

The effect of strenuous exercise on alanine concentration in venous plasma

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Abstract

The effect of strenuous exercise on the alanine concentration in venous blood plasma was studied. Adult male subjects were exercised by use of a bicycle ergometer. Blood samples of the subjects were collected from an antecubital vein and analyzed for plasma amino acid concentrations.

Alanine concentration in venous blood showed increasing tendency after exercise. This increment however, was not statistically significant. Urea concentration in serum was not affected by exercise. However, alanine concentration in venous blood after exercise is correlated with alanine concentration in venous blood before exercise. Difference of alanine concentration between before and after exercise correlated with branched-chain amino acid concentration in venous blood plasma before exercise. It was suggested that alanine formation was induced by uptake of branched-chain amino acids and degradation of protein in strenuous exercise.

Key Words : Alanine ; Branched-chain amino acid ; Strenuous exercise

Introduction

Recent studies have demonstrated that blood glucose plays an important role as the fuel substrate of skeletal muscle during exercise. Maintenance of glucose homeostasis in prolonged exercise necessitates an increase in the rate of gluconeogenesis. Several recent reports indicated that exercise may increase the levels of nitrogen excretion⁽¹⁾, amino acid oxidation⁽²⁾⁽³⁾, and gluconeogenesis⁽⁴⁾⁽⁵⁾. Since increased protein break down and increased amino acid utilization during exercise could alter the concentrations of free amino acids in tissues and in plasma, we have investigated the effect of strenuous exercise on free amino acid levels in plasma. In this study, young males were subjected to the strenuous exercise by use of a bicycle ergometer and their blood samples were analyzed for the free

amino acid concentrations. We also determined the urea concentration in plasma which may reflect the total body protein metabolism.

Methods

Subject : Six healthy males, the university students of physical education participated as the experimental subjects. Physical characteristics of the subjects are given in Table 1. The experiment was performed in the morning after an overnight fast.

Procedure : The exercise was carried out by use of a bicycle ergometer (Monark). The subjects exercised at 1 kp for 2 min. by bicycle ergometer with an increase of work load of 0.5 kp per min. with pedaling frequency of 50 revolution per minute.

Oxygen and carbon dioxide concentration were analyzed by SANEI expired gas analyser 1 H 02, Expired gas during pedaling was collected every minute by Douglas bag method. Electrocardiogram was recorded by chest unipolar leading. Heart rate was counted every minute.

In the resting state and immediately after exercise Teflon catheter was inserted in antecubital vein and blood sample of 5 ml was collected from each subject. Blood was deproteinized with sulfosalicylic acid and amino acids concentrations in plasma were determined by the automated ion-exchange chromatography (JLC-8AH amino acid analyzer).

Results

Changes in oxygen uptake during exercise are shown in Fig. 1. Oxygen uptake increased linearly with the increase of work load. Mean oxygen uptake and heart rate for all subjects were 2297 ml/min and 173 beats/min, respectively.

The levels of amino acids concentration in venous blood plasma obtained before and after exercise are shown in Table 2 and Fig. 2-4.

The mean concentration of individual amino acids before exercise are indicated in Fig. 2. The mean concentration of individual amino acids after exercise are indicated in Fig. 3. The concentration of each amino acids before and after exercise were compared. As shown in Fig. 4, concentrations of alanine and arginine rose slightly after exercise. However these increments were not statistically significant. The level of branched-chain amino acid concentration after exercise declined slightly than that

Table. 1 Physical characteristics of subjects.

Subjects	Age (yr)	Height (cm)	Weight (kg)	R. I	Runing time (min)	Heart rate at exercise (beats/min)
Y. S	21	165	58	129	8' 05"	154
R. S	25	168	61	129	7' 56"	152
Y. O	26	170	65	132	9' 00"	164
S. O	21	173	62	120	8' 02"	212
S. Y	28	171	64	128	8' 00"	179
Mean	24	169	61	126	8' 10"	173

before exercise. Urea concentration in plasma did not change by exercise, as shown in Fig. 5. The work-dependent change in the serum alanine concentration significantly correlated with branched chain amino acid concentration in venous plasma before exercise (Fig. 6). Alanine concentration in venous blood after exercise correlated with alanine concentration in venous plasma before exercise (Fig. 7).

Discussion

In the present study, it is shown that alanine concentration in venous plasma increases slightly after exercise but this increment is not statistically significant. Alanine is formed by the transamination of glutamate and pyruvate through a reaction catalyzed by alanine aminotransferase (6). Goldberg et al. have shown that the amino group for alanine synthesis in muscle is derived from the catabolism of the branched-chain amino acids (leucine, isoleucine and valine) (7). Accumulation of branched-chain amino acids would lead to an increase in glutamate and subsequently to a release of alanine by a linked transamination (8).

In the present study, the level of branched-chain amino acids in venous plasma slightly declined after exercise.

Furthermore, this decline of branched-chain amino acids is correlated with increment of alanine concentration after exercise.

It is also suggested that amino group of alanine formation derived branched-chain amino acids in blood.

Urea production is a reflection of total body protein metabolism. Urea concentration in plasma,

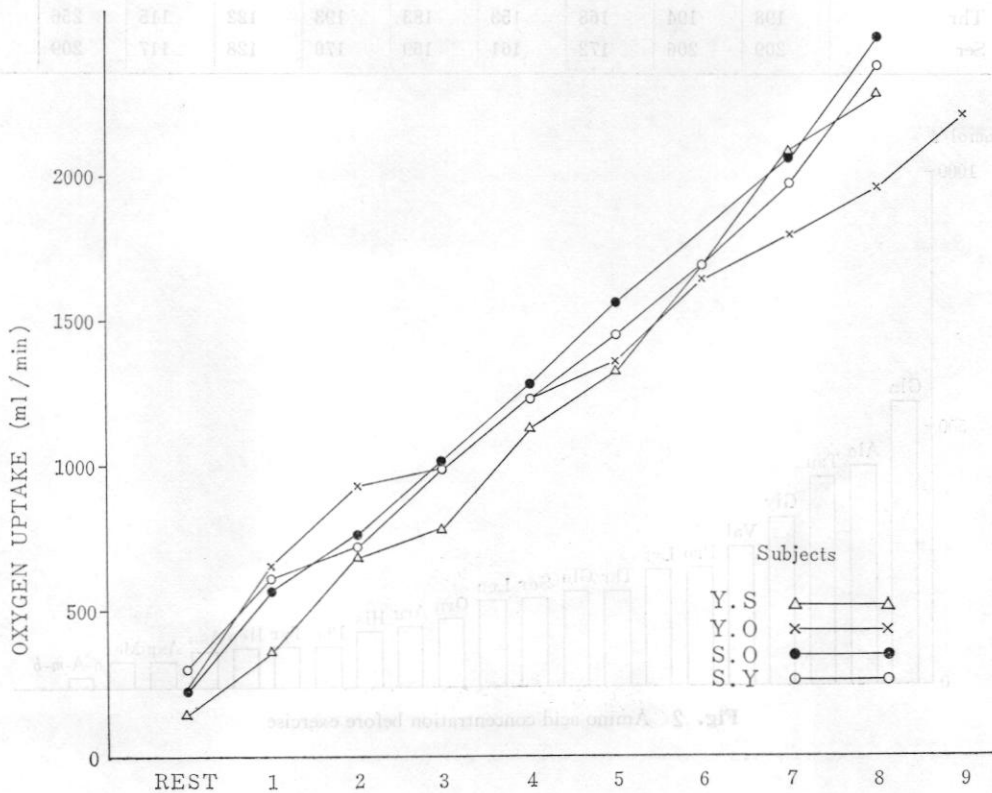
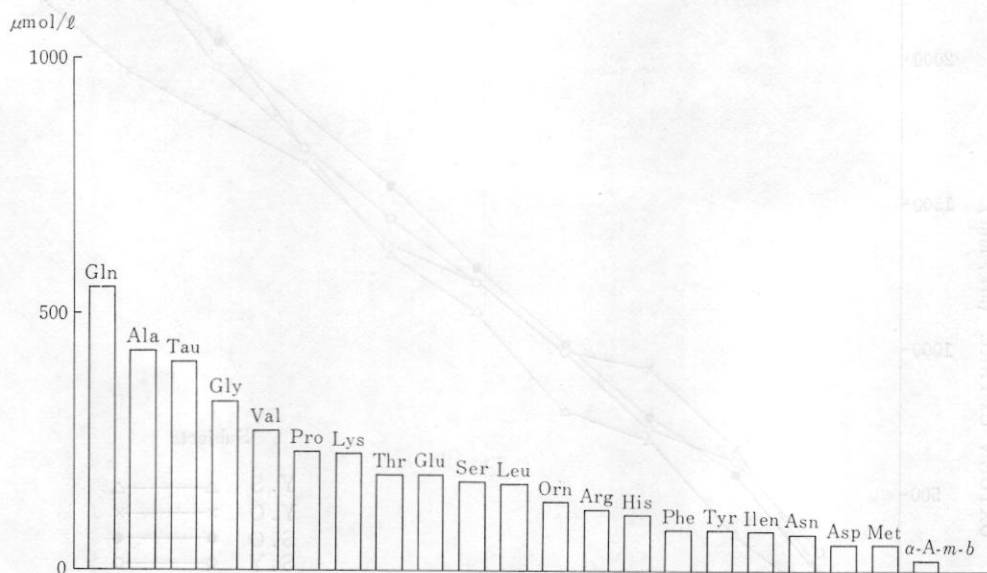


Fig. 1 Changes in oxygen uptake during exercise.

Table. 2 Amino acid concentration before and after exercise running

Amino acid $\mu\text{mol/l}$	Y. S		R. M		Y. O		S. O		S. Y	
	before	after	before	after	before	after	before	after	before	after
Tau	188	217	382	283	391	391	392	353	664	292
Urea	5748	6028	4545	5000	5909	5688	2168	2023	5967	13524
Asp	40	38	50	41	51	50	33	44	41	55
Asn	70	81	61	62	61	59	50	51	75	140
Glu	257	243	109	116	155	168	130	163	256	525
Gln	480	667	578	511	345	323	736	687	589	849
Pro	204	203	189	161	394	415	190	183	130	444
Gly	346	368	319	302	327	337	245	252	391	708
Afa	500	606	271	329	411	533	354	441	573	817
α -A-n-b	26	25	22	21	19	18	13	12	33	57
Val	281	286	226	219	258	278	222	188	360	654
Met	36	33	35	34	28	27	25	27	52	83
Ileu	76	80	68	63	87	89	63	58	101	206
Leu	174	180	143	139	159	159	136	123	187	386
Tyr	69	70	77	72	62	68	69	66	120	205
Phe	90	100	86	88	82	89	66	63	94	186
Orn	188	181	123	124	118	109	90	78	162	295
Lys	212	223	275	275	182	197	161	156	283	555
His	113	121	98	87	96	94	85	87	133	228
Arg	75	85	129	148	111	121	103	109	169	310
Thr	198	194	168	153	183	193	122	115	256	439
Ser	209	206	172	161	169	170	128	117	209	436

**Fig. 2** Amino acid concentration before exercise

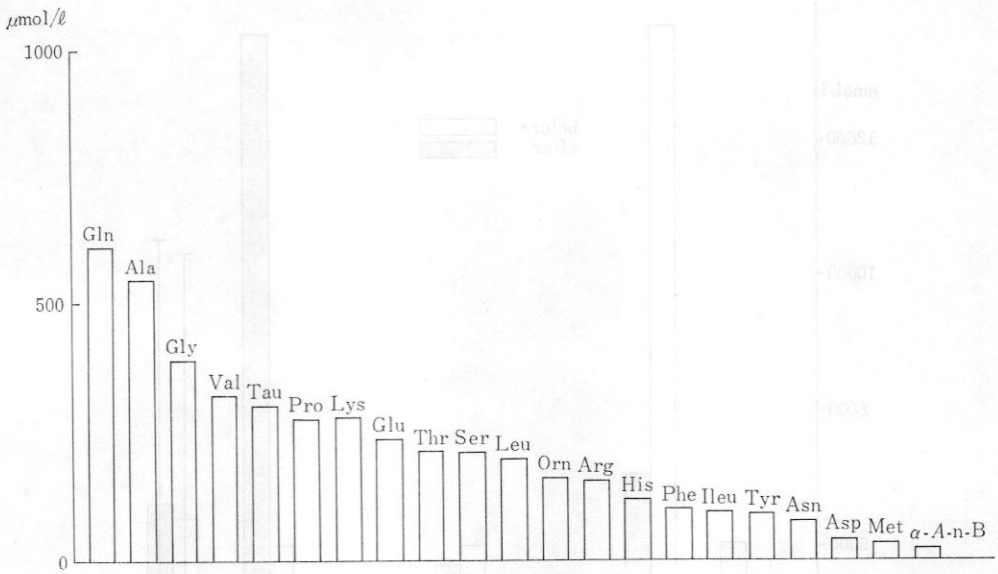


Fig. 3 Amino acid concentration after exercise

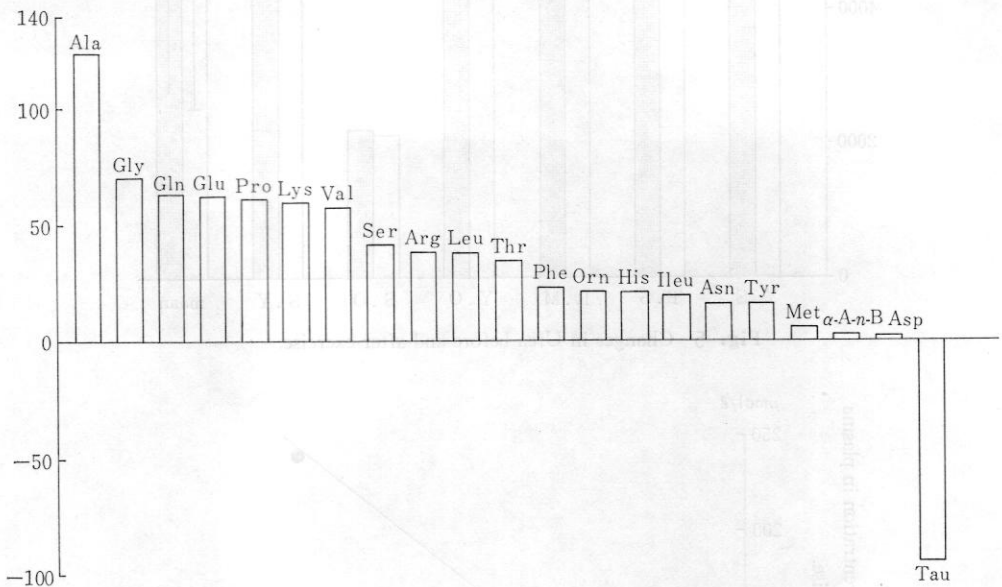


Fig. 4 Difference of amino acid concentration in plasma between before and after exercise.

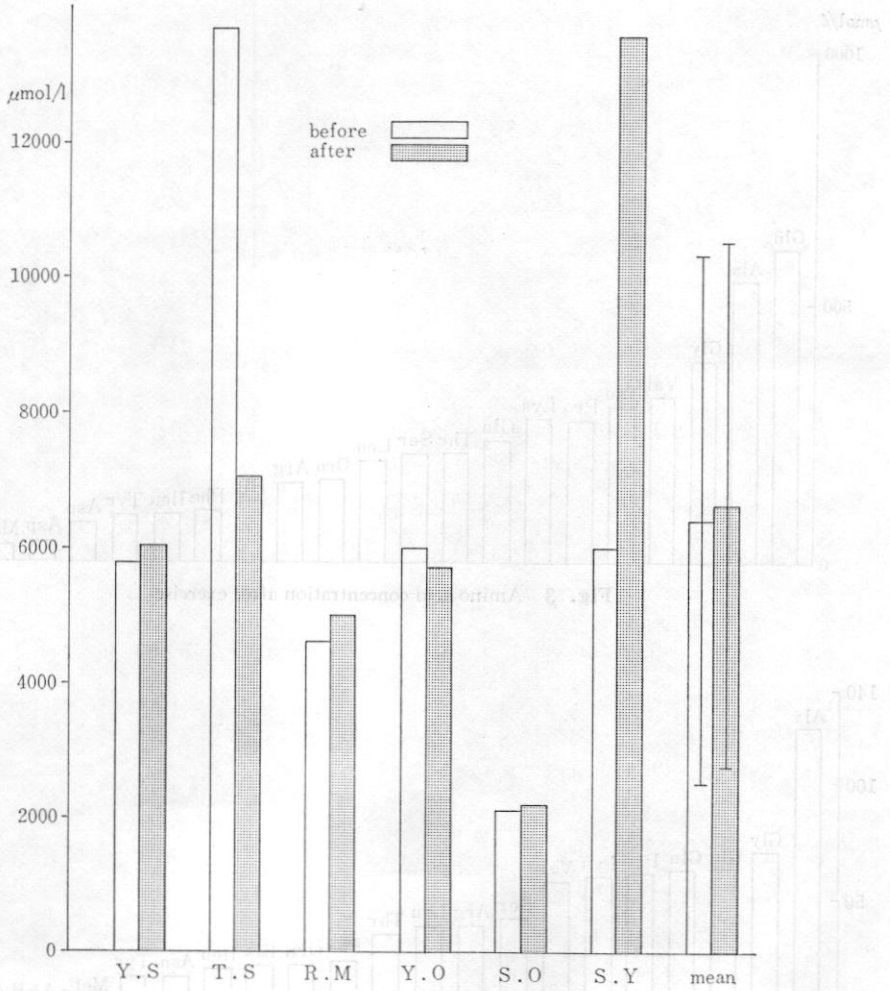


Fig. 5 Changes in Urea before and after exercise.

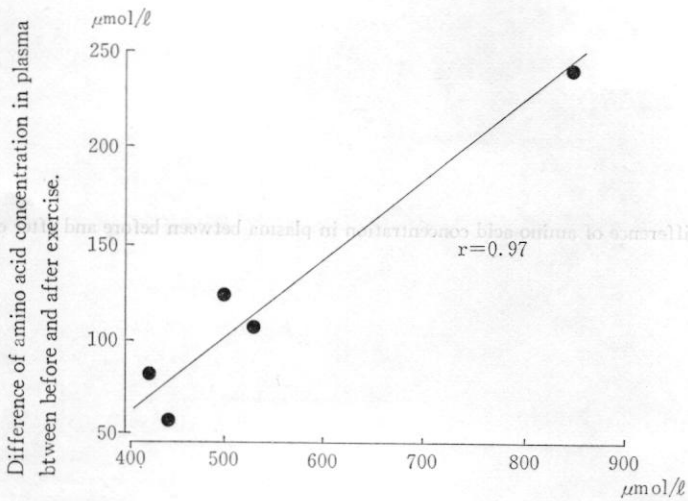


Fig. 6 Branched chain amino acid concentration before exercise

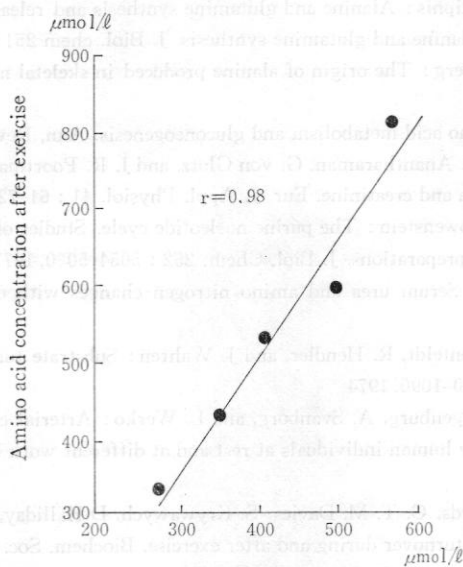


Fig. 7 Amino acid concentration before exercise

serum or sweat has been reported to increase during exercise by some investigators (9) (10) (11). These results suggest the possibility of increased protein breakdown during exercise. In some cases, however, argument has arisen that the site of protein catabolism may not be muscle. Ahlborg et al. have shown that there is an increased output of branched-chain amino acids from the splanchnic bed during prolonged light-intensity exercise (12).

In the present study urea concentration in serum increased slightly after exercise but this increase is also statistically insignificant.

Under the prolonged light-intensity work, the concentrations in plasma of many amino acids, including alanine, tend to fall, whereas those of the branched-chain amino acids rise. When the exercise is of moderate to heavy intensity (60% $\dot{V}O_2$ max or greater) but of short duration, the plasma level of amino nitrogen, alanine and branched-chain amino acids increase. During work of longer duration (2 hours), however, the concentrations of amino nitrogen, total amino acid and branched-chain amino acids decline. In the present study, exercise received by the subjects is of light to heavy intensity and of short duration. Under the present condition, however, alanine, branched chain amino acids in serum did not show any statistically significant increment after exercise. Therefore, further detailed experiments may be necessary to clarify effect of exercise on alanine synthesis.

Reference

1. Berg, A., and J. Keul : Serum alanine during lasting physical exercise. *Int. J. Sports. Med.* 1 : 199-202, 1980
2. Lemon, P. W. R., and J. P. Mullin : Effect of initial muscle glycogen levels on protein catabolism during exercise. *J. Appl. Physiol.* 18 : 624-629, 1980
3. Dohn, G. L., A. L. Hecker, W. E. Brown, G. J. Klain, F. R. Puente, E. W. Askew, and G. R. Beecher : Adaptation of protein metabolism to endurance training. *Biochem. J.* 164 : 705-708, 1977
4. Felig, P : The glucose-alanine cycle. *Metabolism* 22 : 179-207, 1973
5. Felig, P : Amino acid metabolism in exercise. *Ann. N. Y. Acad. Sci* 301 : 56-63, 1977

6. Garber, A. J., I. E. Karl and Kipnis : Alanine and glutamine synthesis and release from skeletal muscle-the precursor role of amino acids in alanine and glutamine synthesis. *J. Biol. chem* 251 : 836-843, 1976
7. Chug, T. W., and A. L. Goldberg : The origin of alanine produced in skeletal muscle. *J. Biol. chem.* 253 : 3677-3684, 1978
8. Ruderman, N. B. : Muscle amino acid metabolism and gluconeogenesis. *Ann, Rev, Med.* 26 : 2450258, 1975
9. Decombaz, J., P. Reinhardt, K. Anantharaman, G. von Glutz, and J. R. Poortmans : Biochemical changes in a 100 km run : Free amino acids urea and creatinine. *Eur. J. Appl. Physiol.* 41 : 61-72, 1979
10. Goodman, M. N., and J. M. Lowenstein : The purine nucleotide cycle. Studies of ammonia production by skeletal muscle in situ and in perfused preparations. *J. Biol. Chem.* 252 : 5054-5050, 1977.
11. Haralambie, G., and A. Berg : Serum urea and amino nitrogen changes with exercise duration. *Eur. J. Appl. Physiol.* 35 : 39-48, 1976
12. Ahlborg, G., P. Felig, L. Hagenteldt, R. Hendler, and J. Wahren : Substrate turnover during prolonged exercise in man. *J. Clin. Invest.* 53, 1080-1090, 1974
13. Calsten, A., B. Hallgren, R. Jagenburg, A. Svanborg, and L. Werko : Arterial concentrations of free fatty acids and free amino acids in healthy human individuals at rest and at different work loads. *Scand. J. Clin. Lab. Invest.* 185-191, 1962.
14. Rennie, M. J., R. H. T. Edwards, C. T. M. Davies, S. Krywawych, D. Halliday, J. C. Waterlow, and D. J. Millward : Protein and amino acid turnover during and after exercise. *Biochem. Soc. Trans.* 8 : 499-501, 1980
15. Refsum, H. E., L. R. Gjessing, and S. B. Stromme : Changes in plasma amino acid distribution and urine amino acids excretion during prolonged heavy exercise. *Scand. J. Clin. Invest.* 39 : 407-413, 1979.

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References

1. Berg, A. and J. Karl : Serum alanine during fasting physical exercise. *Int. J. Sports Med.* 1 : 100-103, 1980
2. Hanson, P. W. Jr. and J. P. Molina : Effect of initial muscle glycogen levels on protein catabolism during exercise. *J. Appl. Physiol.* 18 : 629-631, 1977
3. John, G. H., A. L. Heller, W. E. Brown, G. J. Klein, R. E. Franco, R. W. Askew, and G. R. Keckler : Adaptation of protein metabolism to endurance training. *J. Appl. Physiol.* 44 : 707-709, 1977
4. Felig, P. : The glucose-alanine cycle. *Metabolism* 22 : 179-207, 1973
5. Felig, P. : Amino acid metabolism in exercise. *Ann. N. Y. Acad. Sci.* 343 : 56-73, 1977