

**Effects of Vespa Amino Acid Mixture (VAAM) Isolated from Hornet Larval Saliva
and Modified VAAM Nutrients on Endurance Exercise in Swimming Mice
— improvement in Performance and Changes of Blood Lactate and Glucose —**

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Abstract

For endurance exercise in swimming mice. 1.8% VAAM (Vespa amino acid mixture) which has the same amino acid-components as hornet (*Vespa mandarinia*) larval saliva, 1.8% casein amino acid mixture (CAAM), 10% glucose, or amino acid mixtures in which the amino acids were varied while maintaining the same molar ratio as VAAM were administered orally to mice. Mice receiving 1.8% VAAM showed significantly longer maximum swimming times than mice receiving other nutrients. Among these nutrients, mixtures of proline, glycine, and essential amino acid mixture (EAAM) from the VAAM component, showed maximum times near those with VAAM. In swimming exercise in mice bearing of 0.3g tail weight, mice administered 1.8% VAAM showed lower blood lactate concentrations and higher blood glucose concentrations than mice receiving other nutrients. Mice receiving 1.8% VAAM also had lower lactate concentrations in muscle as well as blood. This suggests that VAAM suppresses lactate production and glucose catabolism during exercise. The effects of hornet larval saliva were stronger than those of VAAM. VAAM therefore showed the major effect of the -saliva. The results suggest that VAAM improves physiological condition during endurance exercise. A positive correlation was observed between the blood concentrations of lactate and glucose in exercising mice administered various nutrients ($r=0.779$). This suggests metabolic equilibration between glucose and lactate during exercise. A positive correlation ($r=0.507$) was also found between the maximum swimming time and blood glucose concentration. Maximum swimming times were high, low (Ca. 2.5 mMol) and high (Ca. 4.0 mMol) blood lactate concentrations in high blood glucose concentrations. These facts support that glucose-homeostasis is important in prolonged exercise.

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key words : Endurance exercise, Blood lactate, Blood glucose, Hornet larval saliva, Amino acid nutrient

Introduction

Much is known about the influence of foods on exercise activity. In a recent study of endurance exercise by MacLean et al.¹², a high carbohydrate (CHO) diet was found to produce prolonged exercise with high concentrations of glucose and lactate in the blood. In many cases of endurance exercise^{6,14,17}, the concentration of blood lactate increases rapidly just before exhaustion, while blood glucose levels increase temporarily at the

start of exercise, then gradually decrease to exhaustion. Excess production and accumulation of lactate in either muscle or blood brings about acidosis⁶, while a decrease in blood glucose levels directly suppresses the functioning of the central nervous system. Either situation lead to the inability to continue exercise and thus can be said to promote fatigue conditions. It is therefore important to preserve glucose homeostasis and lactate degradation in order to increase exercise activity.

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Supplementation with amino acids, especially branched chain amino acids (BCAA), improves exercise activity, apparently by preventing the catabolism of muscular proteins during exercise⁴). However, it is not known whether supplementation with specific components of other amino acids will improve physiological condition as well as exercise performance.

There are some living creatures that consume amino acid mixtures in nature. One example is the family of hornets. Adult hornets are not able to consume solid foods because of their constricted trunks. The meat boal of insects to be preyed on by adult hornets is brought back to the nest where it is fed to the larvae. A nutritional exchange between the meat boal and larval saliva, that is trophallaxis, is performed between adults and larvae. The giant hornet, *V. mandarinia*, covers an area with a radius of 2 Km in hunting a prey. Adult hornets continue their hunting at flying velocities over 30 Km/hr all day long, resulting in daily flight distances of about 100 Km; Their wings must support a weight of over 3g if they carry a meat boal, since their body weights, heavy among flying insects, are commonly over 2g. The question arises then as to how they produce the flight energy for their hunting, and how they reduce the fatigue brought on by heavy endurance exercise. The answer may exist in the ecological habits of their social life, for example, trophallaxis. Larval saliva may contain the secret that sustains hornet flight. Our studies show clearly that larval saliva consists mainly of amino acids, the composition of which is similar among the five hornet species found in Japan¹) In this study, we prepared an amino acid mixture identical to that in the larval saliva of *V. mandarinia*, and analyzed the nutritional effect of these amino acid nutrients on endurance exercise in swimming mice.

Materials and Methods

Materials

Tryptophan and perchloric acid (FCA) were purchased from Wako Chemical Co. (Tokyo, Japan). Diagnostic kits and reagents for measuring blood lactate and glucose were purchased from Sigma Chemical Co. (St. Louis, MO., USA) and Boehringer Mannheim (Mannheim, Germany), respectively. Glucose and -Haemo-sol were from Iwaki -Pharm. Co. (Tokyo, Japan) and Haemo-Sol Co. (Baltimore, MD, USA), respectively. All amino acids except tryptophan were from Kyowa Hakko Kogyo Co. (Tokyo, Japan). Hornet larval saliva was collected from *V. mandarinia* larvae by the method previously reported⁶), and frozen at -80°C until used.

Methods

Preparation of nutrients

An amino acid mixture with the composition of hornet larval saliva from *V. mandarinia* was prepared as 1.8% VAAM (Vespa amino acid mixture) -as shown in Table 1. As a positive control, 1.8% CAAM (casein amino acid mixture), with the same composition as the major casein component (C 1 á) from cow milk was prepared as shown in Table 1.

Effects of VAAM administration on the changes of blood lactate and glucose by exercise:

Animals

Untrained mice (male; ddY), aged 4 to 10 weeks, were fasted for 16 hrs at room temperature (24C) and then administered various nutrients.

Optimum dose of nutrients by oral administration

Solution containing 1.8% VAAM at 0, 12.5, 25.0, 37.5, and 50.0 µ ml per gram body weight were each administered to five 5 week-old mice

Table 1. Amino acid compositions of nutrients (Mol %)

Amino acids	VAAM	EAAM	EAAM 1	EAAM2	EAAM3	EAAM4	CAAM
Asp	0.2	-	-	-	-	-	7.5
Thr	7.2	-	-	-	-	-	2.5
Set	2.5	-	-	-	-	-	8.0
Glu	3.2	-	-	-	-	-	19.6-
Pro	18.0	-	-	-	-	-	8.5
Gly	19.1	-	-	-	-	-	4.5
Ala	6.0	-	-	-	-	-	4.5
Va.	5.9	13.5	22.7	-	19.4	14.9	5.5
Cys	-	-	-	-	-	-	0.4
Met	0.5	1.2	-	3.0	1.6	-	2.5
lie	4.5	10.2	17.1	-	14.8	11.4	5.5
Leu	6.2	14.2	23.8	-	20.4	15.7	8.5
Tyr	6.0	13.7	23.0	-	19.7	15.2	5.0
Phe	3.8	8.7	-	21.5	12.5	-	4.0
Lys	8.6	19.6	-	48.5	-	21.8	7.0
Ttp	2.2	5.0	-	12.4	-	5.6	1.0
His	2.6	5.9	-	14.6	-	6.6	2.5
Arg	3.5	8.0	13.4	-	11.5	8.9	3.0

previously fasted for 16 hrs. The mice were then allowed to rest for 60 min at room temperature (24°C). Several seconds before the start of a swim, the mice were rinsed and washed with 1% Haemo-Sol solution to deaerate the skin hair. Mice administered different doses of 1.8% VAAM were started to placed at 5 mm intervals in a river pool containing 0.01% Haemo-Sol at 40°C with a constant water flow of 8 m/min (Fig. 1). A maximum of five mice were in the pool at any time. A swimming exercise was stopped when the mouse sank to the bottom of the pool with air bobbling from its nose. The optimum doses of 1.8% VAAM were found to be 25.0 μ l and 37.5 μ l/g body weight as shown in Fig. 2. In the following experiments, 37.5 μ l/g body weight was chosen for administration.

Optimum mouse age

Either 37.5 μ l/g body weight of 1.8% VAAM or distilled water (DW) was administered to 4 (is 18g), 5(17~21g), 8(26~30g) and 10(32~35g) week-old fasted mice (5 mice per age group). The mice were allowed to rest for 60 min at room

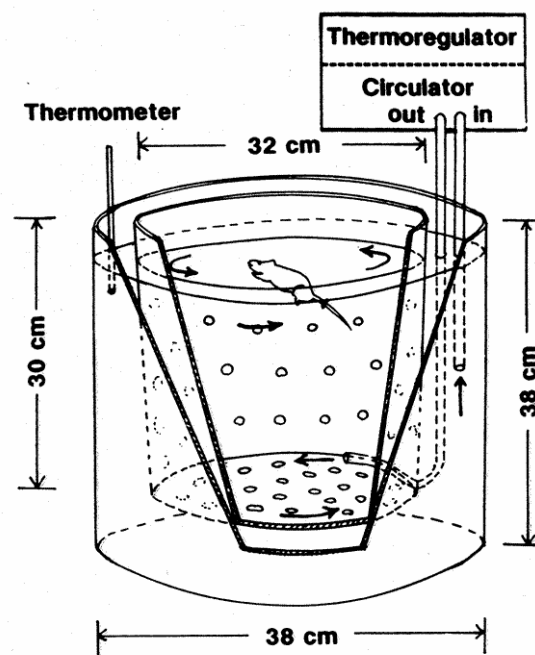


Fig. 1. River pool used for endurance exercise was set at the optimum rate of 8 m/min and maintained at the optimum temperature of 35°C. For details concerning the swimming conditions, see Materials and Methods.

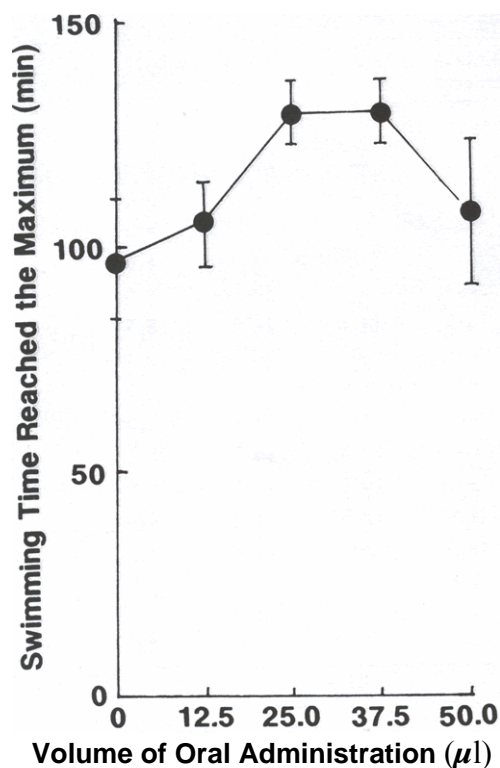
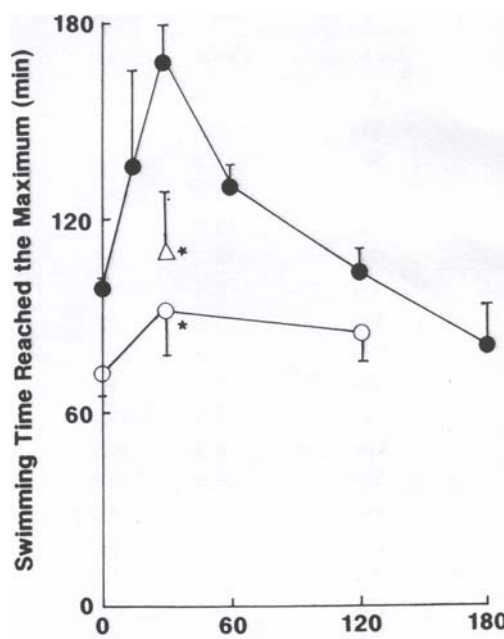


Fig. 2. Optimum doses of orally administered nutrients. Values are mean \pm S. E. M. Significant difference was not found among those values. For details of the experimental conditions, see Materials and Methods.

temperature and the swimming exercise was performed as described above. For mice administered VAAM, the mean swimming times were 101 min in 4 week-old mice, 171 min in 5 week-old mice, 50 min in 8 week-old mice and 93 min in 10 week-old mice. The mean times in mice administered DW were for 53 min in 4 week-old mice, 91 min in 5 week-old mice, 58 min in 8 week-old mice and 69 min in 10 week-old mice. Thus, it was shown that 5 week-old mice were able to swim for the longest time.

Optimum resting time after administration of nutrients

Five week-old fasted mice were administered 37.5 μl/g body weight of 1.8% VAAM, 1.8% CAAM, or DW. The mice were then allowed to



Resting Time After Oral Administration (min)

Fig. 3. Optimum resting times after oral administration of nutrients to mice. ●: 1.8% VAAM Δ:1.8% CAAM ○: DW. Values are mean \pm S. E. M. 1.8% VAAM showed significant differences ($p < 0.05$) to 1.8%CAAM (*) and DW (*) at 30 min. For details, see Materials and Methods.

rest for 0, 15, 30, 60, 120 or 180 min at room temperature prior to being placed in the pool. Swimming times were then measured as described above. The optimum resting time was found to be 30min for mice receiving 1.8% VAAM or DW as shown in Fig. 3. The resting time was therefore fixed at 30 min in subsequent experiments.

Optimum temperature for swim

Thirty minutes before swimming, 5 week-old fasted mice were administered 37.5 μl/g body weight of 1.8% VAAM or DW (n = 5) and placed in the river pool at 25, 30, 35, 40 or 45°C The optimum swimming temperature was found to be 35°C as shown in Fig. 4. At 45°C, the mice stopped swimming within a couple minutes. Based on these results, the swimming conditions in our experiments were set as follows; five week-old mice

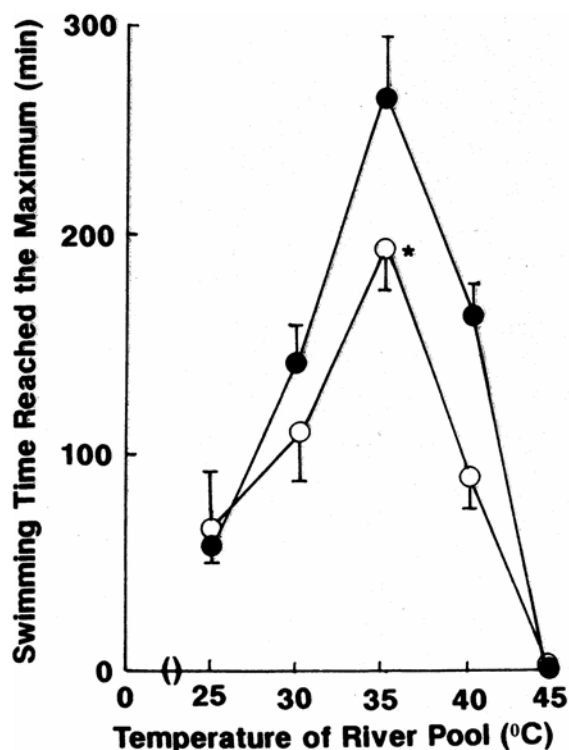


Fig. 4. Optimum water temperature ● 1.8% VAAM O;DW. Values are means ± S.E.M.* Significant difference between 1.8%VAAM and DW ($p < 0.05$). For details, see Materials and Methods.

were administered nutrients at 37.5 μ l/g body weight allowed to the rest for 30 min after administration, and placed a water temperature at 35°C

Assay for blood lactate

In order to assay of blood lactate and glucose levels, mice were administered nutrients, then a weight (0,3 g) was attached onto the tail. The mice were then placed in the river pool for 30 min under the conditions described above. Under these conditions, mice administered DW were exhausted in about 60 min. After the swimming session, the mice were quickly anesthetized with ether, and blood was obtained from the abdominal vein within 1 min. Fifty microliters of the blood was mixed with 100 μ l of 6 % perchloric acid (PCA), mixed

well, and centrifuged at 2,000 rpm for 10 min. One hundred microliters of the supernatant was reacted with 900 pi of lactate dehydrogenase solution containing nicotinamide adenine dinucleotide for 30 min at 37°C using Sigma diagnostic kit. Absorbance at 340nm was measured by a Shimadzu UV-150-02 spectrometer.

Assay for blood glucose

Twenty microliters of blood was mixed well with 40 μ l of 6 % PCA, and the mixture was centrifuged at 2,000 rpm for 10 min. Thirty microliters of the supernatant was reacted with 900 μ l of an enzyme solution containing hexokinase, glucose-6-phosphate dehydrogenase, and nicotinamide adenine dinucleotide phosphate using a diagnostic reagent kit (Boehringer Mannheim). The reaction mixture was incubated for 30 min at 37°C, and the absorbance at 340 nm was measured by a Shimadzu UV -150-02 spectrometer.

Assay for muscular lactate

Mice exercised as described above for the assay of blood lactate were quickly exsanguinated and the leg muscles were immediately frozen in liquid nitrogen. The frozen muscles were crushed in a mortar and pestle, then homogenized with a Polytron homogenizer for 2 min. The homogenate was centrifuged at 15,000 X g for 30 min at 4°C . The supernatant was denatured with 6 % PCA and centrifuged again at 2,000 rpm. The supernatant was assayed for lactate described for the blood lactate analysis.

Statistics

All data are means ± SEM, unless otherwise noted. The Student paired t test was used for testing the significance of differences between related samples of the same subject, and for testing the significance of differences between samples of the same subject obtained at different times during the exercise bouts.

Results

Effects of VAAM. CAAM. glucose. DW and amino acid nutrients containing VAAM components on maximum swimming times in mice

The effects of several orally administered nutrients on the maximum swimming times obtained in mice undergoing endurance exercise were measured. The swimming times in mice receiving 0.9% VAAM corresponding to the concentration in hornet larval saliva and 1.8% VAAM (Nut. no. 2) were significantly longer than in mice receiving DW (Nut. no. 26), 1.8% CAAM (Nut. no. 3), or 10% glucose (Nut. no. 25) ($p < 0.05$) as shown in Table 2. The total intake of nutrients by a 20 g mouse was about 75 mg in the case of 10% glucose, but only about 14 mg in the case of the 1.8%

amino acid nutrients. In spite of the smaller amount of VAAM intake, the swimming times were prolonged. In comparison to CAAM, which has the desirable nutritional balance for mammalian growth, VAAM contains large amounts of threonine, proline, glycine and tryptophan, but little aspartic acid, serine, or glutamic acid, and no cystine or methionine. This suggests that there is a fundamental difference in the amino acid requirements between exercise and growth.

It is thus considered that the peculiar amino acid composition of VAAM might be markedly related to the prolongation of swimming times. Swimming times were measured following the administration of several amino acid nutrients in which the compositions were changed from that of VAAM keeping the molar ratios fixed (Table 1).

Table 2. Maximum swimming times of mice administered amino acid nutrients

Nutrient No.	Nutrients	Swimming time (min)	No. of mouse
		Mean±S. E. M.	
2	VAAM (1.8%)	242±16	12
	VAAM (0.9%)	233± 8	12
3	CAAM (1.8%)	173±12*	13
	Proline (0.624 %)	132± 11 *	11
4	Proline (2 %)	130± 18*	11
5	Glycine(2 %)	141±16*	12
	Threonioe (2 %)	136±18*	5
	Alanine (2 %)	174±74	4
	Pro (1 %) + Gly(1 %)	221±28	7
9	EAAM (1.8%)	184 ± 16	23
10	EAAM (0.9%) +Pro (1 %)	23S±24	12
11	EAAM (0.9%)+Gly (1 %)	217±27	12
12	EAAM 1 (1.8%)	165±13*	25
	EAAM 1 (0.9%) +Pro (0.312%)	111±21*	5
	EAAM (0.66%) +Pro (0.66%)	135±24 *	8
	EAAM 1 (0.9%) +Pro (1 %)	146±20*	12
13	EAAM2 (1.8%)	186±21	14
	EAAM 2 (0.9%) Pro (0.312%)	152±19*	6
	EAAM 2 (0.66%) +Pro (0.66%)	166±32*	7
	EAAM2 (0.9%) + Pro (1 %)	173±44	6
	EAAM 3 (0.9%) +Pro (0.312%)	168±23"	8
25	Glucose (10%)	160±33*	4
26	DW	147±10*	16

* Significant difference between VAAM (1.8%) and other nutrients ($P < 0.05$).

However, no nutrients prolonged the swimming times better than VAAM (Table 2). The swimming times in mice receiving proline+glycine, EAAM (Nut. no.9), EAAM + proline (Nut. no. 10) and EAAM + glycine (Nut. no. 11) were close to that of mice receiving VAAM. These results suggest that the prolongation of swimming time is a reinforcement by several amino acids. Furthermore, the molar ratio of the amino acids in VAAM must play an important role in the effect. This inference is supported by the fact that the administration of insoluble VAAM at high concentration did not prolong swimming times (data are not shown).

Effects of VAAM, CAAM, glucose, and DW on blood concentrations of lactate and glucose in exercising mice

The concentration of blood lactate at the start of the swim was influenced by the administered nutrients, and showed slightly little differences as follows: 2.69 ± 0.12 mMol ($n = 35$) for DW, 2.84 ± 0.13 mMol ($n=20$) for 1.8% CAAM and 2.39 ± 0.13 mMol ($n=20$) for 1.8% VAAM. After swimming for 30min with an 0.3 g tail weight, the blood lactate concentration in mice administered 1.8% VAAM was slightly increased (Fig. 5). but was still lower than the starting concentrations in mice receiving other nutrients. However, the post-swim concentrations in both DW and 1.8% CAAM administered mice increased markedly ($p < 0.05$). The administration of 10% glucose or 1.8% VAAM + 10% glucose resulted in an extremely elevated starting blood lactate concentration. Compared with 10% glucose, however, 1.8% VAAM + 10% glucose clearly decreased the post-swim blood lactate concentration despite the presence of glucose (Fig. 5). The ratios of the increases in blood lactate concentrations after exercise in mice by administered different nutrients were 106.1% for 1.8% VAAM, 117.3% for 10% glucose, 117.8% for 1.8% VAAM+10% glucose,

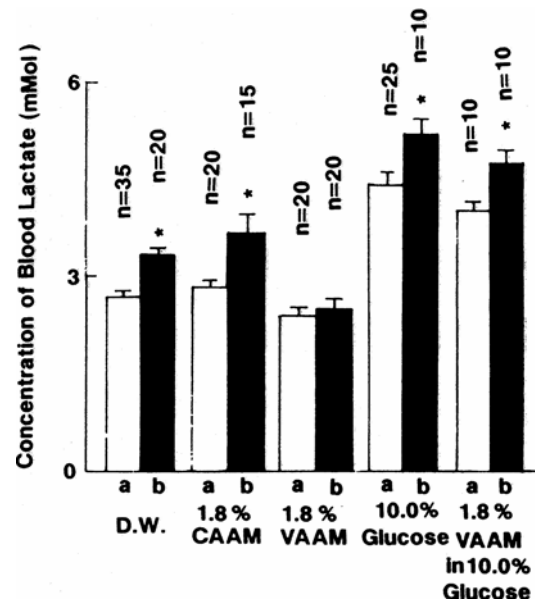


Fig. 5. Changes in blood lactate concentrations in the mice with a 0.3 g tail weight and subjected to endurance exercise after the oral administration of various nutrients. a (open column) is the concentration before exercise; b (closed column) is the concentration after a 30 min swim. Values are means \pm S. E. M. * Significant difference between 1.8% VAAM and other nutrients at post-exercise ($p < 0.05$). Details of the experimental conditions are described in Materials and Methods.

123.2% for DW, and 129.4% for 1.8% CAAM. Lactate production in mice receiving VAAM was definitely lower than in mice receiving other nutrients.

At the same time, blood glucose concentrations were also measured. Pre-exercise blood glucose levels were about 4.5 mMol for DW, 1.8% CAAM and 1.8% VAAM. After exercise, the value decreased slightly for 1.8% VAAM, but largely for DW and 1.8% CAAM (Fig. 6) than those of pre-exercise. In case of 10% glucose and 1.8% VAAM + 10% glucose, pre-exercise blood glucose levels were very high, but the concentrations decreased sharply after swimming, although they were still higher than for nutrients without glu-

cose (Fig. 6). The suppressive effect on the decrease of blood glucose levels by VAAM was also

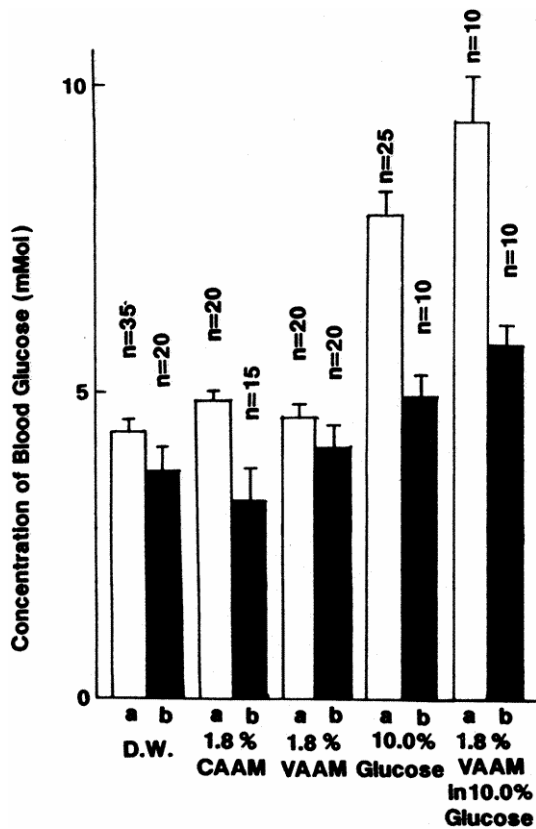


Fig. 6. Changes in blood glucose concentrations in the mice. Values are means \pm S. E. M. Significant difference was not found among those nutrients. Details of the experimental conditions are the same as in Fig.5 and described in Materials and Methods.

present despite the presence or absence of administered glucose. Post-swim blood glucose levels decreased to 85.8% of starting levels in mice administered DW. a comparatively small decrease. However, if it is considered that DW causes simultaneous decreases in swimming times and increases in lactate concentration, the result may be shown less active glucose metabolism than with other nutrients. Following exercise, blood glucose levels in mice receiving 10% glucose and 1.8% VAAM + 10% glucose decreased to 61.2% and 61.8%, respectively, of pre-swim levels. As with the increases in blood lactate. the decrease in blood glucose for these nutrients was very similar. However, for 1.8% VAAM blood glucose levels decreased only to 89.4% of pre-swim levels, very small in comparison with the decrease to 66.3% for 1.8% CAAM. Considering the compositional differences between VAAM and CAAM, acidic and sulfur containing amino acids, such as glutamic acid, aspartic acid, cystine and methionine, present in large amounts in CAAM, but rare in VAAM, may act to suppress maximum exercise times and changes in blood composition during exercise. Glucose homeostasis during exercise brought about by VAAM, as found in these experiments, may prevent hypoglycemia due to exercise. These effects of VAAM would lead to the prolongation of exercise ability.

Table 3. Concentrations of muscle and blood lactate in swimming mice after oral administration of various nutrients

Nutrients	Muscle ($\mu\text{mol/g}$ fresh weight) (mean \pm S.E.M.)	Blood (mMol) (mean \pm S.E.M.)	n
Distilled water	10.16 \pm 0.43	3.269 \pm 0.104	11
1.8% CAAM	10.37 \pm 0.51	2.600 \pm 0.144*	11
1.8% VAAM	9.76 \pm 0.56	5.013 \pm 0.248	11
10.0% Glucose	13.17 \pm 0.35		8

The Concentration of muscular lactate at resting condition was $5.94 \pm 0.10 \mu\text{mol/g}$ fresh weight (n=5) at fast.

* Significant difference of blood lactate between 1.8% VAAM and other nutrients ($p < 0.05$).

Muscular lactate concentration in exercising mice administered VAAM, CAAM, glucose or DW

Concentrations of muscular lactate in the legs of mice undergoing the same swimming exercise were analyzed. Administration of 1.8% VAAM brought about lower muscular lactate concentrations than other nutrients (Table 3). Muscular lactate concentrations correlated with blood lactate concentrations in mice receiving each nutrient.

Differences in blood concentrations of glucose and lactate in exercising mice administered amino acid nutrients containing VAAM components, and relationship between these concentrations

To analyze which amino acids cause the effect of VAAM in exercise, blood concentrations of lactate and glucose were measured after the administration of several amino acid nutrients. Administrations of glycine (Nut. no. 3). EAAM (Nut. no. 9). VAAM - Pro (Nut. no. 16), and VAAM - (Met, Asp, Ser) (Nut. no. 20) produced low concentrations of blood lactate; however, they also produced low concentrations of blood glucose. On the other hand, the administration of EAAM + Pro Nut. no. 10, EAAM + Gly (Nut. no. 11) or EAAM 4 (Nut. no. 15) produced high blood concentrations of both glucose and lactate. As shown in Fig. 7, no nutrient was able to give a similar effect to 1.8% VAAM (Nut. no. 2) or hornet larval saliva (for 60 min swimming) (Nut. no. 1). It is naturally shown that VAAM produced the similar effect to hornet larval saliva. The results of which varying the amino acid composition of VAAM loses its exercise-prolonging effects are suggesting that the special amino acid composition of VAAM must be important and necessary for the effect.

Blood concentrations of lactate and glucose during exercise in mice administered various nutrients were found to correlate ($r = 0.779$) (Fig. 7).

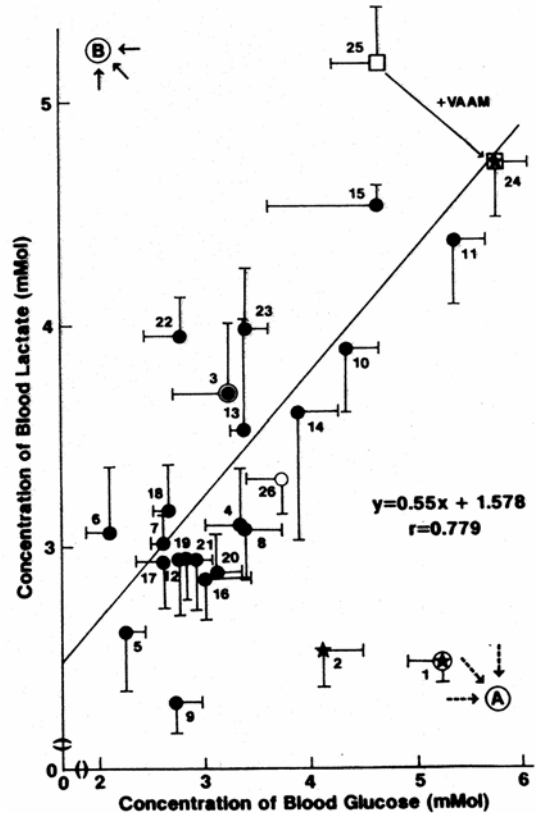


Fig. 7. Differences and correlation between blood lactate and glucose levels in the mouse endurance lactate and glucose levels in the mouse endurance exercise after oral administration of various nutrients. Nutrient nos. are as follows: 1. larval saliva (n = 8); 2. VAAM (1.8%) (n = 20); 3. CAAM (1.8%) (n = 15); 4. Proline (2%) (n = 10); 5. Glycine (2%) (n = 10); 6. Tyrosine (2%) (n = 10); 7. Pro (1%) + Tyr (1%) (n = 5); 8. Pro (1%) + Phe (1%) (n = 10); 9. EAAM (1.8%) (n = 5); 10. EAAM (0.9%) + Pro (1%) (n = 15); 11. EAAM (0.9%) + Gly (1%) (n = 5); 12. EAAM 1 (1.8%) (n = 4); 13. EAAM 2 (1.8%) (n = 5); 14. EAAM 3 (1.8%) (n = 5); 15. EAAM 4 (1.8%) (n = 5); 16. VAAM-Pro (1.8%) (n = 10); 17. VAAM-Gly (1.8%) (n = 16); 18. VAAM-Thr (1.8%) (n = 10); 19. VAAM - (Gly, Thr) (1.8%) (n = 5); 20. VAAM - (Met, Asp, Ser) (1.8%) (n = 8); 21. VAAM - (Met, Asp, Ser, Ala) (1.8%) (n = 5); 22. VAAM - (Met, Asp, Ser, Glu, His) (1.8%) (n = 4); 23. VAAM - (Met, Asp, Ser, Glu, His, Trp) (1.8%) (n = 5); 24. VAAM (1.8%) + Glucose (10%) (n = 10); 25. Glucose (10%) (n = 10); 26. DW (n = 20). Values are means \pm S. E. M. Details of the experimental procedures are described in Materials and Methods.

As shown in Fig. 7, blood glucose decreased in correspondance to the decrease in blood lactate during endurance exercise at the point of ultimate exhaustion according to hypoglycemia. Hypoglycemia in exercise suppresses the active functioning of the brain, and often leads to the disability to continue exercise. Mice receiving either 1.8% VAAM or hornet larval saliva, however, did not fit the correlation. The position of A in Fig. 7 represents the concentrations of glucose and lactate in the blood of resting mice. These concentrations in mice receiving 1.8% VAAM and hornet larval saliva were near the position A despite exercise. These levels are naturally linked to an improvement of the exercise activity. On the other hand, B shows the hypothetical worst condition with very low concentrations of blood glucose and very high levels of lactate, representing undesirable conditions for exercise.

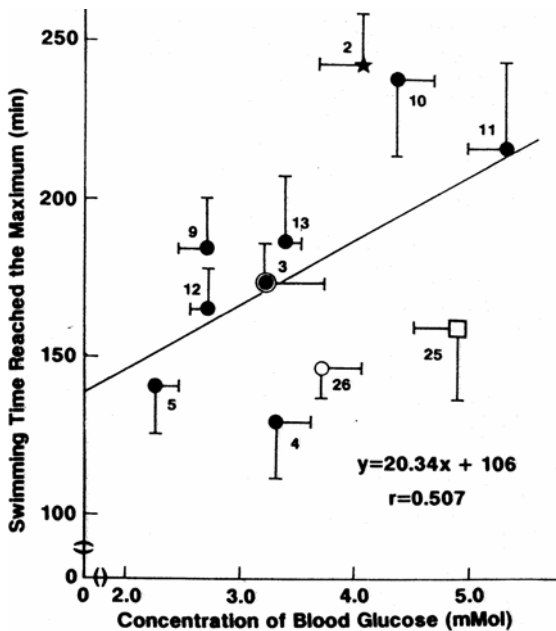


Fig. 8. Correlation between maximum swimming time and blood glucose concentration after oral administration of various nutrients. Values are means \pm S. E. M. Numbers show the nutrient no. as in Fig. 7.

Correlations of the swimming time and the blood concentration of glucose or lactate

In mice administered various nutrients and subjected to endurance exercise, a positive correlation between the maximum swimming time and the concentration of blood glucose was shown ($r=0.507$) (Fig. 8).

On the other hand, the maximum swimming time and the concentration of blood lactate during exercise showed a biphasic relationship (Fig. 9). Swimming times were longest at lactate concentrations of 2.5 mMol and 4.0 mMol at high concentrations of glucose. The fact that higher lactate levels coexisted with higher glucose levels can be explained by the equilibration of the recycling metabolism between lactate and glucose. However, at low concentrations of lactate seen as an effect of VAAM administration, if glucose was used mainly for exercising energy, then the lactate produced by the glucose metabolism must be

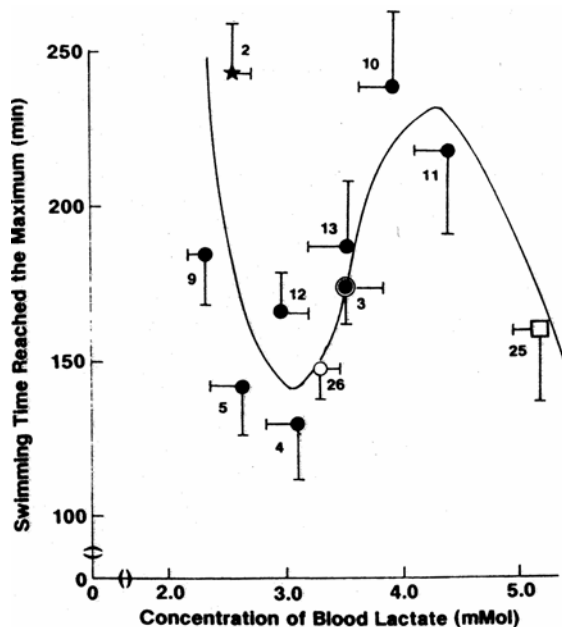


Fig. 9. Correlation between maximum swimming time and blood lactate concentration after oral administration of various nutrients. Values are means \pm S. E. M. Numbers are nutrient nos. as shown in Fig. 7.

resynthesized into glucose very quickly, in order to maintain the low lactate concentration. Alternatively, noncarbohydrates, such as fatty acids, which produce little lactate, must be used as energy sources for exercise. Other possibilities must also be considered.

Discussion

The optimum dose of nutrients was 25.0 to 37.5 μl per g body weight (Fig. 2). The optimum resting time was 30 min in 5 week-old mice (Fig. 3). While, applications of high concentrated VAAM such as 2.7% and 3.6%, did not produce the prolongation of swimming time (unpublished data). The optimum nutrient volume and resting time may reflect therefore the absorption velocity of the alimentary tract.

Homeostasis of blood glucose is important for the prolongation of endurance exercise^{2,11,19}). It is known that running endurance capacity is markedly decreased by the inhibition of gluconeogenesis⁹). If phosphoenolpyruvate carboxylase, a key enzyme in gluconeogenesis, is inhibited by mercaptopicolinic acid, gluconeogenesis significantly decreases. Gluconeogenesis carries out the major role of glucose homeostasis in endurance exercise¹⁷). In our study, the prolongation of the swimming times seen in mice receiving VAAM must be brought about by an improvement in the physiological function or metabolic control of exercise as well as by an activation of energy metabolism. And correlations between glucose and lactate levels in the blood (Fig. 7), or between blood glucose and swimming time (Fig. 8) also support glucose homeostasis as a necessity for prolongation in endurance exercise. This suggests that there is a close linkage between lactate production and gluconeogenesis in endurance exercise. Lactate produced from glucose by anaerobic respiration will be metabolically recycled to glucose through

gluconeogenesis coupling with the Cori cycle; thus blood concentrations of glucose and lactate equilibrate to a stable condition. This also shows that glucose is used to produce energy as well as to maintain brain functions for endurance exercise. It also suggests that the high concentrations of blood lactate produced during exercise do not strongly inhibit exercise ability as long as blood glucose levels remain high. Therefore, the suppression of lactate production and glucose degradation by VAAM is certainly related to improvements in exercise activity and resistance to fatigue.

A high positive correlation between the blood concentrations of glucose and lactate during exercise was shown in our experiments (Fig. 7). Blood glucose and lactate have often measured simultaneously in experiments on exercise in humans and rats, and a correlation between glucose and lactate was recognized under administration of CHO diet^{12,13,18,20}). This correlation also suggests a close connection between glucose and lactate metabolism. This metabolic relationship in endurance exercise implies that exercise can be prolonged if blood glucose concentrations remain high even if blood lactate concentrations are also high^{7,8}). The other metabolic condition produced by VAAM and hornet larval saliva, that is, the low lactate concentration together with the high glucose concentration in the blood, makes for an imbalance in the blood glucose-lactate relationship. The increase in blood glucose caused by VAAM in endurance exercise is induced through the acceleration of glycogenolysis or suppression of glycolysis or through competitive inhibition of glycolysis by other fuel sources than glucose. The decrease in lactate concentration arises through the activation of the Cori cycle⁶) or the suppression of glycolysis. Further analysis of the metabolic mechanism of VAAM is needed to address the above possibilities.

The energy used by muscle comes largely from

glycogen and fatty acids. Amino acids are not always useful energy sources compared with glucose and fatty acids since they release ammonia¹⁵). The role of amino acids in muscular activity has been studied from the point of energy sources as well as metabolic regulators. The catabolism of muscular proteins is activated by exercise, and the produced BCAA are used especially for muscular energy under certain condition. Oral supplementation with BCAA in humans prevents the catabolism of muscular proteins during 30 Km runs and marathons⁴). Changes in the amino acids compositions in blood and muscle during exercise have been investigated^{3, 5, 10}). In exhaustion trials by trained athletes, the levels of some plasma amino acids such as alanine, glycine, valine, α -amino butyric acid, isoleucine, threonine, serine and tyrosine decrease sharply³). Among these amino acids, alanine, glycine and threonine are present large amounts in VAAM. On the other hand, exhaustion causes little change in the levels of aspartic acid, glutamic acid, cysteine, or methionine. These acidic and sulfur containing amino acids are either absent from or present in very small amounts in VAAM. The composition of VAAM suggests therefore, a supplementary effect to amino acid depletion during exercise. The imbalance in VAAM composition must be an important factor in the improvement of exercise endurance, since the complete imbalance would enhance, while the defect in component might inhibit exercise endurance, as shown by our experiments involving the administration of various amino acid nutrients (Table 2 and Fig. 7).

It is very interesting to present this functional aspect of amino acids in ecological systems in nature.

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