

Changes in AAs in human plasma during 6-days ultra-endurance exercise

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Abstract

The study is an analysis of AAs during the 6-days ultra-endurance competition called the Adventure Racing World Championship (ARWC). Six males and one woman participated in the study. The race consisted of an 800 km long predetermined course that the athletes covered by a mixed transport of running, kayaking, cycling, in-line skating, climbing, caving and canyoneering. Blood samples were drawn in the morning the day before the race (PRE), in the middle of the race (72 h), immediately after the end of the race (POST) and 24 hours into recovery (REC). Of twenty AA, sixteen was analysed by reversed phase HPLC. Among these, we have selected the BCAA:s, taurine, glutamine, glutamate, phenylalanine and tyrosine which changes may be of special interest. Overall the AAs behaved as expected at the initiation of the exercise, adopted from earlier studies of endurance exercise, but further into the competition they showed some unpredicted results. For example, the concentrations of the BCAA:s increased by 15-68% between the 72 h- and POST-values which could be of significance for the performance due to the central fatigue hypothesis. The importance of the nutritional intake has been taken into the discussion and could be an important factor for the observed results.

Keywords

Adventure race, BCAA, taurine, glutamine, glutamate, phenylalanine, tyrosine, nutrition

Introduction

The importances of amino acids (AA) in physical performance, and changes during prolonged exercise, have had different meanings among scientists throughout the years. The effects of both short time exercise and endurance exercise reaching over three to four hours are known, but what happens in events longer in time? Changes in AA concentrations ([AA]) do affect several physiological factors as changes in brain monoamine metabolism [1] and the TCA cycle [2], having an impact on the physical fatigue and, thus, the performance.

Adventure racing is in a literal sense an adventure with the basic tenets of a start line, a finish line and a number of checkpoints in between. The participants' task is to cover the distance by foot, bike, kayak, climbing or a number of other alternatives that could be added. The conditions for the athletes in these kinds of events involve an extreme physiological strain, lack of energy and most of the time they are in a state of sleep deprivation. The food itself is not limited, but due to a combination of high energy expenditure and problems associated with intake and logistics, the participants will have an inadequate energy intake [3].

For our purposes the sport of adventure racing is a good opportunity to study due to its extreme length and strain on the participants. The sport is, however, still seen as quite a new area among both the athletes and the scientists. Previous research on AA metabolism during

ultra-endurance exercise and AA involvement is limited and experimental events that have been carried out is restricted to about maximum six hours [4] and a distances of up to 100 km [5]. In addition, only two previous studies have considered the total AAs aspect [6, 7]. But despite these limitations, conducted research gives us a good basis for possible mechanisms and functions in the body that are affected in extreme situations as in adventure racing. Something that should be noted is, nevertheless, that most studies also have been carried out in the fasting state and without supplementation [4]. In our study the athletes are well-fed at the start and have in addition a free access to food during the competition.

But, however, by knowing the alterations in extreme situations, we could not only update the knowledge for the adventure racers, but also get to know a lot about the human body in general. With these aspects in mind our interests lies in how the challenge affect the AA metabolism, and hence how any possible changes may affect the body and the athletes' performance. The aim of the present study is therefore to evaluate the changes in [AA] in human plasma during 6-days of ultra-endurance exercise.

Ultra-endurance exercise and amino acids

Some of the basic knowledge about the AAs is the fact that they are not a major source of energy, which is now widely known. We also know that they nonetheless has a variety of important functions in the body. The exercise will result, inter alia, by an increase in protein turnover in which the main cause of this is the increase in a variety of metabolic processes. Processes that could be mentioned are the hepatic gluconeogenesis and the citric acid cycle [7]. Other features are the importance for the central fatigue, physical fatigue, pain tolerance and mental alertness. There are 20 standard AAs in the body of which sixteen are included in our analysis. Nine of those are further considered indispensable or essential (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) and must come from the diet [8]. But, even if the AA:s all have an important significance for the change in metabolism and associated effects, we have chosen a few AA:s, listed below, which changes may be of special interest.

Branched Chain AAs

Human skeletal muscle are able to oxidize at least eight AAs (alanine, asparagine, aspartate, glutamate, isoleucine, leucine, lysine, and valine), but during exercise leucine, isoleucine and valine, the AAs which are also known as the branched AAs (BCAA), are preferred [8]. They are all essential for the human body and the main degradation is the one that occurs in skeletal muscle. Due to the oxidation during exercise [1, 7] they must be replenished by the diet [9] and supplementation has, in turn, been proposed to reduce protein degradation in muscles during exercise and affecting the brain by reducing fatigue. The explanation to the latter is supposed to be by decreasing the ratio between free tryptophan (unbound to albumin) and BCAA in plasma, suggesting that an increase in plasma BCAA can reduce the synthesis of 5-TH in the brain and enhance performance (The Central fatigue hypothesis [9]). The BCAA:s have earlier also been suggested to work as a fuel during exercise [10] but after that the enzymes involved in the oxidation of BCAAs were shown to be too low to allow a major contribution of BCAA:s to energy expenditure [11, 12] this proposition was taken back. Due to interesting results in animal studies another aspect of the BCAA:s is the relation to overtraining symptoms. The increased synthesis of 5-TH in specific areas of the brain, due to the changed plasma tryptophan/BCAA ratio, plays a role in the induction of sleep by affecting

motor neurons and specific releasing factors from the hypothalamus. This is a phenomenon seen in overtrained athletes and therefore discussed to be a possible explanation for a part of the overtraining syndrome [13].

Knowing that the BCAA requirement is increased by exercise, the next question to ask is whether a supplementation before, during and/or after exercise would be beneficial for decreasing exercise-induced muscle damage and promoting muscle-protein synthesis. Supplementation has proved to be safe to use when given in a ratio similar that of animal protein (i.e., 2:1:1 leucine: isoleucine: valine ratio) [14]. However, studies trying to clarify the effects of BCAA ingestion have been done, both to evaluate the effects on muscle damage and central fatigue. The results are indicating that supplementation of BCAA reduces the perceived exertion and mental fatigue during exercise and improves cognitive performance after the exercise [1]. In addition, in some situations ingestion of BCAA might also improve physical performance during endurance exercise by attenuate factors as muscle damage [A].

Taurine

Taurine, 2-aminoethane sulphonic acid, is a non-essential AA which is unusual in several aspects. For example, biochemically the amino group is located in the α -position and the AA is therefore not present in proteins. Taurine can form di- and tripeptides with different AA in, among others, the brain and parathyroidea, but these peptides are often very low in concentrations. In many tissues taurine is the dominant free AA, especially in skeletal muscle [16], and synthesized from cysteine via oxidation and decarboxylation [17]. Taurine is engaged in a wide variety of biological reactions [18] but it is still unknown whether the influence of their impact is intra- or extra cellular. A reason to why the functions of taurine could not be better defined is the difficulty to influence the endogenous levels. During heart muscle contraction taurine seems to have a clear role in the regulatory mechanism of Ca^{2+} homeostasis as well as increasing the sensitivity of force generating myofilaments to calcium [19]. An interaction with head groups of neutral phospholipids has also been discussed to mediate membrane conformational changes [20] and taurine could be able to change the muscle membranes capacity to respond to stimuli by changing the membrane chloride conductance [21].

In a study done by Ward et al. [16] an increased level of plasma taurine during ultra-endurance events was measured. The primary determining factor for this was thought to be the intensity rather than the duration of the exercise. The reason for this increase is not known but may reflect muscle damage, muscle fatigue or some adaptation to changes in osmolarity within the blood. In contrast to this study earlier studies have shown different results by a decrease in the plasma taurine concentration [7] respective no change [6].

Very limited research has been done in the area of taurine and endurance exercise and due to low coherence, our expectations was not clear before the analysis of the samples in this study. An interesting aspect is, however, the popularity of the use of taurine as an additive in energy drinks. There are no explanations for this supplement or proofs of the claimed effects on performance in the present literature. Since taurine have been shown to increase during exercise and, thus, an increased catabolism is proposed, we could suppose that the idea of the supplementation is to be able to reduce muscle fatigue and damage and have a positive effect on the osmolarity in the blood.

Glutamine and glutamate

Glutamine is a naturally occurring nonessential AA that together with glutamate is the base for other AA:s by promoting them with amino groups. It is also an important constituent of proteins and has significance for the transport of nitrogen between tissues [22]. Glutamine is the most abundant free AA in human muscle and plasma. Its effects have been described as anabolic and immunostimulatory [23]. Prolonged exercise (e.g. marathon), that is associated with a fall in the plasma concentration of glutamine, has therefore been hypothesized to impair immune function, a situation that also is associated with immunosuppression in endurance athletes in periods of very heavy training [24].

Glutamate is, in turn, a transamination product of almost all AA that in the next reaction forms alanine together with pyruvate [25], or aminated via glutamine synthase to form glutamine [23]. It clearly plays an important role in neuronal differentiation, migration and survival in the developing brain via facilitated Ca^{2+} transport. Glutamate also plays a critical role in synaptic maintenance and plasticity [26].

Previous studies have shown that short-term exercise results in a decrease in the concentration of glutamate and an increase in glutamine concentration in plasma. In prolonged exercise the overall results yet show a more stable level or slightly decreased concentration of glutamate [7] and a decreased concentration of glutamine [27]. The accelerated AA oxidation and purine nucleotide catabolism that produce an increased nitrogen load must, however, be either incorporated into other compounds or released as free ammonia. For preventing a potentially toxic hyperammonemia the nitrogen in the end product is incorporated into AAs, where glutamine is one of the primary ones [28]. The unchanged ratio of glutamine observed could, however, be explained by a simultaneous decreased release from glutamine sources other than exercising muscle, as lung or liver [23, 28].

Measurements of the glutamine and glutamate concentration have also been found to be interesting by using the ratio between them. It is proposed that glutamine concentration reflects the tolerance to volume of work and glutamate concentration reflects tolerance to high intensity training. The glutamine/glutamate ratio has been proposed to be able to represent overall tolerance to training [29].

Phenylalanine and Tyrosine

Phenylalanine is an essential AA while tyrosine is referred to as a semi essential AA, because it can only be synthesized by the hydroxylation of phenylalanine. Other than this biochemical action, phenylalanine and tyrosine can only be attained from nutritional intake [30]. Since neither of them could be formed or broken down in the muscle they are often used as measures of protein turnover by measuring how their concentration in muscle changes. With this method, it can not be distinguished, however, whether it is a decreased synthesis or an increased degradation that has occurred [31].

Materials and methods

Subjects

Six males and one woman participated in the study whose characteristics are presented in table 1. The subjects were well-trained Swedish ultra-endurance athletes with experience from several years of training and competition at elite level in event with duration over 48 hours. All subjects were fully informed about the procedure, possible discomfort involved, and their right to terminate the experiment at any point. Written informed consent was obtained from all subjects. The design of the study was conformed to the Declaration of Helsinki, and approved by the Regional Ethics Committee in Stockholm, Sweden.

Pre-tests

Two to four weeks before the race, Adventure Racing World Championship (ARWC), the athletes completed both lactate threshold test (L-test) and all-out tests for determining maximal oxygen uptake (VO_{2peak}) during running, kayaking and cycling. All subjects were well acquainted with all tests methods. The tests in different exercise mode were separated by at least 24-h. Running was performed on a treadmill (Rodby Electronics, Vansbro, Sweden), kayak on a kayakergometer (Dansprint aps, Hvidovre, Denmark) and cycling on a cycle ergometer (Monark ergomedic 839 E, Monark exercise AB, Sweden). Exercise protocol was similar in all the three disciplines and started with a non-exhausting warm up period for 10 min in a self selected pace. Thereafter the incremental five bout L-test was performed. After 30 min of rest and a five min re-warm up an incremental all-out test was performed. Heart rate (HR) was measured using Polar S610i (Polar electro oy, Kempele, Finland), and VO_2 was analysed using an automatic system (AMIS 2001, Innovision A/S, Odense, Denmark). Before each test temperature, humidity and barometric pressure were measured. The gas analyser was calibrated against a high precision gas mixture (16.00 ± 0.04 % O_2 and 4.00 ± 0.1 % CO_2 , Air Liquide, Kungsängen, Sweden). During the one min rest period between work bouts in the L-test finger tip capillary blood samples were obtained analyzed for blood lactate concentration ([HLA]) using an enzymatic method (Biosen C-line sport, EFK diagnostics GmbH, Germany).

Experimental Procedures

Due to the fact that the ARWC was an actual competition the subjects prepared themselves according to their normal systems. The race thereafter consisted of an 800 km long predetermined course that the athletes covered by a mixed transport of running, kayaking, cycling, in-line skating, climbing, caving and canyoneering. Before, during (approximately every 24th h) and immediately after the race the athletes carried out individual standardized tests for 6 min on a cycleergometer at a standardized work rate at 125, 150 or 175 W depending on the subject's VO_{2peak} . Blood samples were drawn in the morning the day before the race (PRE), in the middle of the race (72 h), immediately after the end of the race (END) and 24 h into recovery (POST24h). However, it should be noted that the tests in the middle of the competition were taken at the same place for all participants, but that both harvested time and distance varied because of the different choices of journey and the participants' performance. Of the same reason the two latter samples were drawn on random time of the day depending on the individual time point for completion of the race. Samples were kept on

ice for 1 h to allow for clotting before obtaining serum by centrifugation. Serum samples were stored at 70°C. No direct connections to the food intake could unfortunately be drawn due to the fact that food intake was ad lib and not recorded.

Biochemical methods

For the AA measurements, the plasma samples were deproteinized with 5% trichloro acetic acid (TCA; 1:5) and centrifuged at 9,000 g for 2 min, and the supernatant was stored at -80°C until analyzed. The concentration of AAs in plasma was measured by reversed-phase HPLC as described by Pfeifer et al. [32], with orthophthaldialdehyde as the derivatizing agent. Two extracts from each blood sample was made in order to minimize the methodological error. The mean value of the duplicate analyses was used in the results and statistics. The methodological variation between duplicates is presented in table 3.

Statistical analysis

All data are presented as mean \pm SD. Data were tested for normal distribution using the Shapiro-Wilks W-test before performing parametric statistics. Repeated measures ANOVA were used for changes over time for AAs, Hct and [Hb]. When a main effect was found a post hoc analysis was made using the Tukey HSD test. Significance was accepted at $P < 0.05$, and trends were considered at $0.05 < P < 0.1$.

Results

The average work intensity for the entire race, including sleep and rest periods, was approximately about 40 % of VO_{2peak} . During the study body mass did not change ($-0,2 \pm 2,7$ kg). The mean of total EE including rest and sleep periods was 80 075 kcal, corresponding to an EE of more than 500 kcal per total race hour. Fig 1 shows the heart rate recording of subject 3 during the race and typifies the variety of intensity.

There were no significant changes in [Hb] or Hct. The values of the AA analysis and their significance are presented in table 2.

All results from the analysis of the AA are presented in Fig 2 a-h, and the ratio between glutamine and glutamate in Fig 3.

Branched chain amino acids

Valine, leucine and isoleucine (fig. 2a, 2b respectively 2c) showed a slight but unanimous decline in plasma concentration during the first half of the competition by 23%, 13% and 16% respectively. In the second half the similarity was continuous but all AA:s were raised by 15%, 60% and 68%. The concentration of valine only attained a value slightly higher than the basal value whereas leucine and isoleucine dose were ascended by a major extent. The level of valine manages to fall back to baseline (+2%) after the recovery period while leucine and

isoleucine were on the way down (+23% and +15%). Worth mentioning are the people deviating from the mean. Subject A and F showed a raised concentration of all BCAAs even after the finish (POST) and increased the concentration in plasma above the initial level.

Taurine

The subjects follow a similar pattern of change in the plasma concentration of taurine (fig. 2d). However, there are a couple of individual variations. A trend of decline could be seen in the first half of the competition among all subjects by a mean of 22%. During the second part, from the middle to the goal, there was a slight increase by 41%, except for subject E and F. Those two subjects show a major increase that differs radically from the baseline by 126% and 117% respectively. All values returned back to initial values after 24-hour of recovery.

Glutamine and Glutamate

Glutamine (fig. 2e) shows a generally unchanged concentration throughout the competition and even during the recovery. The middle (72h), end of the race (POST) and recovery (REC) shows a mean change compared to pre-values of no more than -5%, +10% and -11% respectively. At the individual level there were some values that dropped slightly at the middle test but then rose to pre-values at the finish and then stayed stable. One person also shows a continuously rising line where both the value at the end of the competition and the one after the recovery is higher than the pre-value. Interesting is yet the subject with a relatively stable concentration of about 100 $\mu\text{mol/l}$, compared to a normal level of around 550 $\mu\text{mol/l}$.

The plasma concentration of glutamate (fig. 2f) has an overall profile of a descending value at the first half of the competition with a mean decline of 10%. The second half showed an increase in concentration of 36% from the pre-value. During the recovery the concentration decreased and after 24h the concentration was 15% above pre-value. The same subjects as seen differ in the BCAA values and taurine (Subject A, E and F) have here a profile with a lower value than average by the finish and a concentration that continues to increase even throughout the time of recovery.

The ratio between glutamine and glutamate (Fig 3) showed a small increase during the first half of the competition and the value was 7% over the pre-value at 72 h. In the second part from the 72 h-measurement to the finish (POST-value), the ratio decreased with 26%. By time of the 24 h recovery-value, the ratio was 8% less than the pre-value.

Phenylalanine and tyrosine

The aromatic AAs phenylalanine and tyrosine both increase during the whole competition. In the first half phenylalanine increases with 18% and tyrosine with 14%. In the second half, after passing the finish, the levels increased by as much as 54% and 75% respectively. During the recovery the concentration starts to decrease and after 24 h the levels were 35% and 43% respectively above pre-values ($P < 0.05$).

Discussion

A fascinating issue that concerns all who are interested in the human physiology is the question of what limits the human endurance. This has inspired people in over a century and by optimising the conditions for a good result and studies of the limit for what the human body is capable of doing is constantly increasing. Only from a physiologists view there are an almost boundless edition of aspects that could be explored and improved. The AA:s are only one of those. Because of their ability to affect so many functions in the body, they still are an important factor to take into account. In order to be able to affect the changes in a positive way and to understand the human body, a basic knowledge is necessary. In the end we, however, need more than just facts and the goal is simply to understand. Why do the AA change and what can we do to improve the levels in the body and hence to optimize performance?

Since earlier facts in the area of this kind of ultra-endurance exercise do not exist it was hard to predict the results in this study. All the AA:s listed above demonstrate, however, a relatively similar overall change (however, not included phenylalanine and tyrosine). The levels in the initial part of the competition until half time all show a more or less decreasing occurrence. This result was expected for the BCAA:s but not as much for the rest. The very small decrease in the glutamine level was, however, not really expected. The reasons for this could have many explanations. As mentioned earlier, the glutamine production could be limited by a decrease in the availability of glutamate, which in turn could occur because of both an increased transamination to alanine and a depletion of the TCA-cycle intermediate α -ketoglutarate [28]. The decreased glutamate level in first half of the competition is also supporting this theory. The fact that glutamine has been shown to be an important fuel for cells of the immune system and also for mucosal cells of the intestine, have attract attention of the sports nutrition market despite limited scientific studies. Glutamine is therefore used as a supplement to improve nitrogen balance and is suggested to support gut function and immune function even if there are not proofs for a working mechanism in athletes as seen in patients [8]. The above mentioned reasons that could create a decreasing value might be a reason to the very low value that could be seen in Subject E. Decreases in glutamine levels mostly occur after trauma or major burns, postoperatively, and in seriously ill patients [33, 34]. There might be other possible reasons, but there is no obvious explanation on the basis of our results.

The next part of the competition was of big interest since they now reached far into the unexplored area of ultra-endurance exercise. Few things could be predicted although some explanations to the found results could be made. Due to a decrease in intensity (se fig 1) the athletes now had more time for food intake and the body seemed to be very stimulated to begin the recovery from the performance during the first part. All concentrations of AA:s increased, although the change in glutamine was, as in the first part, very small. An overall reflection on the unaffected glutamine concentration is, however, that the athletes managed to keep it very stable which is beneficial for the immune functions. The large increases in the glutamate and BCAA, especially leucine and isoleucine have, however, no obvious explanation. A possible cause could although be the food intake since diet supplements are very common among the participants. In today's sports supplements a content of BCAA:s are not unusual and glutamate belongs to one of the most common additives in food. BCAA:s are also found in large quantities in protein [35]. Taurine was also an AA that increased markedly, but only in two individuals. By looking at their self reported food intake we could

note that this was the same athletes that had stated that they used taurine-contained energy drinks during the race. By that information we could probably suppose that the big change was a result of their nutritional intake.

By looking at the results from phenylalanine and tyrosine we could assume that protein degradation has occurred. The concentrations increase in an exponential-like curve and the muscle catabolism seems to increase over time. It is, however, interesting to see that although the intensity was higher in the first part and that the nutritional conditions was better in the second half, the catabolism continues to increase all the way to the finish. As for the AA concentrations in whole it is worth to note that we only can detect the change, but we can not know from where the changes originate.

In addition to the change in the specific AAs it is also interesting to know what can be detected from the calculated glutamine/glutamate ratio. As seen in fig 3 there were no noticeable change in the first half of the competition. In the second half, however, there was a decrease that by the suggested training model would be a measurement of a decreased training status. If that is the case; could this be used for measuring subjects endurance or be an indication on exhaustion? No conclusions could be drawn from the study but could be an interesting matter for further investigation.

One of our limitations in the discussion is our lack of food recording. As mentioned above, our comments about the importance of nutrition could only be speculations, except for the oral information about the energy drinks. Parallel with this study we although have the analysis of a 24-h laboratory trial ongoing where complete data of the nutritional intake have been recorded. We have in addition to this not taken to account the differences in gender where earlier research in endurance exercise has seen differences between men and women [36]. Other aspects that affect the AA concentration are factors as the diurnal rhythm and the sleep deprivation. It is yet reasonable to assume that the diurnal rhythm will have a neglectable impact on the AAs in relation to the extreme strain among the athletes and the inadequate energy intake.

Due to the lack of previous research there where little comparable information. The study also has some limitations, especially in the nutritional area that may be of big importance. Further studies should be needed and should favourable include food recording.

Conclusions

The major finding of this study is the fact that we now have a first insight of what happens with the AAs during adventure racing. No previous research has been done in the area of this kind of ultra-endurance exercise. It is therefore an important step both for the research and the sport itself. Overall, the AA:s behaved as expected, adopted from earlier studies of endurance exercise in the initiation of the exercise, but further into the competition they showed some unpredicted results. The concentrations of the BCAAs, glutamate and tyrosine all increased above pre-values at the second half with no obvious explanation. The importance of the nutritional intake has, however, been taken into discussion and could be an important factor for the observed results.

From a nutritionists point of view it must be emphasized that the choices of food intake and sport supplements with a high degree of confidence have more impact than only to compensate for energy expenditures. With a diet optimised for the challenge the competition could be made better, both in view of performance and also to create less stress and a better recovery for the athlete. To take the issue one step further, however, it would require further studies in the field.

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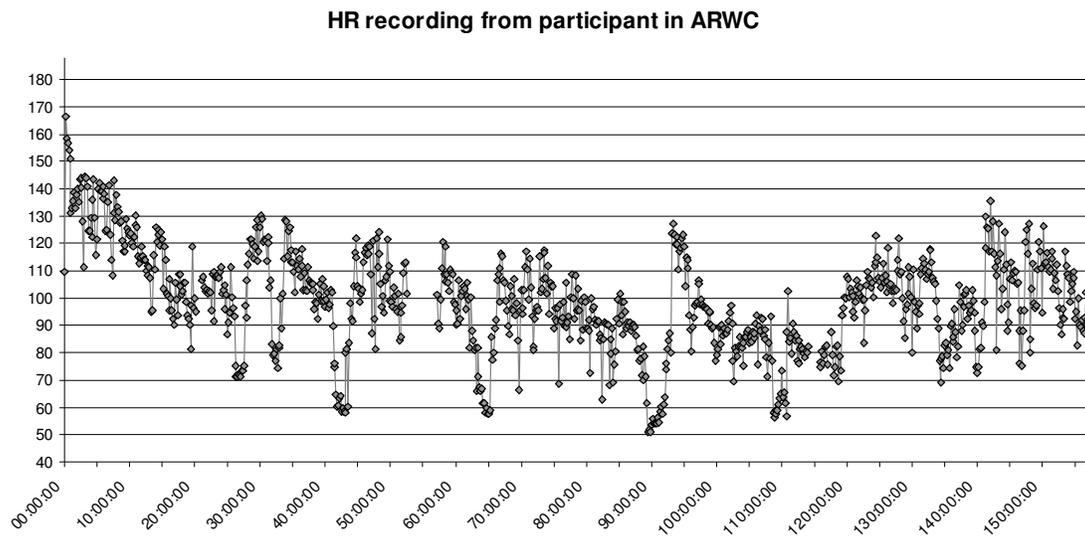
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Figures

Figure 1



Continuous recording of heart rate (HR) from one subject, measured with Polar S610i (Polar electro Oy, Kempele, Finland). The subject's mean heart rate for the entire race was 99 beats/min with a top value of 166 beats/min (mean during 1 min). Maximal heart rate was 188 beats/min and resting value 45 beats/min.

Figure 2 – Individual values of the plasma concentration of eight amino acids

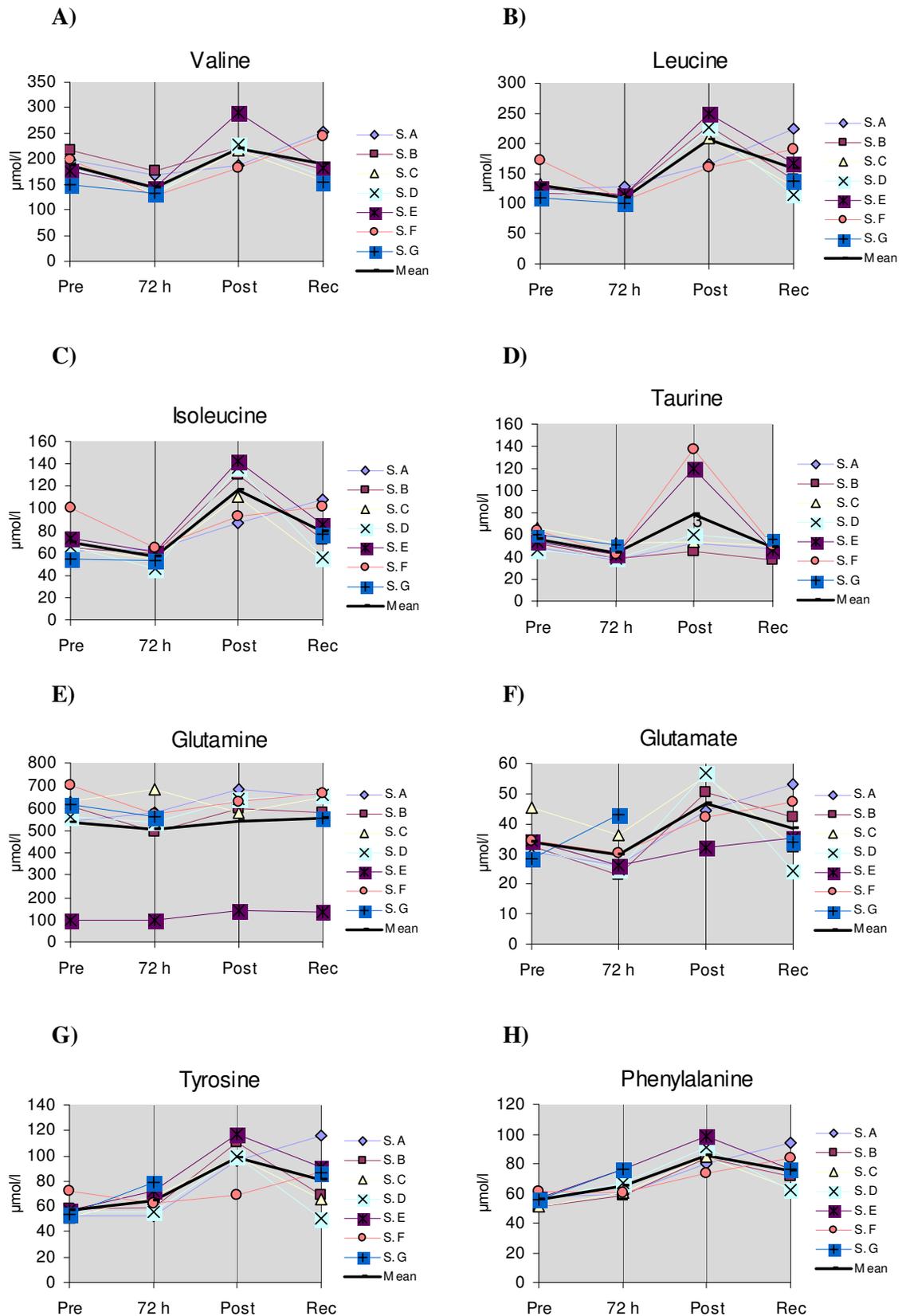
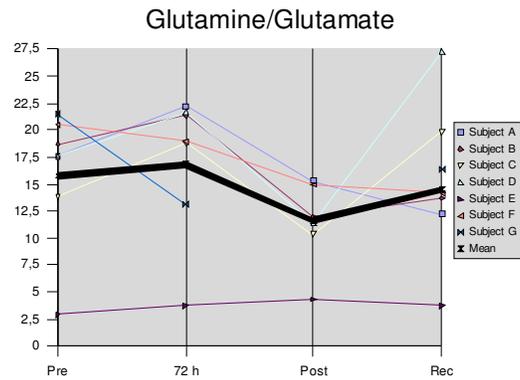


Figure 3 – The ratio between glutamine and glutamate



It is proposed that glutamine concentration reflects the tolerance to volume of work and glutamate concentration reflects tolerance to high intensity training. The glutamine/glutamate ratio has based on this been proposed to be able to represent overall tolerance to training [29]. The figure represents the change in ratio of the seven subjects in this study.

Tables

Table 1 - Subject characteristics

	Subjects (n=7)
Age (years)	30 ± 5
Height (cm)	178 ± 9
Body mass (kg)	76,9 ± 10,1
BMI (kg · m ⁻²)	24,2 ± 1,7
Body fat (%)	18,9 ± 5,7
VO _{2-peak} (L·min ⁻¹)	4,65 ± 0,68
VO _{2-peak} (ml·kg ⁻¹ ·min ⁻¹)	60,4 ± 3,1

Anthropometric values for the subjects participating in the study, incl. six males and one woman.

Table 2 - Mean values from the analysis of the AAs

	Pre	72 h	Post	Rec
Valine	188 ± 22	145 ± 20 *	221 ± 39 #	191 ± 41 #
Leucine	129 ± 20	111 ± 9	207 ± 36 * #	157 ± 38 #
Isoleucine	69 ± 15	57 ± 7 *	117 ± 23 * #	79 ± 21 #
Taurine	56 ± 8	43 ± 6 *	78 ± 40	49 ± 6
Glutamine	537 ± 199	504 ± 188	545 ± 202	556 ± 191
Glutamate	34 ± 5	30 ± 7	47 ± 9 * #	38 ± 10
Tyrosine	57 ± 7	64 ± 9	99 ± 116 * #	81 ± 21 *
Phenylalanine	56 ± 3	66 ± 8 *	85 ± 9 * #	75 ± 11 *

* $P < 0.05$ vs Pre; # $P < 0.05$ vs 72h; † $P < 0.05$ vs Post

Blood samples were drawn in the morning the day before the race (PRE), in the middle of the race (72 h), immediately after the end of the race (END) and 24 h into recovery (POST24h). Samples were analysed and the table shows the values (± SD) of the AA concentrations in µmol/l and their significance.

Table 3 - Control of methodological error in the apparatus and duplicates of samples

<u>Coefficiene of varience (%)</u>	
Valine	4,4
Leucine	4,0
Isoleucine	3,8
Taurine	3,9
Glutamate	4,0
Glutamine	4,5
Tyrosine	3,8
Phenylalanine	3,5

Two extracts from each blood sample was made in order to minimize the methodological error. The mean value of the duplicate analyses was used in the results and statistics. The methodological variation between duplicates is presented in table.