is higher than that of the noninjury-prone players). The applied statistical method was random regression modeling. Bold values represent significant findings

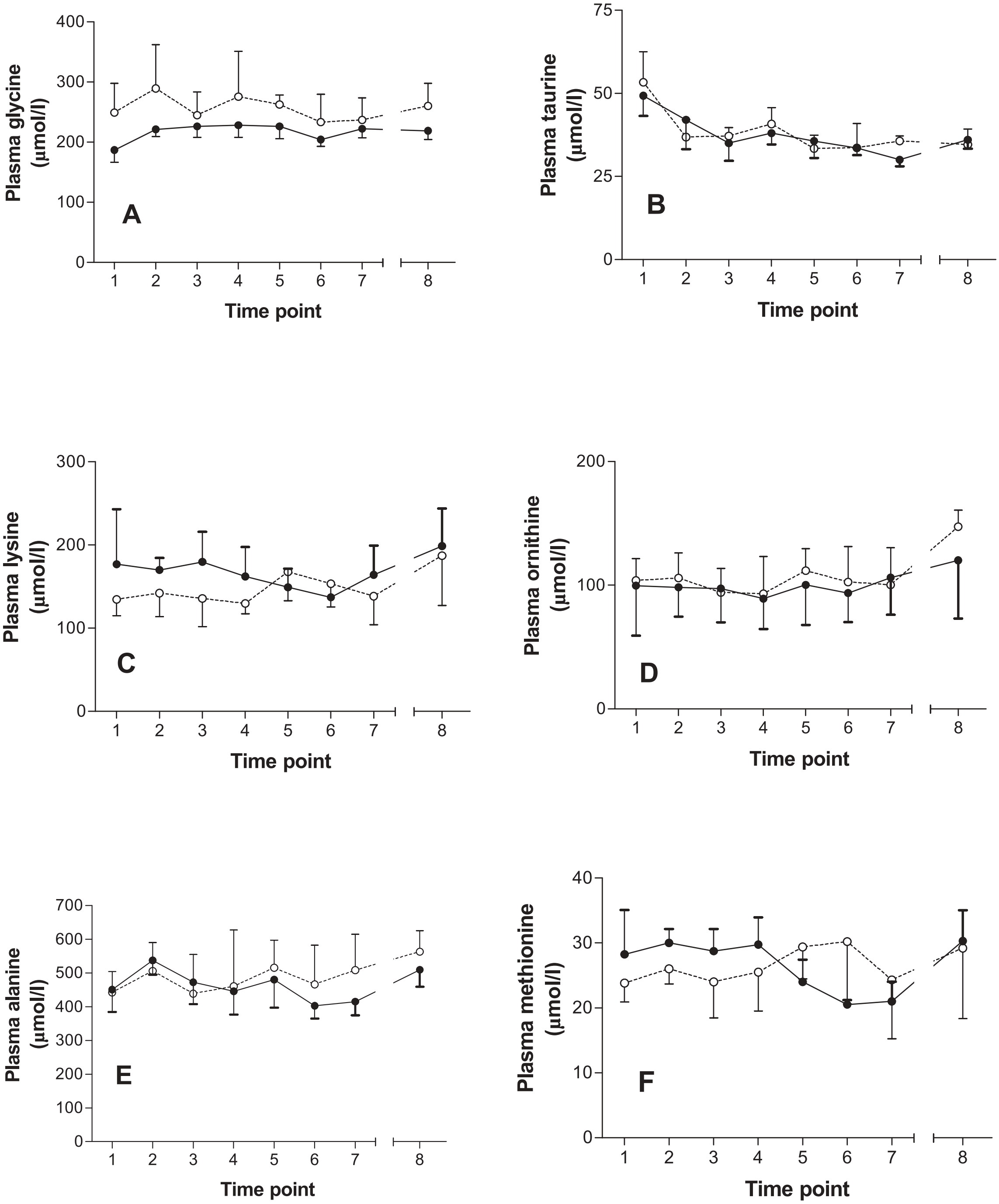

Fig. 1. Plasma concentrations of glycine (**A**), taurine (**B**), lysine (**C**), ornithine (**D**), alanine (**E**) and methionine (**F**) in injury-prone (•, $n = 4$) and noninjury-prone soccer players (○, $n = 6$). Values (means ± standard deviation) were measured from November (time point 1) until next August (time point 8)

When the data obtained from the continuous monthly samples (November to May) were analyzed, thus excluing the last time point (time point 8), the plasma levels of methionine in the injury-prone group turned out to be statistically significant higher ($P = 0.02$) than in the non-injury-prone group. With respect to the time trend, the significant alterations for the amino acids ornithine and lysine disappeared after exclusion of time point 8.

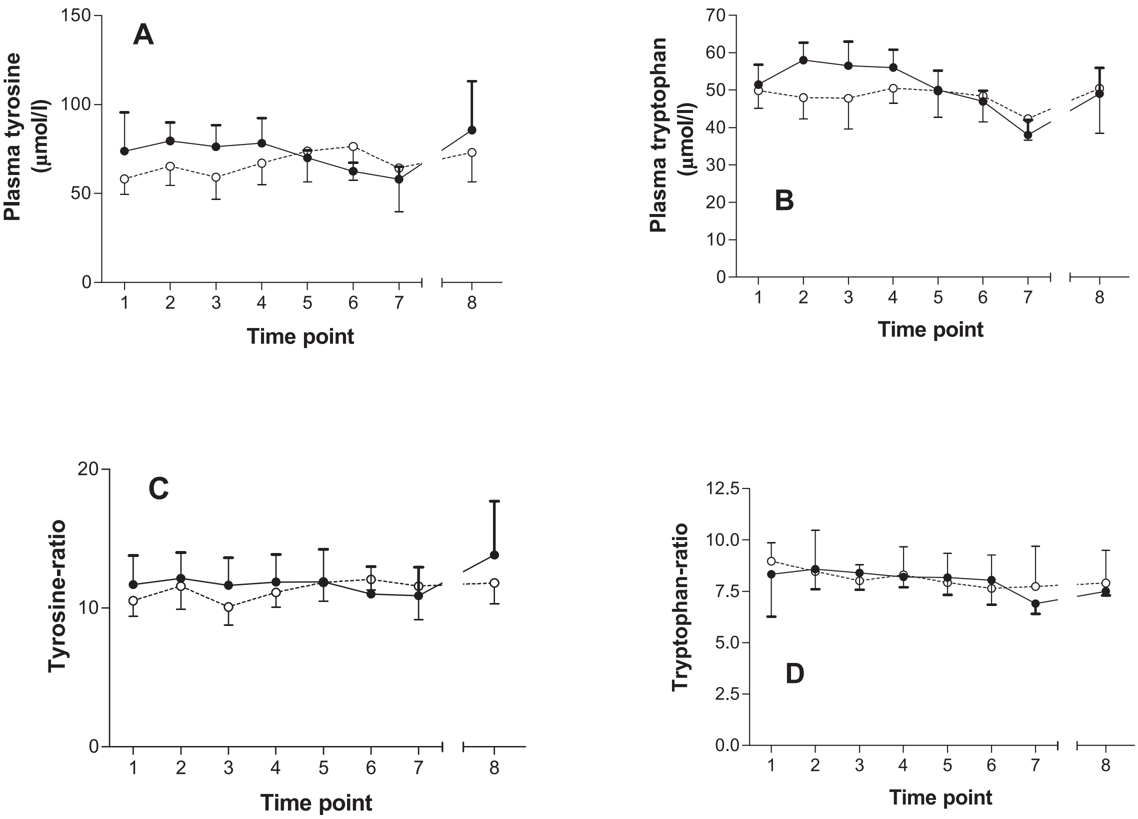

Fig. 2. Plasma concentrations of tyrosine (**A**) and tryptophan (**B**), and the plasma tyrosine- (**C**) and tryptophan-ratios (**D**) in injury-prone (•, $n = 4$) and noninjury-prone soccer players (○, $n = 6$). Values (means ± standard deviation) were measured from November (time point 1) until next August (time point 8)

Discussion

In order to obtain a better insight into how changes in the concentrations of various amino acids may lead to a greater propensity to injury, the plasma concentrations of several amino acids were assayed in young soccer players during a period of 10 months and correlated with injury-proneness. The major finding in this study was that the injury-prone soccer players exhibited a significantly lower plasma level of glycine during the whole period. Glycine is the main inhibitory neurotransmitter in the brainstem and spinal cord, where it participates in a variety of motor and sensory functions (Paul, 1995). Provided that the concentration of glycine in the peripheral blood is a reflection of its concentration in the central nervous system, the lower glycine levels in injury-prone soccer players may result in less inhibition of afferent stimuli entering the spinal cord. It may then be postulated that in individuals with low glycine levels the signals leaving the central nervous system contain more interfering noise. This may result in a less accurate reaction of the soccer player, ultimately increasing the risk of getting injured or injuring another person.

Although the concentrations of both tryptophan and tyrosine were higher in the plasma of the injury-prone group compared to the other group throughout the whole period, their respective ratios showed no difference (Tables 1 and 2, Fig. 2). Thus, our data do not suggest any difference between the two groups for 5-HT, dopamine or noradrenaline at a central level. This is in line with a study of Strüder et al. (1996), who showed that altering the peripheral level of tryptophan is of no influence on the central 5-HT level. Nevertheless, the increased plasma levels of tryptophan and tyrosine in the injury-prone group eventually may result in an increase in the peripheral amount of 5-HT and noradrenaline. As a consequence of this increase, vasoconstriction of all vessels by noradrenaline and of the large arteries by 5-HT, and vasodilatation of the arterioles by 5-HT may occur, resulting in a peripheral pooling of blood and subsequently an increase in heart rate and blood pressure (Guyton and Hall, 1996). All these physiological changes may give a sensation of stress, e.g. warm skin, pounding of the heart. We expect that this stress sensation decreases the mental comfort and alertness and, as a result, increases the chance of obtaining a sport's injury. Interestingly data in the literature has identified significantly positive correlations between plasma tryptophan concentrations in males and scores on extraverted aggression (Møller et al., 1996). This observation is in line with our finding of higher plasma tryptophan levels in injury-prone soccer players, assuming that these subjects indeed are more aggressive and that the supposed higher aggression ultimately will result in more injuries.

In conclusion, in a longitudinal study design we found a difference between the plasma concentrations of some amino acids in injury-prone and noninjury-prone soccer players. The combination of lower plasma glycine levels, higher plasma tryptophan and tyrosine levels may be responsible for the higher incidence of injuries. Further experiments with larger numbers of subjects and the inclusion of psychological questionnaires measuring aggression are clearly warranted to confirm these observations. In future studies, it is also worthwhile to examine whether glycine substitution has any effect on the incidence of sports injuries in soccer players.

Acknowledgements

The authors are grateful to Margriet Edelman for expert technical assistance.

References

Bailey SP, Davis JM, Ahlborn EN (1992) Effect of increased brain serotonergic activity on endurance performance in the rat. Acta Physiol Scand 145: 75–76

Bailey SP, Davis JM, Ahlborn EN (1993a) Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue. J Appl Physiol 74: 3006–3012

Bailey SP, Davis JM, Ahlborn EN (1993b) Serotonergic agonists and antagonists affect endurance performance in the rat. Int J Sports Med 14: 330–333

Barchas JD, Freedman DX (1963) Brain amines: response to physiological stress. Biochem Pharmacol 12: 1232–1235

Blomstrand E, Perrett D, Parry-Billings M, Newsholme EA (1989) Effect of sustained exercise on plasma amino acid concentrations and on 5-hydroxytryptamine metabolism in six different brain regions of the rat. Acta Physiol Scand 136: 473–481

Chaouloff F (1989) Physical exercise and brain monoamines: a review. Acta Physiol Scand 137: 1–13

Curzon G, Sarna GS (1984) Tryptophan transport to the brain: new findings and older ones reconsidered. In: Schlossberger HG, Kochen W, Linzen B, Steinhart H (eds) Progress in tryptophan and serotonin research. Walter de Gruyter, Berlin New York, pp 145–157

DeMyer MK, Shea PA, Hendrie HC, Yoshimura NN (1981) Plasma tryptophan and five other amino acids in depressed and normal subjects. Arch Gen Psychiatry 38: 642–646

Fekkes D, van Dalen A, Edelman M, Voskuilen A (1995) Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated derivatization with *o*-phthaldialdehyde. J Chromatogr B 669: 177–186

Fernström JD, Wurtman RJ (1972) Brain serotonin content: physiological regulation by plasma neutral amino acids. Science 178: 414–416

Guyton AC, Hall JE (1996) Textbook of medical physiology, 9th edn. WB Saunders Company, Philadelphia, pp 205–207, 210–213

Mann JJ (1995) Violence and agression. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology: the fourth generation of progress. Raven Press, New York, pp 1919–1928

Møller SE, Mortensen EL, Breum L, Alling C, Larsen OG, Bóge-Rasmussen T, Jensen C, Bennicke K (1996) Agression and personality: association with amino acids and monoamine metabolites. Psychol Med 26: 323–331

Paul SM (1995) GABA and glycine. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology: the fourth generation of progress. Raven Press, New York, pp 87–94

Statistical Analysis Systems (1991) SAS Campus Drive, Cary NC

Strüder HK, Hollmann W, Platen P, Duperly J, Fisher HG, Weber K (1996) Alterations in plasma free tryptophan and large neutral amino acids do not affect perceived exertion and prolactin during 90 min of treadmill exercise. Int J Sports Med 17: 73–79

Van Praag HM, Plutchic R, Conte H (1986) The serotonin hypothesis of (auto)agression. Critical appraisal of the evidence. Ann N Y Acad Sci 487: 150–167

Verbeke G, Molenberghs G (1997) Linear mixed models in practice. Springer, New York Berlin Heidelberg

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