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Exercise-induced Changes in Muscle and Plasma Amino Acid Levels in the Standardbred Horse

A. R. PÖSÖ,[1] B. ESSÉN-GUSTAVSSON, A. LINDHOLM and S. G. B. PERSSON

Department of Medicine and Surgery, Swedish University of Agricultural Sciences, S-75007 Uppsala, Sweden and [1]Department of Biochemistry, College of Veterinary Medicine, SF-00581 Helsinki, Finland

ABSTRACT. Concentrations of amino acids in the gluteus muscle and in venous plasma were analysed in six Standardbred horses that performed an incremental standardised exercise tolerance test (SET) and also a submaximal exercise test to fatigue (SEF) on a treadmill. The intramuscular concentration of phenylalanine increased after SET suggesting net protein breakdown during exercise. Alanine accumulated in the muscle in SET and an increasing tendency was observed in SEF. In both tests the increase in alanine concentration correlated with the decrease in glutamate concentration. Concentration of glutamine in the muscle increased only after SEF test. Concentration of alanine after both SET and SEF and glutamine after SEF also increased in plasma indicating that plasma concentrations reflect the changes in the muscle. The results support the view that amino acid metabolism in the working muscle is enhanced and related to both intensity and duration. It is suggested that the *de novo* synthesis of alanine may be a physiologically important pathway for the removal of the carbohydrate-derived pyruvate

Key words Alanine; glutamate; glutamine; branched-chain amino acids; phenylalanine; horses.

INTRODUCTION

Amino acid and protein metabolism during and after exercise have been extensively reviewed in man, dog and rat.[3,17] The rate of protein synthesis has been reported to decrease during exercise, whereas the breakdown of proteins increases. In the muscle, the oxidation of leucine and the synthesis of alanine increase and changes in the steady-state concentrations of many amino acids have been reported. These changes have been shown to depend mainly on intensity and duration of the exercise, but the dietary state, the training status and the species studied may also contribute.[3,17]

The concentration of alanine has been shown to increase in horse muscle[20] and plasma[18] during exercise, but the response of other amino acids is not known. The aim of this study was to investigate the amino acid concentrations in skeletal muscle of Standardbred horses after short intense exercise and prolonged submaximal exercise. Plasma amino acid concentrations were measured to evaluate their suitability as indicators of intracellular changes in protein and amino acid metabolism during exercise. Amino acids discussed here will be limited to the branched-chain amino acids (leucine, isoleucine and valine) that are readily metabolised in the muscle, aromatic amino acids (tyrosine and phenylalanine) that are not oxidised in the muscle, alanine and glutamine that are released by the muscle during exercise, and glutamate that is the key link between energy and amino acid metabolism.[3,17]

METHODS

Horses. Six clinically healthy Standardbred horses with the mean age of 8 years (range from 5 to 14 years) were studied.

Exercise tests. All horses performed an incremental standardised exercise tolerance test (SET) on a treadmill at 6.25% slope.[16] During SET the speed was increased at 2 min intervals until a heart rate of 200 bpm was reached. The starting speed was 5 m s^{-1} for 3 horses and 6 m s^{-1} for the other 3 horses. Two of the horses finished at 8 m s^{-1} and 4 at 9 m s^{-1}. During the submaximal exercise test to fatigue (SEF) the horses were allowed to trot at 7 m s^{-1} until they could not keep pace with the treadmill. The heart rate during this test was between 140–170 bpm and other conditions as described by Essén-Gustavsson et al.[5]

Muscle biopsies. Biopsies of the gluteus medius muscle were taken at rest and immediately after each exercise test as described earlier.[12] The biopsies were frozen in liquid nitrogen and stored at −80°C until analysed.

Blood samples. The SET protocol included venous blood samples from the jugular vein at rest, during the final 15 seconds of each speed and 15 min after the test. In SEF, venous blood samples were taken at rest, at 20 min during the exercise, at the end of the exercise and 10 min after the exercise. The blood was centrifuged and plasma was separated and stored at −80°C until the analysis.

Analysis of amino acid. For the analysis of amino acids, muscle biopsies were lyophilised, blood, fat and connective tissue were dissected off and a weighed sample was homogenised in 0.9% NaCl. The homogenates were deproteinised with methanol.[8] The plasma samples were treated as the muscle homogenates. Two HPLC methods for amino acid analysis were used. The first involved derivatisation with dansyl chloride followed by separation of the derivatives at ambient temperature by a high-speed C^{18} reversed phase column (Chrompack, 100×4 mm) with fluorescence detection (ex. 340 nm, em. 540 nm) as described by Työppönen.[21] The other method involved derivatisation with FMOC (9-fluorenylmethyl-chloroformate), removal of reagent excess by ADAM (1-aminoadamantane) and separation of derivatised amino acids at 45°C by a C^{8} reversed phase column (Merck Supersphere, 250×4 mm) with fluorescence detection (ex. 265 nm, em. 305 nm) as described by Gustavsson and Betnér.[9] The concentrations were calculated with external standards. A number of plasma samples were analysed by both techniques and with the exception of tryptophan the results of the two methods were within the limits of methodological error. Plasma lactate was analysed with a lactate analyzer (Analox, Analox Instruments Ltd, London, UK). Muscle lactate was analysed as described by Essén-Gustavsson et al.[5]

Statistics. Analysis of variance, paired *t*-test and regression analysis (Statgraphics®, Statistical Graphics Corporation) were used to calculate the statistical significances.

RESULTS

Standardised exercise tolerance test. In the muscle the total amino-N increased by 14.1% during SET. The concentrations of leucine, isoleucine, phenylalanine and alanine increased significantly and the concentration of glutamate decreased (Table 1). The largest change (91%) was observed in the concentration of alanine which in molar terms was equal to the decrease in the glutamate concentration. The negative linear correlation between these changes was statistically significant ($df=10$; $r=-0.792$; $p<0.01$). There was no correlation between the exercise-induced changes in alanine and lactate concentrations (Table 1).

During SET the concentrations in the plasma of leucine, isoleucine, and alanine increased with speed (Table 2). However, only the increase in the concentration of alanine was linear with the trotting speed ($df=29$; $r=0.446$; $p<0.05$). For the other amino acids the increase was apparent at the beginning of the test (at 5 or 6 m s^{-1}) and was not significant thereafter. During the recovery phase amino acid concentrations in the plasma did not change significantly. The exercise-induced changes in plasma amino acid

Table 1. *Amino acid and lactate concentrations in the gluteus muscle before and after the standardised exercise tolerance test (SET) and the submaximal exercise test to fatigue (SEF)*

Biopsies of the gluteus muscle were taken before and immediately after the tests. The results are means ±SEM of 6 horses for SET and 4 horses for SEF. The results are expressed as mmol kg^{-1} dry weight

	SET		SEF	
	Before	After	Before	After
Leucine	2.1±0.2	2.7±0.1[b]	1.7±0.2	3.3±0.7
Isoleucine	1.3±0.1	1.6±0.1[b]	1.1±0.2	1.8±0.4
Valine	4.8±0.4	5.3±0.3	3.7±0.3	5.3±0.7
Phenylalanine	1.4±0.1	1.7±0.1[a]	1.3±0.1	2.1±0.4
Tyrosine	0.87±0.10	1.6±0.2	0.78±0.08	0.90±0.15
Glutamate	30.0±3.9	17.2±2.0[a]	22.6±3.1	14.2±3.6
Glutamine	25.4±5.7	25.7±6.4	15.0±2.0	27.7±3.6[a]
Alanine	19.0±3.1	36.3±4.6[c]	10.8±1.1	21.0±3.4
Lactate	20.3±1.9	49.7±7.1[b]	11.3±2.5	23.0±3.5

[a] $p<0.05$, [b] $p<0.01$ and [c] $p<0.001$ from the respective value before the test (paired *t*-test).

concentrations did not correlate with the respective changes in the muscle concentrations.

Submaximal exercise test to fatigue. In this test the horses were allowed to trot on the treadmill at 7 m s^{-1} until fatigue which caused the following individual variation in the duration of the test: 4 horses 50 min, 1 horse 60 min and 1 horse 75 min. Muscle biopsies were available from 4 horses only. Total amino-N in the muscle biopsies taken immediately after SEF was 37% higher than the concentrations before the test. The only significant change that was found was an increase in the concentration of glutamine (Table 1). The decrease in the concentration of glutamate and the increases in the concentrations of alanine, leucine, isoleucine, valine, tyrosine, and phenylalanine were similar to those observed after SET, but not sig-

Table 2. *Plasma amino acid concentrations during and after the standardised exercise tolerance test*

The results are means ± SEM of 6 horses and they are expressed as μmol l^{-1}

	Before	5–6 m s^{-1}	8–9 m s^{-1}	Recovery
Leucine	79±9	98±11	104±11[c]	109±10
Isoleucine	54±7	62±8	61±6[a]	65±6
Valine	169±16	191±18	191±16	191±13
Phenylalanine	64±4	72±5	71±3	73±3
Tyrosine	103±8	112±8	109±6	116±5
Glutamate	24±5	34±6	43±13	39±10
Glutamine	501±54	536±38	557±44	498±24
Alanine	204±25	244±28	320±38[c]	352±29

[a] $p<0.05$, [c] $p<0.001$ (ANOVA).

Table 3. *Plasma amino acid concentrations after submaximal exercise to fatigue*

Blood samples were taken before, at 20 min, at the end and 10 min after the test. The results are means ± SEM of 6 horses and they are expressed as μmol l^{-1}

	Rest	20 min	Immediately after	Recovery
Leucine	64±3	88±11	144±14[b]	149±17
Isoleucine	34±3	41±8	68±8[b]	69±8
Valine	176±9	183±22	234±15	238±16
Phenylalanine	47±2	56±7	85±6[b]	80±5
Tyrosine	68±6	69±7	91±3[a]	92±5
Glutamate	22±3	28±3	39±4[a]	30±1
Glutamine	471±15	492±53	563±26	495±30
Alanine	214±19	286±38	426±40[b]	386±32

[a] $p<0.05$, [b] $p<0.01$ (ANOVA).

nificantly different from the values before the exercise. As in SET, a negative linear correlation was found between the decrease in glutamate concentration and the increase in alanine concentration ($df=6$, $r=-0.829$; $p<0.01$). No correlation was found between the exercise-induced increases in muscle alanine and lactate concentrations (Table 1).

With the exception of glutamine and valine the plasma concentrations of all amino acids increased during SEF (Table 3). The changes in the plasma concentration of leucine correlated linearly with the increases in muscle concentrations ($df=6$; $r=0.996$; $p<0.01$). During the 10 min recovery period no significant changes in the concentrations were observed.

DISCUSSION

The changes seen in the concentrations of amino acids after SET and a similar tendency seen after SEF can be taken as an indication that both the intensity and the duration of the exercise enhance the metabolism of amino acids in horse muscle. At rest, the concentrations were found to be comparable to values in man,[19] rat[2,4] and dog,[14] although the comparison is hampered by the differences in the expression of the data. The concentrations of alanine, glutamate and the branched-chain amino acids were similar to the respective concentrations in dog,[14] but somewhat higher than those in man[19] and rat.[2,4] The concentration of glutamine was markedly lower than that reported for man[11] and dog.[14]

Phenylalanine and tyrosine are not oxidised in the muscle and thus the changes in their concentrations can be used as indicators of the balance between the synthesis and degradation of intramuscular proteins. Accordingly, the observed increase in the intramuscular concentration of phenylalanine (Table 1) can be taken as an indication that the rate of protein synthesis during intense exercise was lower than the rate of protein degradation.

Branched-chain amino acids are readily transaminated in the muscle[11] and their further oxidation is greatly enhanced during exercise.[10,19,23] During SET the concentrations of these amino acids increased in the muscle which is in accordance with earlier findings in rats.[2,4] The plasma concentrations of the branched-chain amino acids were elevated in both tests which may have been due to increased output by the liver, where proteolysis has been shown to accelerate during exercise[3,17] and where the metabolism of the branched-chain amino acids is very low. Both the net protein breakdown and in-

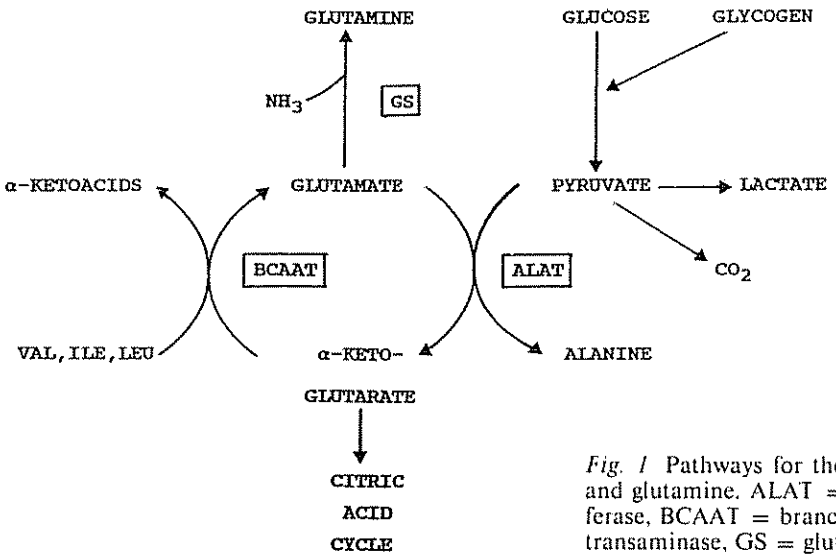

Fig. 1 Pathways for the synthesis of alanine and glutamine. ALAT = alanine aminotransferase, BCAAT = branched-chain amino acid transaminase, GS = glutamine synthetase.

creased uptake may have contributed to the observed increase in the intramuscular concentration of the branched-chain amino acids. The uptake of leucine by the muscle has been shown to increase with plasma concentration,[10] which is supported by the observed linear correlation between the increases in the plasma and muscle leucine concentrations. Unfortunately, the measurement of concentrations does not provide information on the flux of leucine or other branched-chain amino acids through various metabolic pathways. Thus, on the basis of these results, it is not possible to draw any conclusion on the rate of oxidation of the branched-chain amino acids in horse muscle during exercise.

Accumulation of alanine in the muscle was observed after SET and the concentration tended to increase also after SEF. Experiments with rats have shown that the carbon chain of alanine originates from the carbohydrate-derived pyruvate[7] and at rest the availability of pyruvate controls alanine formation.[1] In this study there was a linear correlation in both exercise tests between the increase in the concentration of alanine and the decrease in the concentration of glutamate and the changes in molar terms were nearly equal. This can be taken as an indication that nearly all alanine could have been formed in the reaction catalysed by alanine aminotransferase, ALAT (Fig. 1).

The increase in the intramuscular alanine concentration after SEF was almost as high as the increase after SET. Thus, the behaviour of alanine differs from the observed increases in the intramuscular lactate concentrations which were high after SET and remained low after SEF test. It can be speculated that pyruvate is mainly transaminated to alanine in the oxidative fibers which are recruited both during aerobic and anaerobic exercise[22] and can readily regenerate glutamate via their more active transamination of leucine.[10] This view is further supported by the fact that the other product of the reaction catalysed by ALAT, namely α-ketoglutarate, is an intermediate of the citric acid cycle and therefore of vital importance for the working oxidative fibers. Thus the synthesis of alanine both eliminates pyruvate and increases the concentration of one intermediate of the citric acid cycle.

Glutamine was formed in the muscle only after SEF test. The precursor of glutamine in the muscle is glutamate (Fig. 1), which in the reaction catalysed by glutamine synthetase

binds free ammonia. The concentration of free ammonia has been shown to limit the rate of this reaction.[1] During similar submaximal exercise to fatigue some of the muscle fibers become depleted of glycogen,[5,22] which may induce the degradation of AMP to IMP and free ammonia.[15] At fatigue the concentration of IMP in the muscle was increased (Pösö et al., unpublished observation) and the plasma concentration of free ammonia was elevated.[5] It can be speculated that the observed accumulation of glutamine was coupled to the increased availability of ammonia for glutamine synthesis.

In connection with the intramuscular changes in amino acid concentrations it was of interest to study how these changes were reflected in the plasma. It should, however, be kept in mind that the plasma samples were taken from the jugular vein where the concentrations are not equal to those in veins coming directly from the working muscles. Furthermore, the portion of amino acids transported by the red blood cells[6] together with hemoconcentration and the small decrease in plasma volume may have influenced the concentrations in plasma. The influence of these factors further complicates the comparisons of muscle and plasma concentrations. Only the concentration of alanine increased in both muscle and plasma after both tests which allows the conclusion that venous plasma samples can be used to demonstrate increases in the muscle concentration. The size of the intramuscular change cannot, however, be estimated on the basis of plasma concentration. The changes in the muscle and plasma glutamine concentrations during SEF indicate a similar relationship. On the other hand, the plasma concentrations of aromatic amino acids do not give a valid estimate of the protein turnover in the muscle.

In conclusion, these data suggest that amino acid metabolism in the working muscle is enhanced. Net protein breakdown is supported by the accumulation of phenylalanine. The increases in the intramuscular concentrations of alanine and glutamine are reflected in the plasma. It is suggested that during exercise the synthesis of alanine from pyruvate is physiologically important for aerobic energy metabolism.

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