The effect of time of feeding on plasma amino acids during exercise and recovery in horses

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ABSTRACT: Feeding management in horses suggests feeding horses in advance of exercise, particularly the grain portion of the diet. Plasma amino acids (**AA**) peak at 3 to 6 h postfeeding depending on the AA. The timeframe between feeding and exercise may affect the availability of AA during and after exercise. The purpose of this study was to observe the differences in plasma AA in horses fed prior to exercise or after exercise. Eight light type horses were fed a diet with adequate protein and AA for horses in light to moderate exercise. After an adjustment period, horses completed a standardized exercise test (**SET**). Relative to the SET, horses were fed either 2 h prior (**PRE** horses) to the SET, 1 h after completing the SET (**POST** horses), or horses remained fasted throughout the sampling period (**FASTED** horses). Plasma was drawn prior to exercise, at the peak of exercise as well as at 1, 2, 4, and 7 h postexercise. Plasma was analyzed for AA, glucose, lactate, creatinine, creatine kinase, ammonia, urea-N, and 3-methylhistidine. After completion of the SET and sampling period, horses entered a 1-wk recovery period, which was followed by another SET. The protocol repeated until horses rotated through all feeding protocols in the study (three SETs). The majority of the plasma AA were elevated in PRE horses compared with POST horses prior to the SET until 2 h postexercise where POST horses' plasma AA concentrations became elevated and remained elevated until the end of the sampling period. In that same time frame, plasma AA for the PRE group decreased out to the end of the sampling period. The elevation of plasma AA in POST horses would be expected as they were fed at 1 h postexercise, whereas PRE horses were reaching a 4 h postfeeding time frame at this point. This elevation was not observed for plasma concentrations of isoleucine, leucine, methionine, and histidine. Concentrations of these AA initially were greater for POST horses in the postexercise period; however, they declined more rapidly than the other AA. The rapid decrease of some of the plasma AA concentrations may suggest uptake by muscle for recovery. This in conjunction with a decrease in plasma creatine kinase concentrations for POST horses suggests that feeding postexercise may facilitate better muscle protein balance (synthesis vs. breakdown) in the recovery period following exercise.

Key words: amino acids, exercise, feeding, horses, protein, timing

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INTRODUCTION

Exercise alters metabolism based on fuel needs and the duration of exercise (Hiney and Potter, 1996). Protein and amino acids (**AA**) are

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generally not considered large portions of the fuel supply during exercise (Miller-Graber et al., 1991); however, they play a pivotal role in muscle mass development and recovery (Wolfe, 2000). Plasma AA in horses have been observed to peak at 3 and 6 h postfeeding depending on the AA (Johnson and Hart, 1974; Russell et al., 1986). AA have been shown to stimulate an increase in muscle protein synthesis and a decrease in protein degradation in muscle when given after exercise in humans (Wolfe, 2000). Van den Hoven et al. (2010, 2011) also demonstrated increases in muscle free AA as well as decreases in markers of muscle protein breakdown in horses fed a protein supplement after exercise. Muscle protein synthesis was also demonstrated to increase (as measured in the hindlimb) in the horse when given an infusion of glucose and AA intravenously (Matsui et al., 2006).

Traditional feeding management of horses suggests horses be fed well in advance of exercise, particularly the grain portion of the diet to avoid digestive upset (Clark et al., 1990). However, this may limit the supply of AA during exercise as well as during recovery. The objective of this study was to observe the changes in plasma AA concentrations for horses fed prior to exercise compared with those fed after exercise.

MATERIALS AND METHODS

Animals and Feed

Eight horses of light horse type weighing 585 ± 3 kg (in average body condition; score 5 to 6) were used in the study. Horses received routine health care treatments. Care and management of the horses as well as experimental protocols including sampling were approved by the college's IACUC committee. Horses were fed a commercial grain mix (Omolene 100, Purina Mills, St. Louis, MO) and grass hay (timothy/orchardgrass mix). Protein and AA concentrations in the diet are shown in Table 1. During the study, horses were fed to meet the requirements for horses in light to moderate exercise (NRC, 2007). Rations were divided into equal meals per day fed in the morning and early evening. Horses participated in the College's riding program consisting of light to moderate exercise during the study exercising 1 to 2 h per day for 5 d a week. Diets were fed for a 4-wk adjustment period prior to an exercise test. Following the adjustment period, horses were assigned to one of three treatments: horses fed 2 h prior to exercise (**PRE**), horses fed 1 h after exercise (**POST**), and horses who remained fasted (**FASTED**) throughout the sampling period. FASTED horses would have been 12 h without feed prior to any testing. Horses in the POST group were allowed access to feed for 2 h postfeeding to allow for comparable consumption between PRE and POST groups. After completion of the standardized exercise test (**SET**) and sampling, horses entered a 1-wk recovery period in which they were fed the standard ration split equally into two meals daily. After 1-wk recovery, horses completed another SET on a different feeding protocol (PRE, POST, or FASTED). The protocol was repeated until horses rotated through all treatments in the study (three SETs).

Table 1. Essential amino analysis for grain and hay components of diets

Amino acid, %	Grain[1]	Grass hay[2]
Arginine	1.02	0.19
Histidine	0.41	0.08
Isoleucine	0.60	0.17
Leucine	1.11	0.30
Lysine	0.96	0.19
Methionine	0.23	0.06
Phenylalanine	0.71	0.20
Threonine	0.57	0.17
Valine	0.83	0.24

[1]Omolene 100, Purina Mills, St. Louis, MO.
[2]Timothy/orchardgrass mixture.

Standard Exercise Test

After the initial 4-wk adjustment period and subsequent 1-wk recovery periods between each standard exercise test (**SET**), horses completed a SET. The SET consisted of 5 min at the walk (1.5 m/s), 5 min at the trot (3.5 m/s), 3 min at the canter (5 m/s), and 2 min at the hand gallop (7 m/s) followed by 10 min at the walk (1.5 m/s). The SET was performed under saddle with riders familiar with the SET protocol. Other students assisted as timers to keep track of the pace of the SET as well as the pace of the gait being performed. The same riders rode the same horses in each SET. Horses were fasted overnight prior to performing the SET. Horses were fed according to their assigned group (PRE, POST, and FASTED) the day of the SET. Heart rate was monitored during the exercise test to assess exercise intensity. Blood samples were drawn via jugular puncture just prior to the start of the SET, at the peak of exercise (following the hand gallop), and at 1, 2, 4, and 7 h postexercise. For the PRE group, the postexercise blood samples represent 3.5, 4.5, 6.5, and 9.5 h postfeeding. For

the POST group, the postexercise blood samples represent 0, 1, 3, and 6 h postfeeding.

Analysis

Plasma was separated and frozen for later analysis of AA, 3-methyl-histidine (**3MH**), urea-N, creatinine, lactate, glucose, and creatine kinase (**CK**). Data were summarized as LS Means with standard errors. Statistical analysis was done using a repeated-measures analysis via the PROC MIXED procedure in SAS (version 9.2). A significance level was set at $P < 0.05$. Horse, SET, treatment, and time were used in the model. There was no effect of horse or SET, and they were subsequently removed from the model. Differences were compared as affected by treatment, time, and any interactions.

RESULTS

All horses maintained their body weight and body condition throughout the study. Horses in the PRE group consumed 2.1 ± 0.2 kg grain and 2.0 ± 0.2 kg of hay prior to the start of the SET. Horses in the POST group did not consume any feed prior to the SET but did consume 2.1 ± 0.2 kg of grain and 2.0 ± 0.3 kg hay in the 2 h after the SET. POST horses were only allowed access to feed for 2 h postfeeding on the day of the SET to allow for comparable consumption between PRE and POST horses. The FASTED group did not consume any feed prior to or after the SET until sampling was complete. On a daily basis (during adjustment period and on all non-SET days) all horses consumed 2.1 ± 0.2 kg grain per day and 9.5 ± 0.9 kg hay per day. Average CP intake was 1,024 ± 27 g/d (11.4% CP). Daily AA intakes are shown in Table 2. AA intake during the day of the SET sampling period for horses in PRE and POST groups is shown in Table 3. Differences in AA intake between Table 2 and Table 3 are due to limiting access to feed for the POST horses to have equal feeding times for PRE and POST horses.

Table 2. Daily amino acid intake for all horses during adjustment periods and non-SET days

Amino Acid	g/d	SD
Arginine	30	3.1
Histidine	12	1.1
Isoleucine	19	2.0
Leucine	34	3.1
Lysine	28	2.0
Methionine	8	1.4
Phenylalanine	22	2.0
Threonine	18	1.7
Valine	27	2.5

Treatment Effects

There was no overall effect of treatment on plasma glucose, lactate, 3MH, or CK. There was an effect of treatment on plasma concentrations of plasma urea-N, ammonia, and creatinine ($P < 0.01$). Plasma urea-N was greater for FASTED horses compared with both PRE and POST horses ($P < 0.004$), whereas plasma creatinine was greater for POST horses compared with FASTED and PRE horses ($P < 0.01$). Plasma ammonia concentrations were greater for both FASTED and POST horses compared with PRE horses ($P < 0.05$). Plasma metabolite concentrations by treatment are shown in Table 4.

There was no effect of treatment on plasma concentrations of glycine, isoleucine, leucine, and lysine. There was an effect of treatment on plasma concentrations of alanine, arginine, glutamine,

Table 3. Amino acid intake for PRE (2 h pre-exercise period) and POST horses (2 h postexercise period)

Amino acid	PRE horses, g/d	POST horses, g/d	SD
Arginine	21	23	6.7
Histidine	9	9	2.5
Isoleucine	13	14	4.0
Leucine	24	25	7.3
Lysine	20	21	6.2
Methionine	6	6	2.0
Phenylalanine	15	16	4.8
Threonine	12	13	3.7
Valine	18	19	5.4

Table 4. Plasma metabolite concentrations for PRE, POST, and FASTED horses (treatment effect)

Metabolite	PRE horses	POST horses	FASTED horses	SE
Ammonia, mmol/L	112.20[a]	152.60[b]	151.30[b]	4.90
Creatinine, mmol/L	138.90[a]	159.30[b]	141.60[a]	5.30
Creatine kinase, U/L	209.60	202.00	227.00	10.80
Glucose, mmol/L	6.38	6.36	6.31	0.09
Lactate, mmol/L	0.78	0.64	0.71	0.05
3-Methyl-histidine, mmol/L	11.00	10.70	10.90	0.40
Urea, mmol/L	4.76[a]	5.25[a]	5.95[b]	0.20

[a,b]Superscripts in the same row are different $P < 0.05$.

histidine, methionine, ornithine, phenylalanine, serine, threonine, tyrosine, and valine ($P < 0.05$). The majority of concentrations of AA in plasma were greater for PRE horses compared with FASTED and POST horses. Plasma AA concentrations by treatment are shown in Table 5.

When changes in plasma concentrations were calculated compared with resting plasma concentrations, there was no effect of treatment for plasma CK or lactate. There was an effect of treatment on plasma concentrations of ammonia, creatinine, glucose, 3MH, and urea-N. Changes in plasma ammonia, glucose, 3MH, and urea-N were positive for POST horses, whereas changes in plasma concentrations of creatinine were negative for the POST horses. Changes in plasma ammonia, glucose, and 3MH were negative for the PRE horses, whereas plasma creatinine and urea-N were positive for the PRE horses. Changes in plasma metabolite concentrations are shown in Table 6.

When the plasma AA concentration changes were calculated compared with resting plasma AA concentrations, there was no effect of treatment on changes in plasma glycine concentrations. There was an effect of treatment on plasma concentration changes of alanine, arginine, glutamine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, serine, threonine, tyrosine, and valine ($P < 0.05$). These plasma AA concentration changes were generally positive and significantly greater for the POST and FASTED horses compared with the PRE horses with the exception of plasma glutamine and methionine. Plasma AA concentration changes were generally negative for PRE horses with the exception of glutamine, histidine, and phenylalanine. Changes in plasma AA concentrations by treatment are shown in Table 7.

When changes in plasma concentrations from rest were calculated as a percentage change from resting concentrations, plasma ammonia, CK, and glucose were positive and significantly greater for POST and FASTED horses compared with PRE horses, whereas percentage changes in plasma concentrations of

Table 5. Plasma amino acid concentrations for PRE, POST, and FASTED horses (treatment effect)

Amino acid, mmol/L	PRE horses	POST horses	FASTED horses	SE
Alanine	221.3[a]	198.8[a]	165.5[b]	5.8
Arginine	110.8[a]	106.1[a]	82.3[b]	3.1
Glycine	780.9	745.9	713.0	18.7
Glutamine	1,205.3[a]	1,656.5[a]	4,454.7[b]	54.5
Histidine	81.6[a]	73.4[b]	69.9[b]	1.6
Isoleucine	72.4	71.4	73.3	1.7
Leucine	128.1	119.4	127.4	4.4
Lysine	146.6	142.6	134.5	4.4
Methionine	43.6[a]	43.4[a]	34.4[b]	1.5
Phenyl-alanine	76.4[a]	68.6[b]	73.1[c]	1.1
Ornithine	80.8[a]	78.3[b]	86.8[a]	3.0
Serine	259.4[a]	240.1[b]	227.4[b]	5.1
Threonine	128.1[a]	108.3[b]	124.2[a]	3.1
Tyrosine	88.6[a]	81.2[b]	85.7[a]	2.0
Valine	231.4[a]	210.9[b]	216.2[b]	4.0

[a,b,c]Superscripts in the same row are different $P < 0.05$.

Table 6. Plasma metabolite concentrations expressed as a change from resting concentrations for PRE, POST, and FASTED horses (treatment effect)

Metabolite	PRE horses	POST horses	FASTED horses	SE
Ammonia, mmol/L	−11.00[a]	25.70[b]	−3.400[a]	5.50
Creatinine, mmol/L	0.44[ab]	−29.20[a]	5.750[b]	10.60
Creatine kinase, U/L	50.90	25.70	14.000	13.70
Glucose, mmol/L	−1.52[a]	0.42[b]	0.011[b]	2.20
Lactate, mmol/L	0.14	0.19	0.290	0.07
3-Methyl-histidine, mmol/L	−0.50[a]	0.80[b]	0.600[b]	0.40
Urea, mmol/L	1.25[a]	0.10[b]	−0.300[b]	0.30

[a,b] superscripts in the same row are different $P < 0.05$.

Table 7. Plasma amino acid concentrations expressed as a change from resting concentrations for PRE, POST, and FASTED Horses (treatment effect)

Amino acid, mmol/L	PRE horses	POST horses	FASTED horses	SE
Alanine	−21.1[a]	83.5[b]	41.9[c]	6.4
Arginine	−5.6[a]	39.0[b]	12.4[c]	3.0
Glycine	43.8	53.6	29.6	11.8
Glutamine	27.5[a]	923.4[b]	122.3[a]	63.2
Histidine	3.4[a]	16.5[b]	8.2[c]	1.7
Isoleucine	−12.8[a]	3.5[b]	1.5[b]	2.3
Leucine	−14.9[a]	14.7[b]	18.4[b]	4.6
Lysine	−30.8[a]	42.9[b]	18.6[c]	4.5
Methionine	−0.2[a]	6.8[b]	1.2[a]	1.4
Phenyl-alanine	2.3[a]	8.1[b]	8.3[b]	1.5
Ornithine	−1.2[a]	15.8[b]	5.9[c]	2.1
Serine	−0.4[a]	45.3[b]	20.1[c]	4.9
Threonine	0.9[a]	27.5[b]	25.1[b]	3.1
Tyrosine	−4.5[a]	14.8[b]	8.1[c]	2.0
Valine	−1.0[a]	30.3[b]	18.9[b]	5.0

[a,b,c] superscripts in the same row are different $P < 0.05$.

ammonia, glucose, and 3MH were negative for PRE horses ($P < 0.05$). There was no effect of treatment on changes when expressed as a percentage for creatinine or lactate. Plasma metabolite changes as a percent of resting concentrations are shown in Table 8.

When changes from rest were expressed as a percent change from resting concentrations, there was no effect of treatment on plasma concentrations of glycine. All other plasma AA concentration changes as a percentage of resting concentrations were affected by treatment ($P < 0.05$). POST and FASTED horses had greater positive change percentages for alanine, arginine, glutamine, histidine, isoleucine, leucine, lysine, phenylalanine, ornithine, serine, threonine, tyrosine, and valine compared with PRE horses. PRE horses had negative change percentages for alanine, arginine, isoleucine, leucine, lysine, and tyrosine ($P < 0.05$). Plasma AA changes as a percent of resting concentrations are shown in Table 9.

Time Effects

Plasma concentrations of creatinine, ammonia, and 3MH were not affected by time. Plasma concentrations of CK, glucose, lactate, and urea-N were affected by time ($P < 0.001$). These metabolites, with the exception of glucose, increased at the peak of exercise followed by a decrease at the 1 to 2 h postexercise sampling time. There was another increase in these metabolites during the 4 and 7 h postexercise sampling time ($P < 0.001$). Plasma glucose was less at the peak of exercise compared with rest and all postexercise time points ($P < 0.05$). Plasma metabolite concentrations by time are shown in Table 10.

Table 8. Plasma metabolite concentrations change from resting concentrations expressed as a percent of resting concentrations for PRE, POST, and FASTED

Metabolite, %	PRE horses	POST horses	FASTED horses	SE
Ammonia	−3.53[a]	26.58[b]	−0.41[a]	5.10
Creatinine	2.60	−1.60	4.80	3.00
Creatine kinase	34.60[a]	14.10[b]	8.70[b]	7.40
Glucose	−19.10[a]	8.10[b]	1.90[c]	1.80
Lactate	19.90	36.10	56.40	13.40
3-Methyl-histidine	−3.90[a]	15.00[b]	9.70[b]	4.70
Urea	44.20[a]	12.90[b]	1.09[b]	7.50

[a,b,c]Superscripts in the same row are different $P < 0.05$.

Table 9. Plasma amino acid concentrations change from resting concentrations expressed as a percent of resting concentrations for PRE, POST, and FAST Horses (treatment effect)

Amino acid, %	PRE horses	POST horses	FASTED horses	SE
Alanine	−5.40[a]	72.90[b]	38.50[c]	5.20
Arginine	−2.40[a]	55.90[b]	20.50[c]	4.10
Glycine	7.70	7.90	4.20	1.80
Glutamine	2.10[a]	25.90[b]	11.50[c]	2.50
Histidine	5.80[a]	28.50[b]	13.20[c]	2.60
Isoleucine	−11.70[a]	5.40[b]	2.30[b]	2.90
Leucine	−5.70[a]	14.00[b]	17.80[b]	3.80
Lysine	−15.90[a]	43.70[b]	17.50[c]	3.80
Methionine	2.00[a]	18.50[b]	3.90[a]	3.10
Phenylalanine	4.60[a]	14.40[b]	12.90[b]	2.30
Ornithine	0.77[a]	23.10[b]	10.00[c]	3.00
Serine	1.10[a]	23.70[b]	9.90[c]	2.30
Threonine	2.80[a]	33.90[b]	24.50[c]	3.10
Tyrosine	−3.20[a]	23.30[b]	10.20[c]	2.50
Valine	2.20[a]	16.90[b]	9.60[c]	2.40

[a,b,c] superscripts in the same row are different $P < 0.05$.

All of the plasma AA concentration were affected by time ($P<0.001$). The general trend was for the plasma AA concentrations to be greater at the peak of exercise (with the exception of histidine, methionine, serine, and tyrosine) compared with rest. The trend that followed was for plasma concentrations to decrease by the 1 h postexercise sampling time and remain decreased throughout the 7 h postsampling period ($P < 0.001$) compared with the peak of exercise. Plasma concentrations of histidine and serine became greater at the 2 h postexercise sampling time and remained elevated through the 7 h postexercise time point, whereas plasma concentrations of methionine and tyrosine only became greater in the 4 and 7 h postexercise sampling time compared with the other time points ($P < 0.001$). Plasma AA concentrations by time are shown in Table 11.

When changes in plasma metabolites from resting concentrations were calculated, there was no effect of time for plasma concentrations of ammonia, creatinine or 3MH. There was a significant effect of time on plasma concentration changes for CK, glucose, lactate, and urea-N ($P < 0.05$). Changes in these concentrations were generally positive at the peak of exercise and became negative at the 1 h postexercise sampling time. Plasma urea-N changes plateaued by the 2 h postexercise sampling time, whereas plasma creatinine changes became negative at the 2 h postexercise sampling time. Changes in plasma metabolite concentrations by time are shown in Table 12.

Table 10. Plasma metabolite concentrations at rest, the peak of exercise, 1, 2, 4, and 7 h postexercise (time effect)

Metabolite	Rest	Peak exercise	1 h postexercise	2 h postexercise	4 h postexercise	7 h postexercise	SE
Ammonia, mmol/L	137.90	130.90	138.40	145.80	139.60	137.30	6.50
Creatinine, mmol/L	153.10	150.45	145.14	148.68	141.60	141.60	7.10
Creatine kinase, U/L	193.40[a]	243.60[b]	188.40[a]	214.50[a]	229.20[b]	242.40[b]	16.40
Glucose, mmol/L	6.63[a]	5.63[b]	6.23[ab]	6.88[a]	6.40[b]	6.34[b]	0.13
Lactate, mmol/L	0.57[a]	0.90[b]	0.66[a]	NA[1]	NA	NA	0.05
3-Methyl-histidine, mmol/L	10.80	11.80	10.60	11.40	11.20	11.50	0.48
Urea, mmol/L	4.99[a]	5.64[ab]	4.75[b]	5.16[a]	5.22[ab]	5.85[b]	0.25

[1]NA = not applicable.

[a,b]Superscripts in the same row are different $P < 0.05$.

Table 11. Plasma amino acid concentrations rest, the peak of exercise, 1, 2, 4, and 7 h postexercise (time effect)

Amino acid, mmol/L	Rest	Peak exercise	1 h postexercise	2 h postexercise	4 h postexercise	7 h postexercise	SE
Alanine	176.5[a]	220.3[b]	186.3[a]	208.4[ab]	197.2[a]	196.4[a]	7.6
Arginine	97.1[a]	108.7[b]	109.5[b]	119.3[b]	104.9[a]	97.2[a]	4.1
Glycine	712.1[a]	779.4[b]	743.1[b]	754.8[b]	759.3[b]	784.0[b]	24.7
Glutamine	2,118.5[a]	2,490.1[b]	2,187.0[a]	2,489.9[b]	2,374.1[b]	2,300.3[b]	72.2
Histidine	71.6[a]	74.3[a]	73.5[a]	80.7[b]	78.3[b]	78.6[b]	2.2
Isoleucine	75.1[a]	83.4[b]	73.4[a]	76.3[a]	61.4[c]	63.8[c]	2.3
Leucine	120.1[a]	153.0[b]	123.1[a]	127.8[a]	104.8[c]	113.3[c]	4.4
Lysine	141.3[a]	163.6[b]	145.6[a]	157.6[b]	129.6[a]	120.9[c]	5.8
Methionine	39.7[ab]	42.7[a]	44.0[a]	44.6[a]	37.8[b]	35.8[b]	1.9
Phenylalanine	68.7[a]	79.8[b]	72.5[ac]	73.0[c]	66.0[a]	69.2[a]	1.5
Ornithine	79.1[a]	94.9[b]	81.5[ab]	88.6[a]	82.5[a]	78.1[b]	3.9
Serine	232.7[a]	248.0[ab]	238.9[ab]	257.5[b]	248.0[ab]	252.2[ab]	7.1
Threonine	111.6[a]	125.0[b]	120.7[a]	127.4[b]	116.6[ab]	112.6[a]	4.1
Tyrosine	83.0[ab]	89.9[a]	91.9[a]	93.1[a]	78.4[b]	75.4[b]	2.6
Valine	210.6[a]	234.0[b]	219.6[b]	228.8[ab]	205.3[a]	204.3[a]	5.3

[a,b]Superscripts in the same row are different $P < 0.05$.

Table 12. Plasma metabolite concentration changes from resting concentrations at the peak of exercise, 1, 2, 4, and 7 h postexercise (time effect)

Metabolite	Peak exercise	1 h postexercise	2 h postexercise	4 h postexercise	7 h postexercise	SE
Ammonia, mmol/L	0.63	−6.84	12.17	2.99	6.61	7.90
Creatinine, mmol/L	−2.66	−7.97	−4.43	−11.50	−11.50	14.16
Creatine kinase, U/L	50.20[a]	−5.00[b]	21.10[a]	35.80[a]	48.90[a]	17.70
Glucose, mmol/L	−0.99[a]	−0.40[b]	0.25[c]	−0.22[b]	−0.28[b]	0.16
Lactate, mmol/L	0.33[a]	0.08[b]	NA[1]	NA	NA	
3-Methyl-histidine, mmol/L	0.92	−0.26	0.97	0.66	1.03	0.57
Urea, mmol/L	0.70[a]	−0.13[ab]	0.23[ab]	0.15[ab]	1.30[b]	0.41

[1]NA = not applicable.

[a,b,c]Superscripts in the same row are different $P < 0.05$.

When change from rest was calculated for plasma AA concentrations, there was no effect of time on plasma concentrations of glycine and threonine. There was an effect of time on changes in plasma concentrations of alanine, arginine, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, ornithine, serine, tyrosine, and valine ($P < 0.001$). Changes in the concentrations of the plasma AA were all positive at the peak of exercise as well as at the 2 h postexercise sampling time. Changes at the 1 h postsampling time were positive for alanine, arginine, glutamine, histidine, lysine, methionine, phenylalanine, ornithine, tyrosine, and valine but

negative for isoleucine, leucine, and serine. In the 4 and 7 h postexercise sampling time, changes were positive for alanine, arginine, glutamine, histidine, ornithine, and serine but negative for isoleucine, leucine, lysine, methionine, and tyrosine ($P < 0.001$). Plasma AA concentration changes by time are shown in Table 13.

When plasma metabolite concentration changes from resting concentrations were expressed as a percentage change, there was no effect of time on plasma ammonia, creatinine, or 3MH. There was an effect of time on plasma concentration change percentages for CK, glucose, lactate, and urea-N ($P < 0.001$). Change percentages were positive for plasma CK, lactate, and urea-N from the peak of exercise throughout the postexercise sampling period. Change percentages were negative for plasma glucose at the peak of exercise as well as at the 4 and 7 h postexercise time points. Plasma metabolite concentration changes as a percent of resting concentrations are shown in Table 14.

The percent change for plasma concentrations compared with resting concentrations of all of the AA were affected by time ($P < 0.001$) with the exception of glycine. Change percentages were positive throughout the sampling period for alanine, arginine, glutamine, histidine, lysine, ornithine, and threonine. The percentage changes were initially positive but became negative as the time postexercise progressed for plasma isoleucine, leucine, phenylalanine, tyrosine, and valine, particularly at the 4 and 7 h postexercise sampling times. Plasma AA concentration changes as a percent of resting concentration are shown in Table 15.

Treatment by Time Interaction Effects

There was no time by treatment interaction for plasma concentrations of lactate and 3MH. There was a time by treatment interaction for plasma concentrations of ammonia, creatinine, CK, glucose, and urea-N ($P < 0.01$). Plasma ammonia was greater for FASTED and POST horses at the 2 through 7 h postexercise sampling time points ($P < 0.05$). Plasma creatinine was greater for POST horses compared with PRE and FASTED horses at rest and the peak of exercise. Plasma glucose was greater for PRE horses compared with POST and FASTED horses from rest through 2 h postexercise. Plasma urea-N was generally less for PRE horses compared with FASTED horses as well as compared with POST at a few time points ($P < 0.05$). Plasma metabolite concentrations by treatment over the sampling points are shown in Table 16.

There was a time by treatment interaction for plasma concentrations of alanine, arginine, glutamine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, serine, threonine, tyrosine, and valine ($P < 0.05$). There was no time by treatment interaction for plasma concentrations of glycine. PRE horses generally had greater concentrations of plasma concentrations of alanine, arginine, histidine, isoleucine, leucine, lysine, phenylalanine, serine, threonine, tyrosine, and valine compared with POST and FASTED horses

Table 13. Plasma amino acid concentration changes from resting concentrations at the peak of exercise, 1, 2, 4, and 7 h postexercise (time effect)

Amino acid, mmol/L	Peak exercise	1 h postexercise	2 h postexercise	4 h postexercise	7 h postexercise	SE
Alanine	52.1[a]	6.9[b]	36.2[a]	18.9[b]	32.6[a]	9.0
Arginine	16.3[a]	7.0[b]	25.7[a]	9.8[b]	8.9[b]	4.3
Glycine	73.5	29.4	52.1	49.4	89.4	16.6
Glutamine	477.7a	71.3[b]	497.3[a]	340.4[a]	324.5[a]	89.4
Histidine	4.9[a]	1.9[a]	10.3[b]	6.4[a]	9.4[b]	2.4
Isoleucine	11.2[a]	−4.3[b]	3.9[a]	−13.2[b]	−7.6[b]	3.4
Leucine	37.5[a]	−2.2[b]	12.2[b]	−16.6[bc]	−1.5[b]	6.5
Lysine	29.1[a]	2.9[b]	27.1[a]	−3.1[b]	−4.8[b]	6.4
Methionine	4.7[a]	3.5[b]	6.3[a]	−0.8[b]	−1.6[b]	1.9
Phenylalanine	13.4[a]	3.8[b]	7.2[b]	−2.1[b]	3.5[b]	2.2
Ornithine	17.2[a]	0.2[b]	11.3[a]	4.2[b]	3.8[b]	3.0
Serine	21.5[a]	−1.4[b]	27.2[a]	13.6[a]	27.9[a]	7.0
Threonine	17.8	7.0	17.9	6.9	8.1	4.4
Tyrosine	9.9[a]	7.3[a]	13.4[a]	−2.1[b]	−2.3[b]	2.8
Valine	29.9[a]	4.3[b]	22.8[a]	−6.5[b]	0.1[b]	7.1

[a,b,c]Superscripts in the same row are different $P < 0.05$.

Table 14. Plasma metabolite concentration changes from resting concentrations expressed as a percent of resting concentrations at the peak of exercise, 1, 2, 4, and 7 h postexercise (time effect)

Metabolite, %	Peak exercise	1 h postexercise	2 h postexercise	4 h postexercise	7 h postexercise	SE
Ammonia	6.10	−0.30	13.60	6.40	8.00	7.30
Creatinine	5.50	1.90	3.90	−0.80	−0.90	3.90
Creatine kinase	31.90[a]	0.20[b]	14.90[a]	21.70[a]	26.80[a]	9.50
Glucose	−11.80[a]	−5.10[b]	5.10[b]	−1.10[b]	−2.30[b]	2.30
Lactate	60.20[a]	14.70[b]	NA[1]	NA	NA	10.90
3-Methyl-histidine	13.10	0.10	13.70	10.90	13.70	6.70
Urea	26.30[a]	5.90[a]	13.70[a]	17.50[a]	41.00[b]	10.60

[1]NA = not applicable.

[a,b,c]Superscripts in the same row are different $P < 0.05$.

Table 15. Plasma amino acid concentration changes from resting concentrations expressed as a percent of resting concentrations at the peak of exercise, 1, 2, 4, and 7 h postexercise (time effect)

Amino acid,%	Peak exercise	1 h post-texercise	2 h post-texercise	4 h post-texercise	7 h post-texercise	SE
Alanine	40.10[a]	8.90[b]	33.80[a]	30.50[a]	42.50[a]	7.30
Arginine	22.50[ab]	8.50[a]	38.10[b]	20.50[a]	20.80[a]	5.70
Glycine	11.10	4.70	8.00	7.90	13.30	2.50
Glutamine	24.40[a]	4.90[b]	15.60[ab]	6.70[b]	7.10[b]	3.50
Histidine	7.60[a]	3.20[a]	16.40[b]	13.40[ab]	17.60[b]	3.70
Isoleucine	17.10[a]	−4.30[b]	8.80[a]	−14.40[b]	−8.60[b]	4.10
Leucine	35.40[a]	0.55[b]	16.60[bc]	−9.50[b]	1.90[bc]	5.30
Lysine	25.50[a]	3.60[b]	31.50[a]	10.50[b]	5.30[b]	5.40
Methionine	13.30[a]	9.90[ab]	16.10[a]	0.50[b]	−2.40[b]	4.40
Phenylalanine	21.00[a]	6.40[ab]	12.90[a]	−1.30[b]	6.10[a]	3.30
Ornithine	24.20[ab]	1.10[b]	16.70[a]	8.90[b]	8.80[b]	4.30
Serine	10.20[a]	−0.50[b]	13.90[a]	8.70[a]	15.80[a]	3.30
Threonine	18.80[a]	7.70[b]	21.30[a]	12.00[a]	12.60[a]	4.40
Tyrosine	13.90[a]	9.70[a]	20.90[b]	1.44[bc]	−0.46[bc]	3.50
Valine	15.70[a]	2.90[b]	13.40[a]	−0.60[ab]	1.90[ab]	3.50

[a,b,c]Superscripts in the same row are different $P < 0.05$.

at rest, the peak of exercise, and 1 h postexercise ($P < 0.05$). Plasma concentrations then generally became elevated for POST horses compared with PRE and FASTED horses for plasma alanine, arginine, histidine, lysine, methionine, phenylalanine, serine, tyrosine, and valine postexercise particularly during the 2 and 4 h postexercise sampling time ($P < 0.05$). At the 7 h postexercise sampling time, plasma concentrations of isoleucine and leucine were less for POST horses compared with PRE and FASTED horses ($P < 0.05$). There were no differences between treatments at the 4 h postexercise time point for plasma methionine and threonine. Plasma concentrations of glutamine were significantly elevated for POST horses compared with PRE and FASTED horses at all sampling time points ($P < 0.05$). Plasma AA concentrations by treatment and time are shown in Tables 17 and 18.

When changes in plasma metabolite concentrations were compared with resting concentrations, there was no time by treatment interaction for 3MH or creatinine. There was a time by treatment interaction for plasma concentration changes for ammonia, CK, glucose, lactate, and urea-N ($P < 0.05$). The changes in plasma concentrations were negative

Table 16. Plasma metabolites at rest, during SET, and postexercise sampling times for PRE, POST, and FASTED horses

	NH_3, mmol/L	CRT, mmol/L	CK, U/L	GLU, mmol/L	LAC, mmol/L	3MH, mmol/L	Urea, mmol/L
PRE horses							
Rest	120.9	138.1[a]	173.4	7.64[a]	0.69	11.49	3.70[a]
Peak exercise	133.4	146.9[a]	283.6[a]	4.00[a]	0.52	12.18	5.48[a]
1 h postexercise	115.0	139.8	186.8	6.90[a]	0.51	10.75	4.27
2 h postexercise	114.4[a]	133.6	200.5	7.29[a]	na	11.11	4.65[a]
4 h postexercise	100.0[a]	133.6	208.9	6.15[a]	na	10.81	4.98
7 h postexercise	101.7[a]	139.8	241.8	6.29	na	11.58	5.41[a]
SE	13.0	12.4	28.5	0.22	0.09	0.97	0.50
POST horses							
Rest	130.6	183.2[b]	186.1	6.00[b]	0.52	9.99	5.16[b]
Peak exercise	130.6	155.8[b]	190.4[b]	6.26[b]	0.81	11.18	4.80[b]
1 h postexercise	136.0	154.0	175.1	5.67[b]	0.60	10.02	5.07
2 h postexercise	170.4[b]	172.6	223.8	7.22[a]	na	11.69	4.79[a]
4 h postexercise	177.8[b]	146.9	243.8	6.89[b]	na	11.69	5.03
7 h postexercise	162.4[b]	142.5	226.1	6.11	na	11.43	7.33[b]
SE	13.0	12.4	28.5	0.22	0.09	0.97	0.50
FASTED horses							
Rest	154.1	137.2[a]	220.7	6.29[b]	0.51	10.40	6.18[b]
Peak exercise	143.6	148.7[a]	256.8[a]	6.63[b]	1.05	11.29	6.78[c]
1 h postexercise	134.7	142.5	203.2	6.11[b]	0.56	10.34	5.31
2 h postexercise	157.5[b]	139.8	219.4	6.13[b]	na	11.97	6.29[b]
4 h postexercise	142.0[b]	142.5	235.0	6.18[a]	na	11.36	5.43
7 h postexercise	161.4[b]	141.6	259.3	6.63	na	11.96	6.17[a]
SE	13.0	12.4	28.5	0.22	0.09	0.97	0.50

[1]NH_3 = ammonia, CRT= creatinine, CK= creatine kinase, GLU = glucose, LAC = lactate, 3MH = 3-methyl-histidine.

[a,b,c]Superscripts in the same column for the same time point are different $P < 0.05$.

Table 17. Plasma essential amino acid concentrations (mmol/L) at rest, during SET, and postexercise sampling times for PRE, POST, and FASTED horses

	ARG[1]	HIS	ILE	LEU	LYS	MET	PHE	THR	VAL
PRE horses									
Rest	115.5[a]	78.6[a]	82.9[a]	139.9[a]	172.9[a]	43.6	74.3[a]	127.0[a]	231.2[a]
Peak exercise	126.6[a]	85.7[a]	84.4	158.7	191.8[a]	48.0	86.9[a]	142.3[a]	254.0[a]
1 h postexercise	133.7[a]	82.0[a]	78.4[a]	134.0[a]	176.8[a]	50.5[a]	80.1[a]	137.4[a]	242.9[a]
2 h postexercise	114.2[a]	86.0[a]	62.2[a]	100.2[a]	138.1[a]	44.0[a]	69.7[a]	130.3	221.9[a]
4 h postexercise	90.3[a]	72.7[a]	54.8	96.5	98.7[a]	37.6[a]	66.6	105.5	200.5
7 h postexercise	79.9[a]	72.3[a]	67.1[a]	121.1[a]	99.8[a]	38.3	74.5[a]	102.4	202.9
SE	8.2	4.4	4.6	8.9	11.7	3.9	2.9	8.1	10.5
POST horses									
Rest	72.6[b]	59.3[b]	68.4[b]	106.8[b]	105.7[b]	37.5	61.7[b]	84.7[b]	184.9[b]
Peak exercise	89.4[b]	62.0[b]	82.2	147.4	135.4[b]	42.6	74.1[b]	99.1[b]	212.3[b]
1 h postexercise	73.3[b]	58.9[b]	64.8[b]	105.2[b]	106.3[b]	38.8[b]	64.5[b]	86.3[b]	183.9[b]
2 h postexercise	145.5[b]	79.7[a]	100.1[b]	171.0[b]	214.1[b]	57.4[b]	82.7[b]	125.7	253.5[b]
4 h postexercise	128.2[b]	82.1[b]	65.1	104.3	168.8[b]	45.3[b]	64.5	119.1	211.2
7 h postexercise	127.6[b]	85.7[b]	55.2[b]	94.8[b]	141.3[b]	39.4	61.6[b]	112.7	201.4
SE	8.2	4.4	4.6	8.9	11.7	3.9	2.9	8.1	10.5
FASTED horses									
Rest	71.7[b]	63.0[b]	72.1[b]	111.7[b]	118.5[b]	33.4	65.9[b]	102.6[b]	200.0[b]
Peak exercise	92.6[b]	67.8[b]	90.4	164.9	157.4[b]	38.1	80.8[b]	126.3[a]	239.6[a]
1 h postexercise	73.9[b]	65.5[b]	67.3[b]	112.8[b]	122.8[b]	35.8[b]	68.9[b]	111.5[c]	202.0[b]
2 h postexercise	77.1[c]	66.0[b]	72.9[a]	123.7[a]	126.1[a]	32.1[c]	71.1[a]	112.0	209.0[a]
4 h postexercise	71.1[a]	65.6[a]	67.5	114.8	121.2[a]	30.1[a]	65.6	112.7	192.7
7 h postexercise	79.2[a]	71.0[a]	78.3[a]	138.1[a]	141.7[b]	32.0	76.2[a]	123.6	211.9
SE	8.2	4.4	4.6	8.9	11.7	3.9	2.9	8.1	10.5

[1]ARG = arginine, HIS = histidine, ILE = isoleucine, LEU = leucine, LYS = lysine, MET = methionine, PHE = phenylalanine, THR = threonine, VAL = valine.

[a,b,c]Superscripts in the same column for the same time point are different $P < 0.05$.

for PRE and FASTED horses for ammonia and glucose from the 1 h postsampling time through the 4 h postsampling time compared with POST horses. POST horses had positive plasma concentration changes throughout the sampling period for ammonia but negative changes for urea-N ($P < 0.05$). Concentration changes for CK were greater for PRE and FASTED horses compared with POST horses at the peak of exercise while lactate concentrations were greater for POST and FASTED horses compared with PRE horses at the peak of exercise ($P < 0.05$). Plasma metabolite changes by treatment and time are shown in Table 19.

When changes in plasma concentrations were calculated compared with resting concentrations, all plasma AA changes were affected by a time by treatment interaction ($P < 0.05$). The plasma concentration changes were positive for alanine, arginine, glycine, glutamine, histidine, lysine, methionine, ornithine, serine, threonine, tyrosine, and valine for POST horses in the 2 h through 7 h postexercise sampling times compared with PRE and FASTED horses. The PRE horses had mostly negative changes in the same time frame for these same AA ($P < 0.05$). Plasma concentration changes from rest for PRE horses were significantly greater (albeit negative changes) for isoleucine and leucine compared with POST and FASTED horses ($P < 0.05$). Plasma concentration changes for histidine and phenylalanine were positive and greater for POST horses compared with negative changes for PRE horses. FASTED horses had positive plasma concentration changes but to a lesser extent than POST horses. Plasma AA concentration change from rest by treatment and time are shown in Tables 20 and 21.

When changes from rest were expressed as a percent change (of resting concentrations), there was no treatment by time interaction for creatinine or 3MH. Plasma ammonia percentage changes were greater for POST horses in the 4 and 7 h postexercise sampling times compared with both PRE and FASTED horses, whereas plasma glucose percentage change was less for POST horses compared with PRE and FASTED horses ($P < 0.05$). Plasma urea-N and creatinine percentage changes were greater for PRE horses at the peak of exercise as well as during the 4 and 7 h postexercise sampling times for urea-N compared with POST and

Table 18. Plasma nonessential amino acid concentrations at rest, during SET and postexercise sampling times for PRE, POST, and FASTED horses

	ALA[1]	GLY	GLN	ORN	SER	TYR
PRE horses						
Rest	238.2[a]	705.0	1,629.4[a]	78.9	259.4[a]	92.3[a]
Peak exercise	276.3[a]	834.7	1,985.2[a]	96.6	283.1[a]	99.0[a]
1 h postexercise	241.8[a]	753.8	1,760.7[a]	83.5	271.6[a]	104.0[a]
2 h postexercise	218.4[a]	773.9	1,699.8[a]	78.3[a]	263.4[a]	91.7[a]
4 h postexercise	189.7[a]	752.8	1,478.8[a]	68.8[a]	242.1[a]	72.0
7 h postexercise	158.4[a]	737.7	1,374.8[a]	61.4[a]	235.5[a]	71.8
SE	15.3	49.4	144.4	8.0	14.3	5.3
POST horses						
Rest	127.2[b]	667.1	3,663.1[c]	73.3	201.3[b]	68.5[b]
Peak exercise	180.3[b]	710.5	4,427.6[b]	86.5	217.3[b]	78.8[b]
1 h postexercise	136.5[b]	678.5	3,695.9[b]	70.3	189.5[b]	72.4[b]
2 h postexercise	246.7[a]	736.1	5,047.7[b]	104.7[b]	276.9[a]	106.6[b]
4 h postexercise	230.4[a]	777.6	4,921.9[b]	99.7[b]	262.5[b]	86.0
7 h postexercise	249.9[b]	818.9	4,855.0[b]	97.3[b]	285.2[b]	77.9
SE	15.3	49.4	144.4	8.0	14.3	5.3
FASTED horses						
Rest	129.6[b]	755.6	1,100.5[b]	75.7	210.2[b]	78.7[b]
Peak exercise	194.6[b]	803.0	1,413.5[a]	96.2	234.9[b]	91.6[a]
1 h postexercise	137.3[b]	783.7	1,150.3[a]	74.7	205.5[b]	85.1[b]
2 h postexercise	138.7[b]	774.0	1,137.5[c]	78.7[a]	212.2[b]	81.4[a]
4 h postexercise	139.8[b]	769.6	1,038.5[c]	74.9[a]	208.9[a]	76.6
7 h postexercise	184.5[a]	839.4	1,136.6[a]	80.7[ab]	234.1[a]	83.0
SE	15.3	49.4	144.4	8.0	14.3	5.3

[1]ALA = alanine, GLY = glycine, GLN = glutamine, ORN = ornithine, SER = serine, TYR = tyrosine.

[a,b,c] superscripts in the same column for the same time point are different $P < 0.05$.

FASTED horses ($P < 0.05$). Plasma metabolite changes as a percent of resting concentrations are shown in Figure 1.

When changes from rest were expressed as a percentage (percent change from resting concentrations), there was a time by treatment interaction for plasma alanine, arginine, glycine, glutamine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, serine, threonine, tyrosine, and valine. At the 2 h postexercise sampling time, POST horses had greater (and positive) percentage changes compared with PRE and FASTED horses for alanine, arginine, glutamine, histidine, lysine, methionine, ornithine, phenylalanine, serine, threonine, tyrosine, and valine, which persisted until the 7 h postexercise sampling time with the exception of methionine and phenylalanine, which only persisted until the 4 h postexercise sampling time ($P < 0.05$). Plasma concentration percent changes for isoleucine and leucine were significantly less for POST horses at the 4 and 7 h postexercise sampling time, whereas PRE horses saw a significant decline by 2 h postexercise ($P < 0.05$). PRE horses had a significant negative percentage change in plasma isoleucine and leucine and lysine at the 4 and 7 h postexercise sampling time. Plasma methionine and phenylalanine percentage change returned to baseline by the 4 and 7 h postexercise sampling time. Plasma alanine concentration percent change was significantly less for PRE horses compared with POST and FASTED horses at the peak of exercise and significantly greater through the 2 to 7 h postexercise sampling times for POST horses compared with PRE and FASTED horses ($P < 0.05$). Plasma AA concentration changes as a percent of resting concentrations are shown in Figures 2 and 3.

DISCUSSION

The role of feeding and available fuel sources during exercise in horses has been explored in regard to carbohydrates and fat sources (Hiney and Potter, 1996). Little attention has been paid to AA during exercise because it is believed that they provide very little in terms of fuel during exercise (Miller-Graber et al., 1991). However, availability of AA postexercise have been shown to improve the availability of free AA in muscle which stimulate muscle protein synthesis and reduce muscle protein breakdown (van den Hoven et al., 2010, 2011). Therefore, the timing of feeding with respect to exercise may affect the availability of AA in the recovery period.

In the current study, plasma glucose was reduced during exercise for POST horses as well as those that were in the FASTED group. This is most likely due to the longer period of time between feeding and exercise in these horses. Horses were fasted overnight prior to the SET so horses in the POST and FASTED groups would have gone at least 12 h without feed. Overall, glucose was reduced at the peak of exercise due to the uptake of glucose by the tissues during exercise for the production of energy. Lactate was greatest at the peak of exercise for all groups which would be expected as exercise increases the accumulation of lactate due to increased metabolism (Makai et al., 2007). Overall, FASTED and POST horses had lower plasma urea-N concentrations which again may be related to the length

Table 19. Plasma metabolite concentration changes at rest, during SET and postexercise sampling times for the PRE, POST, and FASTED horses

	NH_3, mmol/L[1]	CRT, mmol/L	CK, U/L	GLU, mmol/L	LAC, mmol/L	3MH, mmol/L	Urea, mmol/L
PRE horses							
Peak exercise	12.50	8.85	110.3[a]	−3.64[b]	0.16[b]	0.70	1.77[a]
1 h postexercise	−6.50	0.89	13.4	−0.74	0.12	−0.70	0.56
2 h postexercise	−6.60[a]	−4.43	27.1	−0.36[a]	NA	−0.04	0.95
4 h postexercise	−26.10[a]	−4.43	35.5	−1.49[b]	NA	−0.07	1.33[b]
7 h postexercise	−19.30[a]	0.89	68.4	−1.35[b]	NA	0.01	1.70[b]
SE	13.70	23.90	30.6	0.28	0.10	1.00	0.71
POST horses							
Peak exercise	0.01	−27.44	4.3[b]	0.25[a]	0.29[a]	1.20	−0.36[b]
1 h postexercise	5.30	−30.10	−11.0	−0.34	0.08	0.02	−0.08
2 h postexercise	39.80[b]	−11.51	37.6	1.22[b]	NA	1.70	−0.36
4 h postexercise	47.20[b]	−36.29	57.6	0.88[c]	NA	1.70	−0.13[a]
7 h postexercise	31.80[b]	40.71	40.0	0.11[a]	NA	1.40	2.17[b]
SE	13.70	23.90	30.6	0.28	0.10	1.00	0.71
FASTED horses							
Peak exercise	−10.60	11.51	36.0[a]	0.41[a]	0.54[a]	0.90	0.61[a]
1 h postexercise	−19.40	5.31	−17.5	−0.12	0.05	−0.01	−0.87
2 h postexercise	3.30[a]	2.66	−1.4	−0.11[a]	NA	1.60	0.11
4 h postexercise	−12.10[a]	5.31	14.3	−0.06[a]	NA	0.90	−0.75[a]
7 h postexercise	7.30[a]	4.43	38.5	0.39[a]	NA	1.60	−0.01[a]
SE	13.70	23.90	30.6	0.28	0.10	1.00	0.71

[1]NH_3 = ammonia, CRT = creatinine, CK = creatine kinase, GLU = glucose, LAC = lactate, 3MH = 3-methyl-histidine.

[a,b]Superscripts in the same column for the same time point are different $P < 0.05$.

Table 20. Plasma essential amino acid concentration (mmol/L) changes from resting concentrations during the SET and postexercise sampling times for the PRE, POST, and FASTED horses

	ARG[1]	HIS	ILE	LEU	LYS	MET	PHE	THR	VAL
PRE horses									
Peak exercise	11.1	7.1	1.5[b]	18.8[b]	18.9	4.4	12.6	15.4	22.9
1 h postexercise	18.2	3.5	−4.5	−5.9	3.9	6.9	5.8	10.5	11.7
2 h postexercise	−1.3[a]	7.5[a]	−20.7[b]	−39.6[b]	−34.8[b]	0.4[a]	−4.6[a]	3.4[a]	−9.2[a]
4 h postexercise	−25.6[b]	−6.3[a]	−31.6[b]	−50.4[b]	−74.9[b]	−6.9[a]	−8.8[a]	−23.7[b]	−38.3[a]
7 h postexercise	−35.6[b]	−6.2[a]	−15.7[b]	−18.8[b]	−73.1[b]	−5.3	0.2	−24.6[b]	−28.3[b]
SE	7.5	4.2	5.9	11.3	11.1	3.3	3.8	7.6	12.4
POST horses									
Peak exercise	16.8	2.7	13.8[a]	40.6[a]	29.7	5.1	12.5	14.4	27.4
1 h postexercise	0.7	−0.4	−3.6	−1.6	0.6	1.3	2.8	1.6	−1.0
2 h postexercise	72.9[b]	20.4[b]	31.6[c]	64.2[c]	108.4[c]	19.9[b]	21.0[b]	41.0[b]	68.5[b]
4 h postexercise	55.5[c]	22.9[c]	−3.4[a]	−2.5[c]	63.1[c]	7.8[b]	2.8[b]	34.4[c]	26.2[b]
7 h postexercise	54.9[c]	26.5[b]	−13.2[b]	−12.0[b]	35.6[a]	1.8	−0.1	27.9[a]	16.5[a]
SE	7.5	4.2	5.9	11.3	11.1	3.3	3.8	7.6	12.4
FASTED horses									
Peak exercise	20.9	4.8	18.4[a]	53.1[a]	38.8	4.7	14.9	23.7	39.6
1 h postexercise	2.2	2.5	−4.7	1.0	4.3	2.4	2.8	8.9	2.0
2 h postexercise	5.4[a]	3.0[a]	0.9[a]	12.0[a]	7.6[a]	−1.3[a]	5.2[a]	9.4[a]	9.0[a]
4 h postexercise	−0.5[a]	2.6[a]	−4.5[a]	3.0[a]	2.6[a]	−3.3[a]	−0.3[a]	10.1[a]	−7.3[ab]
7 h postexercise	7.5[a]	8.1[c]	6.2[a]	26.3[a]	23.2[a]	−1.4	10.3	20.9[a]	11.9[a]
SE	7.5	4.2	5.9	11.3	11.1	3.3	3.8	7.6	12.4

[1]ARG = arginine, HIS = histidine, ILE = isoleucine, LEU = leucine, LYS = lysine, MET = methionine, PHE = phenylalanine, THR = threonine, VAL = valine.

[a,b,c]Superscripts in the same column for the same time point are different $P < 0.05$.

Table 21. Plasma nonessential amino acid concentration changes from resting concentrations during the SET and postexercise sampling times for the PRE, POST, and FASTED horses

	ALA[1]	GLY	GLN	ORN	SER	TYR
PRE horses						
Peak exercise	38.1	129.7[b]	355.8[a]	17.7	23.6	6.8
1 h postexercise	3.6	48.9	131.3	4.6	12.2	11.7
2 h postexercise	−19.8[a]	68.9	70.4[a]	−0.6[a]	3.9[a]	−0.6[a]
4 h postexercise	−56.5[b]	23.7[a]	−175.6[a]	−13.2[a]	−19.3[a]	−21.6[b]
7 h postexercise	−79.7[b]	32.7[a]	−254.6[a]	−17.5[b]	−23.9[b]	−20.4[b]
SE	15.6	28.7	154.8	5.2	12.2	4.9
POST horses						
Peak exercise	53.1	43.4[a]	764.4[b]	13.2	16.0	10.2
1 h postexercise	9.3	11.3	32.8	−3.0	−11.7	3.8
2 h postexercise	119.4[b]	68.9	1,384.5[b]	31.5[b]	75.7[b]	38.0[b]
4 h postexercise	103.1[b]	110.5[b]	1,258.7[b]	26.4[b]	61.2[b]	17.5[c]
7 h postexercise	122.7[c]	151.7[b]	1,191.9[b]	23.9[c]	83.9[c]	9.4[a]
SE	15.6	28.7	154.8	5.2	12.2	4.9
FASTED horses						
Peak exercise	65.0	47.4[a]	313.0[a]	20.6	24.7	12.9
1 h postexercise	7.7	28.1	49.8	−1.0	−4.7	6.4
2 h postexercise	9.1[a]	18.4	37.0[a]	3.0[a]	2.0[a]	2.7[a]
4 h postexercise	10.2[a]	13.9[a]	−62.0[a]	−0.8[a]	−1.2[a]	−2.1[a]
7 h postexercise	54.9[a]	83.8[ab]	36.1[a]	5.0[a]	23.9[a]	4.3[a]
SE	15.6	28.7	154.8	5.2	12.2	4.9

[1]ALA = alanine; GLY = glycine; GLN = glutamine; ORN = ornithine; SER = serine; TYR = tyrosine.

[a,b,c]Superscripts in the same column for the same time point are different $P < 0.05$.

of time since feeding (12 h) resulting in some deamination of some AA for other functions. Plasma CK concentrations were less for POST horses and this was also evident when the plasma concentration was expressed as a change from rest as well as a percent change. CK is typically used as a marker of muscle damage and may be a piece of evidence contributing to less muscle damage or improved muscle recovery when horses are fed after exercise.

Most of the plasma AA concentrations were greater at the peak of exercise. This is in agreement with other studies done in horses (van den Hoven et al., 2010, 2011; Poso et al., 1991). Some of this change (increase) would be due to hemoconcentration during exercise however, when the concentration changes were expressed as a percentage (percent change in concentration from resting concentrations), the shifts were not the same for all AA suggesting some other factors affecting the plasma AA concentrations changes. Several of the AA including alanine, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine, and valine were greater for the PRE horses compared with the POST and FASTED horses at the start of exercise. The concentrations of the AA then reduced through the postexercise period varying between 2 and 4 h postexercise as to when the reduction in AA concentration occurred. These observations are most likely due to the PRE horses being fed 2 h prior to the start of exercise, while the POST and FASTED horses would still have been in a fasted state at the start of the SET. However, when the change in plasma concentrations were calculated as absolute changes as well as expressed as a percent change from rest, these differences in plasma AA concentrations were not as apparent. Looking at absolute change from rest and expressing it as a percent change from resting concentrations may be more meaningful in detecting the effects of the treatments due to the variation that exists in initial (resting) plasma AA concentrations. The positive changes for the POST group and negative changes for the PRE group were again likely due to the time postfeeding since POST horses were in a 1 to 6 h postfeeding time frame compared with a 4.5 to 9.5 h postfeeding time frame for the PRE horses. The increases in plasma AA concentrations in the 2 to 7 h postexercise (1 to 6 h postfeeding) time frame for POST horses are in agreement with other studies showing peak AA concentrations in horses 2 to 6 h after feeding (Johnson and Hart, 1974; Graham-Thiers and Bowen, 2011; van den Hoven et al., 2010).

When AA concentrations were expressed as a percentage change, the POST horses continued

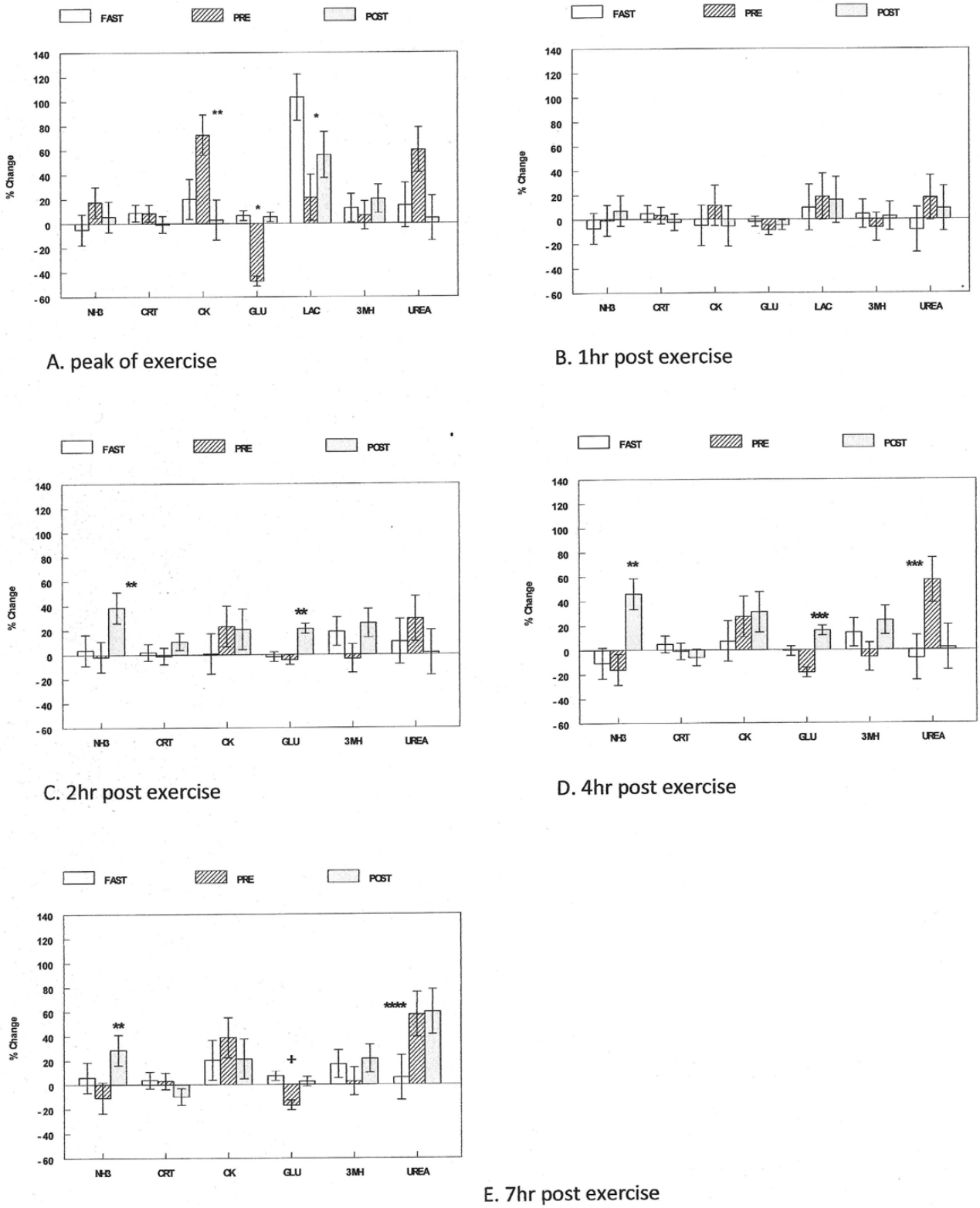

Figure 1. Plasma metabolite changes from rest as a percent of resting concentrations for FASTED, PRE, and POST horses at the peak of exercise (A), 1 h postexercise (B), 2 h postexercise (C), 4 h postexercise (D), and 7 h postexercise (E). NH_3 = ammonia, CRT = creatinine, CK = creatine kinase, GLU = glucose, LAC = lactate, 3MH = 3-methyl-histidine. *PRE < POST and FASTED $P < 0.05$, **POST > PRE and FASTED $P < 0.05$, ***PRE > POST and FASTED $P < 0.05$, ****FASTED < PRE and POST $P < 0.05$, †all treatments different $P < 0.05$.

to have elevated concentration changes compared with the PRE group for arginine, glutamine, ornithine, and valine from the 2 h postsampling time out to the 7 h postsampling time but only remained elevated until the 4 h postsampling time for alanine, lysine, serine, threonine, and tyrosine. Different AA

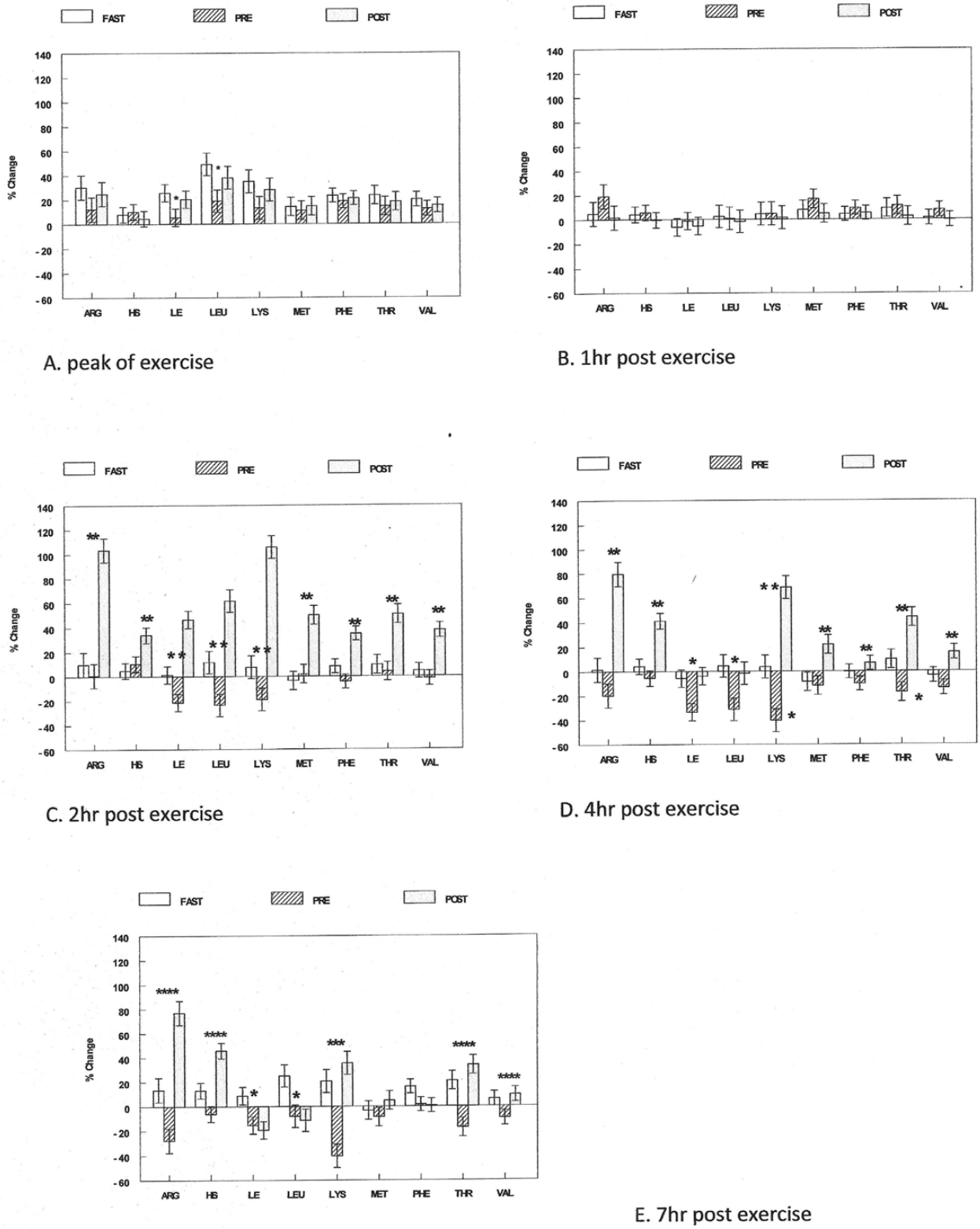

Figure 2. Plasma essential amino acid concentration changes from rest as a percent of resting concentrations at the peak of exercise (A), 1 h postexercise (B), 2 h postexercise (C), 4 h postexercise (D), and 7 h postexercise (E). ARG = arginine, HIS = histidine, ILE = isoleucine, LEU = leucine, LYS = lysine, MET = methionine, PHE = phenylalanine, THR = threonine, and VAL = valine. *PRE < POST and FASTED $P < 0.05$, **POST > PRE and FASTED $P < 0.05$, ***PRE > POST and FASTED $P < 0.05$, ****POST > PRE $P < 0.05$.

have various absorption rates and peak concentrations times depending on absorption competition and various uses by the intestinal epithelium etc. (Bröer, 2008; Woodward et al., 2010). In both cases (absolute changes or expressed as a percent change), POST horses had positive changes, whereas PRE

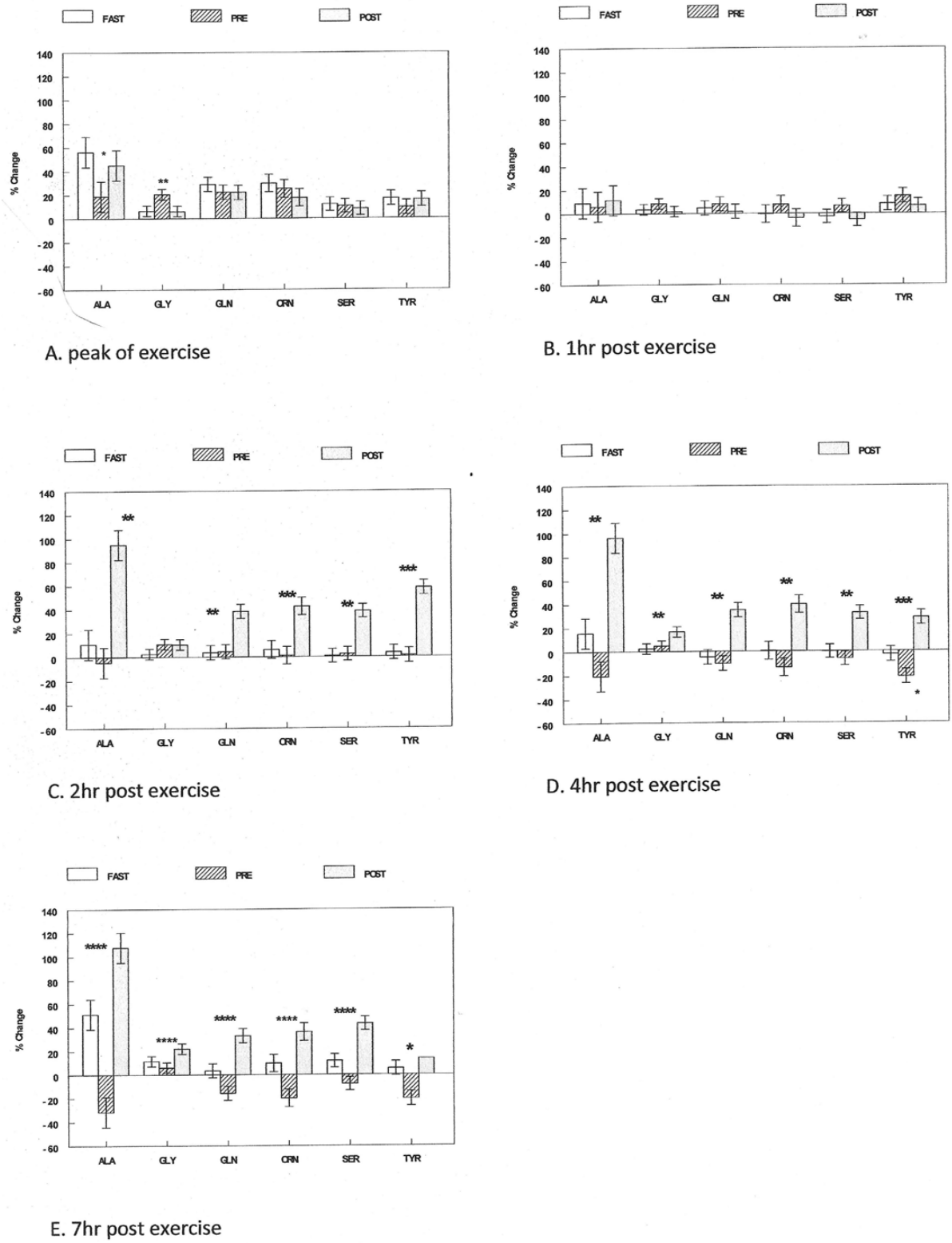

Figure 3. Plasma nonessential amino acid concentration changes from rest as a percent of resting concentrations at the peak of exercise (A), 1 h postexercise (B), 2 h postexercise (C), 4 h postexercise (D), and 7 h postexercise (E). ALA = alanine, GLY = glycine, GLN = glutamine, ORN = ornithine, SER = serine, TYR = tyrosine. *PRE < POST and FASTED $P < 0.05$, **POST > PRE and FASTED $P < 0.05$, ***PRE > POST and FASTED $P < 0.05$, ****POST > PRE $P < 0.05$.

horses had negative changes for the previously mentioned plasma AA concentrations. FASTED horses had changes and percent changes that remained relatively steady throughout the sampling period.

Unlike the majority of plasma AA concentrations previously mentioned, changes and percent change became negative for POST horses as well as PRE horses for plasma isoleucine and leucine.

These changes in isoleucine and leucine may be due to uptake of branch-chain AA (**BCAA**) for use in the muscle (Greer et al., 2007). Leucine has been demonstrated to stimulate muscle protein synthesis in horses (Urschel et al., 2010; Nostell et al., 2012). For the POST horses, isoleucine and leucine had a significant increase at 2 h postexercise, which would be due to being fed at 1 h postexercise; however, they still reached a negative change from rest by 4 h postexercise. Unlike the majority of other AA where the plasma concentrations remained elevated throughout the sampling period, isoleucine and leucine had rapid declines in the 4 and 7 h sampling period. Although PRE horses had decreases in concentrations of isoleucine and leucine earlier in the postexercise period compared with POST horses, this difference would be due to the prolonged period since those horses had received feed compared with the POST horses.

Other AA that had their change from rest become negative were plasma methionine and serine. These AA concentration changes were similar in that PRE horses became negative at postexercise, whereas POST horses remained elevated postexercise. Methionine is an essential AA as well as one of the top limiting AA for swine (NRC, 1998). There is some speculation that the decrease in plasma methionine may be related to its use in muscle protein synthesis. Plasma histidine concentration changes also became negative postexercise PRE. When expressed as a percent change, POST horses' plasma histidine was greater than PRE horses. Graham-Thiers et al. (2012) observed increased muscle free histidine concentrations in exercising horses compared with maintenance horses. This may be related to the buffering of carnosine with exercise in muscle of which histidine is a component.

Plasma concentrations of alanine, glutamine, and ammonia were also significantly elevated in POST horses compared with PRE or POST horses in the postexercise time period. Both alanine and glutamine serve as acceptors of nitrogen which may come from deamination of AA (especially BCAA) and/or ammonia production in the muscle during exercise. These increases observed could potentially be attributed to the deamination of BCAA which were observed to have a significant decrease in plasma concentrations in the postexercise period although the increases were not as significant for PRE horses, which also had significant decreased in BCAA during the recovery period. Other studies have made similar observations in regards to changes in alanine, glutamine and ammonia in exercising horses (Poso et al., 1991; van den Hoven et al., 2010) However, since POST horses were also in a postprandial state, the increase in nitrogen may also come from excess protein for POST horses being fed at 1 h postexercise.

In conclusion, it appears that although PRE horses started the SET with increased plasma AA concentrations, this did not continue during the recovery period, whereas POST horses being fed 1 h postexercise had significantly elevated plasma AA concentrations in the recovery period for most of the measured AA. Specific markers of muscle protein synthesis were not measured in this study; however, other studies have found that feeding protein or an AA supplement following exercise has increased muscle protein synthesis and/or decreased muscle protein breakdown. In this study, the observed elevation in plasma AA concentrations in conjunction with the apparent rapid tissue uptake of BCAA (as evidenced by a decrease in plasma concentrations) along with decreased CK at the peak of exercise would support similar observations in horses that did measure markers of muscle protein synthesis and breakdown. Creating elevated plasma AA concentrations in the recovery period could be beneficial for muscle mass recovery and muscle protein synthesis/degradation balance.

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