

# The Activation of Fatty Acid Metabolism by *Vespa* Amino Acid Mixture (VAAM) and Related Nutrients during Endurance Exercise in Mice

Takashi ABE<sup>1</sup>, Mihoko INAMORI<sup>1</sup>, Kouji IIDA<sup>2</sup>,  
Masahiro TAMURA<sup>1</sup>, Yoshimi TAKIGUCHI<sup>3</sup>, and Kaneaki YASUDA<sup>3</sup>

<sup>1</sup>The Institute of Physical and Chemical Research, Hirosawa 2-1, Wako-Shi, Saitama, Japan 351

<sup>2</sup>Nutritional Laboratory, Central Research Institute, Meiji Milk Products Co., 1-21-3, Sakae-Cho, Higashimurayama-Shi, Tokyo, Japan 189

<sup>3</sup>R&D Lab. I, Nippon Steel Co., 1618 Ida, Nakahara-Ku, Kawasaki, Japan 211

## Abstract

ABE, T. INAMORI, M. IIDA, K. TAMURA, M. TAKIGUCHI, Y. and YASUDA, K. The Activation of Fatty Acid Metabolism by *Vespa* Amino Acid Mixture (VAAM) and Related Nutrients during Endurance Exercise in Mice. *Adv. Exerc. Sports Physiol.*, Vol.3, No.1 pp.35-44, 1997. The action of *Vespa* amino acid mixture (VAAM) on fatty acid metabolism was analyzed as changes in blood biochemical indices during endurance exercise in swimming mice. In response to the oral ingestion of VAAM, but not other nutrients, the concentrations of serum NEFA, blood ketone bodies, and plasma noradrenaline (NA) increased significantly during endurance exercise. The same mice showed the suppression of increase in blood lactate and decrease in blood glucose. Under similar exercise conditions, a relatively low plasma insulin concentration and an increase in the pyruvate/lactate ratio were observed simultaneously compared to other nutrients. A strong correlation ( $r=0.794$ ) was found between the blood glucose and lactate concentrations in mice ingesting various nutrients other than VAAM. The minimal compositional requirement for the induction of serum NEFA during endurance exercise was an amino acid mixture containing proline, alanine, valine, lysine, and isoleucine or leucine in the same compositional ratio as in VAAM, but the effect was not the same as with VAAM. Compositional analyses suggest that the excretion of plasma NA and adrenaline (A) are stimulated by different amino acid compositions, but a constant ratio of both catecholamines was secreted following feeding with either VAAM or VAAM 8. We also showed a high correlation ( $r=0.746$ ) between the induction of serum NEFA and the secretion of plasma NA by various nutrients. These results suggest that VAAM suppresses glucose oxidation, increases fatty acid oxidation, and also enhances the aerobic metabolism through the hormonal activation of NA during endurance exercise.

**Key words:** Catecholamines, NEFA, Acetoacetate, Glucose, Lactate, Endurance exercise, VAAM.

## Introduction

Minimizing fatigue, which significantly limits exercise performance, is one of the most important subjects in exercise sciences. Fatigue during exercise has been mainly attributed to a rise in blood lactate levels, a reduction in blood glucose levels, and the depletion of muscle glycogen.

It is well known that fatigue and exercise performance are markedly influenced by food intake.

Many studies of foods that contribute to energy yield during exercise have been conducted. Many such studies have dealt with carbohydrates, including fructose (17, 26), glucose (17, 25, 26, 32), glucose polymer (25), maltodextrins (10, 32), and corn starch (17). Others have studied fatty acids (10, 32), proteins (6, 20), and amino acids, especially branched chain amino acids (5, 7, 13, 24, 34). A carbohydrate-rich diet results in high levels of both plasma glucose and lactate, but lower plasma NEFA levels during endurance exercise. A fat- and protein-rich diet, however, produces low levels of plasma glucose and lactate, and contrastingly high plasma NEFA levels (23). Protein supplementation prevents the decrease in plasma levels of branched chain amino acids (BCAA), which contributes to energy production during endurance exercise (6, 20). BCAA ingestion also protects muscle proteins from catabolism (7).

On the other hand, very active muscles, such as flight muscles, exist in nature. Hornets, for example, have very strenuous muscles that can be trembled at over 1,000 cycles per min and can lift a weight of over 3 g. The muscle works continuously all daylong and hornets fly distances of over 70 km at 30 km per hr (1). We do not, however, understand the metabolic mechanisms that prevent the occurrence of fatigue during such hard flying exercise. The answer might lie in the special food intake of hornets. Adult hornets, which are among the most developed of social insects, ingest only liquid food comprising an amino acid mixture obtained from larvae during trophallaxis (1). This probably represents a kind of food evolution in which the substances for ingestion change depending on the developmental stage of the animal, progressing from hard solids to soft gels and liquids. Among relatively differentiated animals, such as in-

Address for Correspondence: Takashi ABE  
The Institute of Physical and Chemical Research, Hirosawa 2-1,  
Wako-shi, Saitama, Japan 351

sects, some species ingest mainly liquid diets. In a previous study, we found a major antifatigue component, the amino acid nutrient *Vespa* amino acid mixture (VAAM), from the saliva of *Vespa mandarinia* larvae(1). It has been shown that VAAM suppresses the decrease in blood glucose and the increase in blood lactate concentrations during endurance exercise and elongates swimming time in mice (2). The question arises as to what fuels are used for exercise energy. Blood glucose and lactate changes brought about by exercise after the ingestion of VAAM suggest that glucose is not a major source of energy for exercise(2). As another energy source, plasma NEFA is mainly used during endurance exercise. An increase in plasma NEFA, as well as ketone bodies, is accepted to indicate that the exercise is associated with an increased capacity to oxidize fats, probably caused in part by an increase in the activities of skeletal muscle oxidative enzymes (16). This is in agreement with the hypothesis that the exercise-induced increase in the oxidative capacity of skeletal muscle leads to an increase in the utilization of fatty acids (14). Further, the ability to carry out lipolysis during exercise leads frequently to an improvement in performance; therefore, the induction of blood NEFA during exercise is one of the most important issues for endurance athletes. However, it is not well-understood what nutrients induce lipolysis during exercise. From these points of view, the major effect of VAAM on serum NEFA levels has been analyzed with respect to energy metabolism, including hormonal regulation and the amino acid composition best for the induction of serum NEFA.

## Materials and Methods

### Animals

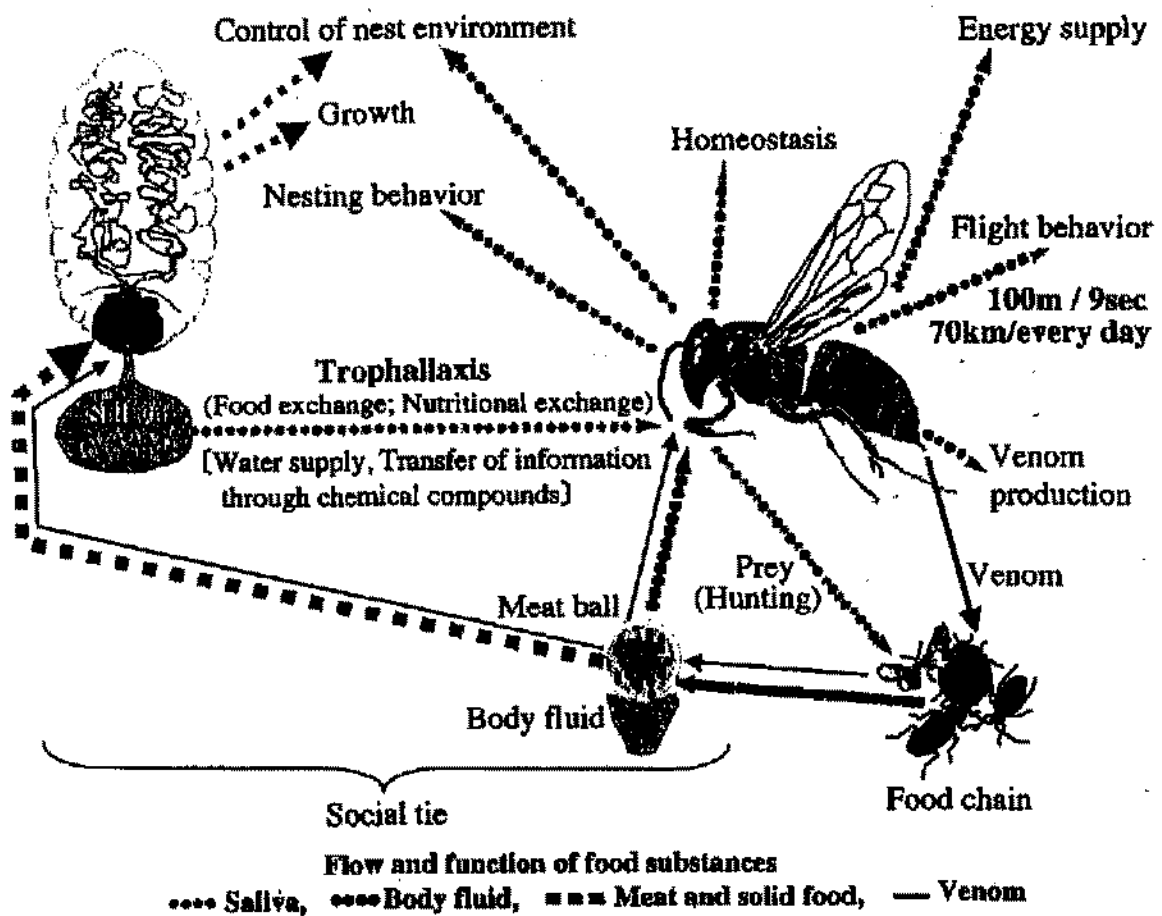
Male ddY strain mice, 6 weeks of age (17~22g body weight, 408 mice) (Saitama Experimental Animals Supply Co., LTD.), were used without any pre-training exercises as in a previous study (2). Treatment of the animals was in accordance with the guidelines of the Institute of Physical and Chemical Research Committee following NIH (USA) guidelines. Swimming was performed at 35°C at a pool flow rate of 5 m/min as in previous experiments (2). The mice had 0.3g weights attached to their tails during swimming. The 16hr fasting schedule and oral administration of nutrients at 37.5  $\mu$ l/g body weight were performed in the same manner as previously described (2). Mice were administered each nutrient 30 min before exercise. Endurance swimming was carried out for 30 or 60 min in the river pool. After swimming, blood was taken quickly from an abdominal vein or artery.

### Preparation of nutrients

VAAM, casein amino acid mixture (CAAM), and essential amino acid mixture (EAAM), and other modified VAAM nutrients used in these experiments are listed in Table 1.

### Blood Assays

Blood concentrations of lactate and glucose after swimming for 60min were analyzed by the lactate dehydrogenase and hexokinase methods, respectively, as in the previous study (2). Blood pyruvate levels just after swimming for 30min were measured by an enzymatic spectrophotometric method using lactate dehydrogenase and a Sigma diagnostics kit, Pyruvate (Sigma Chemical Co., St. Louis, MO, USA). Serum NEFA assays were performed enzymatically just prior to exercise (0 min) and after 30 and 60 min of swimming by a modification of a colorimetric procedure as follows. Forty microliters of mouse serum was mixed with 400  $\mu$ l of 50mMol Na-phosphate reaction buffer, pH 7.0, containing 5mMol MgCl<sub>2</sub>, 1.5mMol 4-aminoantipyrine, 0.73mMol CoA, 4.5mMol ATP, 0.27U acyl CoA synthetase, and 2.7U ascorbate oxidase, and the mixtures were incubated for 10min at 37°C. To the enzyme reaction mixture was added 800  $\mu$ l of dye-enzyme solution containing 1.2mMol 3-methyl-N-ethyl-N-(2-hydroxyethyl)-aniline and 2.92mMol N-ethylmaleimide, 6.8U peroxidase, and 5.5U acyl CoA oxidase, and the mixtures were incubated for 10min at 37°C. Enzyme activity was measured at O.D. 550nm. The amounts of NEFA in mEq were calculated using oleic acid as a standard. Blood ketone bodies in sedentary mice and those swimming for 30min were measured enzymatically with acetoacetate by a modification of the spectrophotometric method followed of Mellanby and Williamson (27). Li-acetoacetate was used as a standard. Serum insulin from mice after 30min of swimming was assayed by an immuno-glass beads-insulin-peroxidase-labeled insulin antibody complex method using the Glazyme Insulin-EIA Test (Wako Chemical Co., Osaka, Japan). Catecholamines, including adrenaline (A) and noradrenaline (NA), after 60min of swimming were determined by high performance liquid chromatography (HPLC) with fluorescence detection. Before HPLC analysis, 1 ml of blood from the carotid artery was mixed with 0.1mmole EDTA-Na, and the plasma was deproteinated with 1N HClO<sub>4</sub>. Plasma catecholamines were adsorbed onto 50 mg of activated alumina packed in a Sepacol mini-column (Seikagaku Kogyo Chemical Co., Tokyo, Japan) under basic conditions, and then extracted with 0.4N acetic acid after the column was washed well with distilled water (yield 80%). The extract was



lyophilized and redissolved in 30  $\mu$ l of 4N acetic acid. Twenty microliters of the sample was applied to ODS-HPLC (4.5x250 mm). The separated A and NA were oxidized with potassium ferricyanate with strong base at 50°C and detected as hydroxyindole fluorescence (Ex. 380nm, Em. 480nm). The minimal detectable levels were 0.1 pmol/ml for both A and NA.

#### Chemicals

Adrenaline (A), noradrenaline (NA), Li-acetoacetate, ATP, peroxidase and D-(-)-3-hydroxybutyrate dehydrogenase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The reduced form of nicotinamide adenine dinucleotide (NADH) and coenzyme A (CoA) or ascorbate oxidase were provided by Oriental Yeast Chemical Co. (Tokyo, Japan). Tryptophane, HClO<sub>4</sub>, 4-aminoantipyrine, 3-methyl-N-ethyl-N-(2-hydroxyethyl)-aniline, EDTA-Na and oleic acid were purchased from Wako Chemical Co. (Osaka, Japan). All amino acids except tryptophan were from Kyowa Hakko Kogyo Co. (Tokyo, Japan). Acetyl CoA synthetase and acetyl CoA oxidase were from Toyobo (Osaka, Japan). N-Ethylmaleimide was from Eastman Kodak Co. (New Haven, CT, USA). Aluminum oxide (Woelm Nutral W-200) was prepared by M. Woelm Pharma (Eshwege, Germany).

#### Statistics

All data are presented as means  $\pm$  SE. The effects of nutrients on swimming time to exercise were assessed by a 1x2 ANOVA. The paired student's t test was used to test the significance of differences between related samples from the same mouse. Repeated measures ANOVA with a subsequent Bonferroni test was used to test the significance of differences in the mean values of blood biochemical indices. The significance level for all analyses was set at  $p < 0.05$ .

#### Results

##### Effects of VAAM, CAAM and Glucose on NEFA induction during swimming exercise

Serum NEFA concentrations, which were  $0.85 \pm 0.03$  mEq/L in resting mice ( $n = 8$ ) after the fasting for 16hrs, were slightly increased to  $0.90 \pm 0.03$  mEq/L by the ingestion of 1.8% VAAM, but not changed by distilled water (DW) ( $0.87 \pm 0.03$  mEq/L) or 1.8% CAAM ( $0.83 \pm 0.04$  mEq/L), and decreased slightly to  $0.65 \pm 0.01$  mEq/L by 20% glucose administered 30min before exercise (Fig. 1). After 30min of continuous swimming, serum NEFA concentrations were significantly increased by VAAM or DW ingestion, while it was increased gradually in CAAM or glucose ingestion. During further swimming up to 60min, serum NEFA concentrations in mice that received VAAM

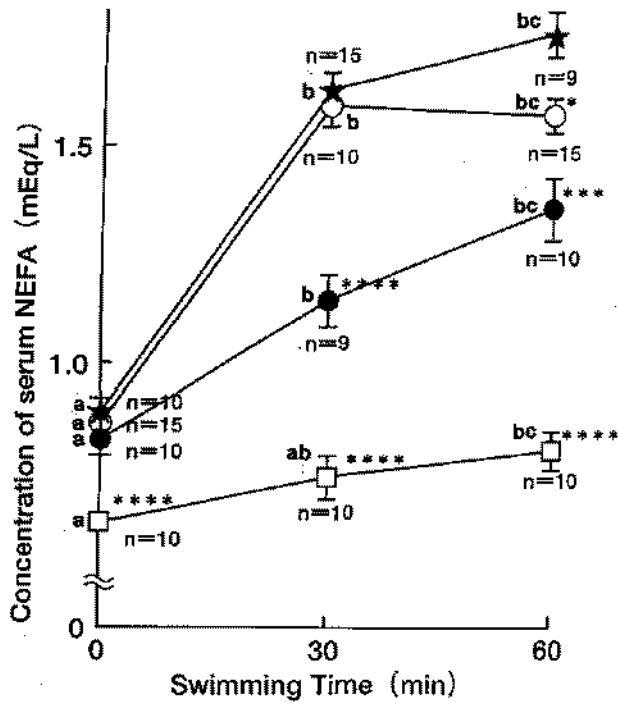


Fig. 1 Changes in serum NEFA concentrations in the blood of mice ingesting 1.8% VAAM (closed stars), DW (open circles), 1.8% CAAM (closed circles), or 20% glucose (open squares) during swimming exercise. Values are means  $\pm$  S.E. *n* is number of mice. Significant differences between swimming times for 0min (a), 30min (b) and 60min (c) are indicated without repetition of symbols ( $p < 0.05$ ). Significant differences from 1.8% VAAM are shown for each swimming time as follows; \* $p < 0.05$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < \text{less than } 10^{-5}$ . Details of the experimental conditions are described in Materials and Methods.

or DW remained constant at about 1.60mEq/L, but the concentrations in mice receiving CAAM or glucose increased continuously to low levels of  $1.15 \pm 0.06\text{mEq/L}$  or  $0.74 \pm 0.06\text{mEq/L}$ , respectively (Fig. 1). Blood concentrations of lactate and glucose were also analyzed in the same swimming mice (Table 2). Blood lactate concentrations were elevated in mice receiving CAAM, glucose or DW, but decreased in mice receiving VAAM. Blood glucose concentrations decreased in mice that ingested DW and CAAM less than in mice receiving VAAM. Both blood lactate and glucose concentrations showed responses similar to those described in our previous study (2). On the other hand, blood levels of ketone bodies formed by the oxidation of fatty acids were analyzed under the same exercise conditions. Following the ingestion of 10% glucose, the resting blood concentration of ketone bodies was very low at  $73.59 \pm 9.30 \mu\text{Mol}$ ; after exercising for 30min, the level was still low at  $119.12 \pm 26.5 \mu\text{Mol}$ , despite the 62.7% increase over the resting level (see Fig. 2). In the case of 1.8% CAAM or DW ingestion, the blood ketone body concentration at rest was  $230.95 \pm 33.83 \mu\text{Mol}$  or  $247.69 \pm 33.95 \mu\text{Mol}$ , respectively. In both cases, there was a very slight increase during exercise of 3.2% and 7.7%, respectively. In mice ingesting 1.8% VAAM, the blood ketone body concentration was  $248.11 \pm 13.35 \mu\text{Mol}$  at rest, and a significant 60.3% increase was observed with exercise. During 60min exercise, as shown in Table 2, plasma insulin concentrations were lower in mice that ingested VAAM than in those receiving CAAM or glucose. On the other hand, the ratio of pyruvate to lactate was about 1.7 times higher in mice ingesting VAAM than in those receiving CAAM.

Table 2 Comparison of serum NEFA and insulin, and blood glucose, lactate, and pyruvate levels of mice ingesting 1.8% VAAM, 1.8% CAAM, 10 or 20% Glucose, and D.W. after 60 min of swimming exercise.

Nutrients	Serum		Blood		Serum		Blood		Blood		Ratio of means Pyruvate/Lactate
	NEFA (mEq/L)	<i>n</i>	Glucose (mMol)	<i>n</i>	Insulin ( $\mu\text{U}$ )	<i>n</i>	Lactate (mMol)	<i>n</i>	Pyruvate (mMol) <sup>d</sup>	<i>n</i>	
1.8%VAAM	$1.74 \pm 0.17^{**}$	9	$3.98 \pm 0.46^{*b}$	6	$4.51 \pm 0.86$	8	$1.02 \pm 0.07^{*c}$	6	$0.170 \pm 0.012^{***e}$	11	0.167
1.8%CAAM	$1.35 \pm 0.22^e$	10	$2.33 \pm 0.48^b$	8	$6.00 \pm 1.93$	8	$1.37 \pm 0.13^c$	8	$0.135 \pm 0.018$	10	0.099
20% Glucose	$1.04 \pm 0.09^f$	9	$4.81 \pm 0.48$	6	$5.01 \pm 0.83^f$	8	$2.12 \pm 0.13^c$	6	$0.256 \pm 0.016^{*f}$	8	0.121
D.W.	$1.57 \pm 0.18$	15	$2.76 \pm 0.10^b$	6	$3.64 \pm 1.35$	8	$1.28 \pm 0.07$	6	$0.142 \pm 0.010$	9	0.111

*n* shows numbers of mice.

Data of significant differences were compared with reference to 1.8% VAAM.

\*a and a, \*b and b, and \*c and c show significant differences ( $p < 0.05$ ).

<sup>d</sup> indicates values after 30min of swimming exercise.

\*\*c and e show a very significant difference ( $p < 0.01$ ).

f is 10% glucose.

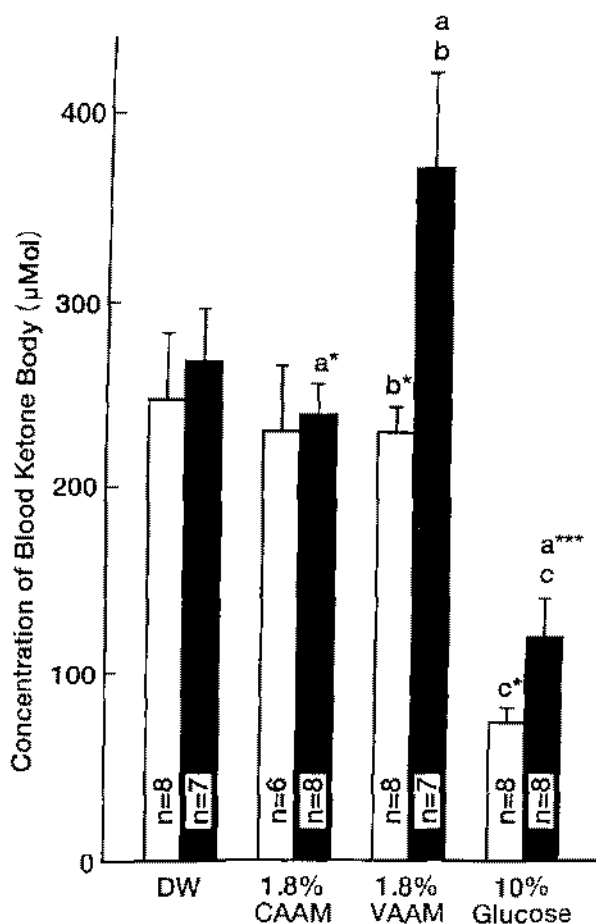


Fig. 2 Differences in the levels of blood ketone bodies with (closed column) or without (open column) swimming exercise in mice ingesting 1.8% VAAM, DW, 1.8% CAAM, or 10% glucose. Values are means  $\pm$  S.E. a\* and a, b\* and b, and c\* and c show significant differences ( $p < 0.05$ ). a\*\*\* and a show extreme differences ( $p < 0.001$ ). Details of the experimental conditions are described in Materials and Methods.

#### Serum NEFA induction by modified VAAM nutrients during swimming exercise

To analyze the amino acid compositions most effective in increasing NEFA concentrations during exercise, modified VAAMs maintaining the original VAAM compositions were fed to mice. Upon endurance swimming for 60min, the major amino acid components, proline, glycine, and threonine, alone induced lower concentrations of serum NEFA than those induced by 1.8% VAAM (Table 3). Proline was especially effective in increasing the NEFA concentration in comparison with EAAM + proline or EAAM. The exclusion of more than one amino acid (VAAM 1, 2, 5) resulted in a decrease in NEFA concentration. It is suggesting that the composition and ratios of VAAM components would influence the in-

crease of serum NEFA concentration during exercise. Further investigations with VAAM 6 and VAAM 7 again showed clearly that proline is the necessary component for maintaining high concentrations of serum NEFA. Finally, in comparative experiments with VAAM 8 and 9, it was found that the most effective amino acids in VAAM for the induction of serum NEFA were proline, alanine, valine, leucine, and lysine. Under the same exercise conditions, the correlation between blood glucose and lactate concentrations was better ( $r=0.794$ ) than the concentration between serum NEFA and blood glucose or lactate concentration ( $r=0.536$  for glucose and  $r=0.526$  for lactate, respectively).

#### Correlation between plasma catecholamine excretion and serum NEFA induction by VAAM and other amino acid nutrients during swimming exercise

The hormonal effect on serum NEFA induction was analyzed with respect to catecholamines. The concentrations of both plasma A and NA were increased under the same swimming conditions as in the NEFA induction experiment (Table 4). The molar ratio in plasma was always lower for A (32~43%) than NA (57~68%) in exercising mice who ingested amino acid nutrients and DW. The increase in the concentration of plasma A and the proportion of A in the total catecholamine content were higher following 1.8% VAAM or DW ingestion (42~43%), but lower following 1.8% VAAM 8 or 1.8% CAAM ingestion (32~36%), as shown in Table 4. The ratio of NA to A was lower in the former case (about 1.3) than the latter (about 2). This difference is caused by the lower level of A, which then produces the low total catecholamine concentration following VAAM 8 or CAAM ingestion. The total catecholamine content showed no correlation to the induction of serum NEFA or the elongation of swimming time (2). The best correlation with NEFA induction was found for NA ( $r=0.746$ ), but was negative for A ( $r=-0.039$ ) (Table 4). The extent of NEFA induction by each nutrient was analyzed for the effect on the ratio of NA to A (Fig.3). The enhancement of NA induction corresponded to an increase in the NA/A ratio as represented by the slope constant. And the parallel induction of both catecholamines was observed quantitatively. At the same time, a larger increase in NA or a greater induction of serum NEFA was related to an increase in the correlation coefficient between A and NA ( $r=0.848$  for VAAM8,  $r=0.862$  for VAAM,  $r=0.544$  for CAAM and  $r=0.145$  for DW). As for catecholamine induction, although the levels of plasma NA and A were similar following either VAAM or DW ingestion, exercise performance was not im-

Table 3 Concentrations of serum NEFA, glucose, and lactate in the blood of swimming mice following ingestion of several modified VAAM nutrients

Nutrients	Serum		Blood		Blood	
	NEFA (mEq/L)	n	Glucose (mMol)	n	Lactate (mMol)	n
VAAM-proline	1.44±0.16	9	3.03±1.22	10	1.19±0.25	10
VAAM-glycine	1.35±0.26	4	2.65±1.06	16	1.23±0.35	16
VAAM-threonine	1.38±0.11	5	2.66±0.43	10	1.32±0.11	10
VAAM-(proline + glycine)	1.52±0.11	5	2.82±0.32	5	1.22±0.17	5
VAAM 1	1.08±0.09	5	N.D.		N.D.	
VAAM 2	1.61±0.20	5	N.D.		N.D.	
VAAM 3	1.87±0.14	5	N.D.		N.D.	
VAAM 4	1.75±0.11	5	4.48±0.13	5	2.68±0.24	5
VAAM 5	1.73±0.12	5	4.47±0.06	5	3.10±0.23	5
VAAM 6	2.41±0.47	5	3.01±0.68	5	1.44±0.21	5
VAAM 7	1.94±0.10	4	4.50±0.35	5	2.32±0.44	5
VAAM 8	2.47±0.23	5	4.12±0.97	5	2.45±0.32	5
VAAM 9	2.28±0.52	5	3.90±0.61	5	2.27±0.25	5
EAAM	1.19±0.16	5	2.74±0.58	5	0.95±0.13	5
EAAM+Proline (1:1)	1.81±0.16	5	4.36±1.26	15	1.62±0.49	15
Glycine+Proline (1:1)	1.36±0.10	5	N.D.		N.D.	

The concentration of each nutrient was 1.8% (w/w) solution.

n shows numbers of mice.

N. D. is no detection.

Table 4 The concentration of plasma catecholamines in swimming mice following oral ingestion of VAAM 8, VAAM, CAAM, or DW.

Nutrients	Adrenaline (nMol)	Noradrenaline (nMol)	Total(A + NA) (nMol)	n
1.8%VAAM 8	17.7±2.4 <sup>**</sup>	38.0±4.5 <sup>*b</sup>	55.7±5.9	10
1.8%VAAM	27.6±5.1 <sup>*a</sup>	36.6±6.6	64.2±11.0	15
1.8%CAAM	15.1±2.9 <sup>a</sup>	26.7±3.5 <sup>b</sup>	41.8±5.6 <sup>c</sup>	12
DW	26.2±2.2 <sup>**a</sup>	36.5±2.4 <sup>*b</sup>	62.7±3.7 <sup>**c</sup>	12
R	-0.039	0.746	0.357	

The concentrations of plasma adrenaline and noradrenaline at rest were 14.9±3.7 nMol (n=5) and 17.6±3.2 nMol (n=5), respectively.

The concentration of each nutrient was 1.8% (w/w) solution.

n shows numbers of mice.

\*a and a, and \*b and b show significant differences (p<0.05).

\*\*a and a, and \*\*c and c show very significant differences (p<0.01).

R is the correlation coefficient versus serum NEFA concentration (see Tables 2 and 3).

proved by DW ingestion. This supports conjecture that VAAM has different effects than D.W., such as the improvement of fat oxidation, an antifatigue effect in brain, etc.

#### Discussion

It is well known that the oxidation of fatty acids

is passively activated by an increase in serum NEFA. In comparison with the ingestion of CAAM, glucose, or D.W., the fact that VAAM induced high serum NEFA and blood ketone body levels, in contrast to the suppression of blood glucose decrease and blood lactate increase (Figs.1 and 2, Table 2)(2), might be expected to encourage lipolysis and the subsequent

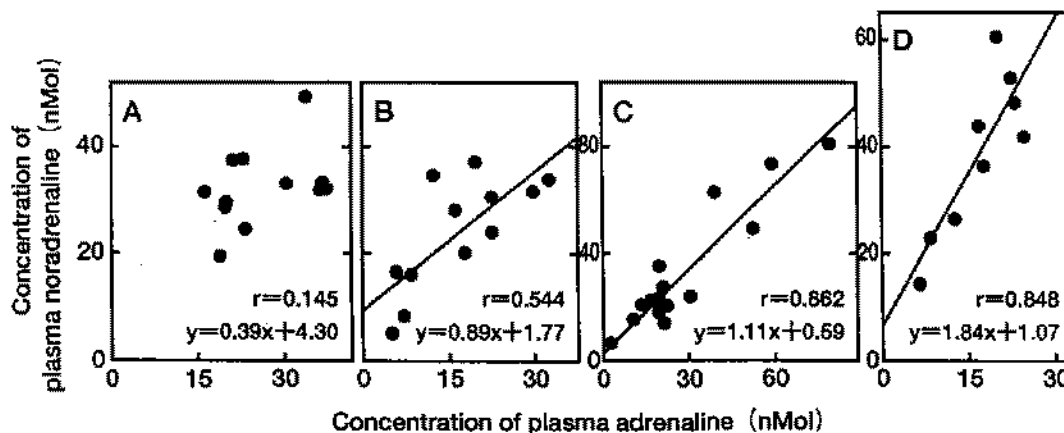


Fig. 3 Correlation between adrenaline and noradrenaline in the blood of swimming exercised mice ingesting DW (A), 1.8% CAAM (B), 1.8% VAAM (C), and 1.8% VAAM 8 (D). Regression lines and correlation coefficients ( $r$ ) are shown for each nutrient. Details of the experimental conditions are described in Materials and Methods.

activation of fatty acid oxidation during endurance exercise. It is to be expected that glucose oxidation does not progress at low plasma insulin levels, so that higher insulin levels cause more glucose oxidation, resulting in low levels of blood glucose. This strongly suggests that the energy supply in endurance exercised mice receiving VAAM depends on fatty acid oxidation but not on the activation of glucose as in the case of CAAM, because the decrease in glucose consumption corresponds to a lower level of insulin, and higher blood glucose and lower blood lactate levels show the suppression of glycolysis. Further, the ratio of pyruvate to lactate in blood reflects an enhancement in aerobic metabolism, which also increases during exercise in mice receiving VAAM (Table 2). These metabolic changes induced by VAAM are analogous to the progressive induction of fatty acid oxidation with training adaptation (5, 19, 33).

The serum NEFA concentrations induced by these selected amino acids (especially, VAAM 6, 8 and 9) are over the risky concentration of 2 mEq/L (9, 29). The serum NEFA inducing effect of these nutrients is, therefore, very close to the physiological maximum of metabolic adaptation of the exercising subject. However, it might be avoid the risk of membrane perturbation because almost all the induced serum NEFA is bound to lipoproteins in blood. Thus, this high concentration of serum NEFA would enhance fatty acid oxidation.

The high serum NEFA levels caused by the ingestion of DW during exercise probably reflects fasting conditions. The concentrations of blood glucose, lac-

tate and pyruvate, and serum insulin were also lower in mice ingesting D.W. than in mice ingesting other nutrients (Table 2). However, the concentrations of serum NEFA and plasma catecholamines were similar to those in mice ingesting VAAM, but the concentration of blood ketone bodies was a little lower. Thus, the physiological conditions in the case of D.W. ingestion represent a state of extreme hunger and excitement following continuous exercise after fasting for 16 hrs. Under starvation conditions, the high blood levels of ketone bodies are remarkably reduced by the injection of glucose. This suggests that glucose supplementation suppresses fatty acid oxidation and/or lipolysis as was observed in previous experiments (2); in other words, glucose takes priority as the energy source for oxidative metabolism over all other nutrients. Despite the depletion of energy stores by strenuous exercise, the ingestion of VAAM brought about higher levels of blood glucose, fatty acid oxidation, and aerobic metabolism, thus producing better performance than other nutrients. Additionally, the same effect of VAAM, that is, the suppression of the increase in blood lactate levels and the decrease in blood glucose levels during exercise, was found with glucose supplementation despite the high blood glucose levels, as shown in the previous study (2). These results suggest that the effect of VAAM is not altered by starvation.

In experiments using various amino acids, the relationship between the concentrations of serum NEFA and blood glucose or lactate was not strong, although similar for both ( $r = 0.536$  for blood glucose,  $r = 0.526$  for blood lactate)(Table 3). However, there

was a good correlation between the concentrations of blood glucose and lactate in this study ( $r = 0.794$ ) (Table 3) and also the previous study ( $r = 0.779$ ) (2). The correlation was generally found regardless of the state of rest or exercise and nutrient ingestion. However, the effect of VAAM goes against this trend, that is, produces high blood glucose and low blood lactate levels during exercise as shown in our previous study (2). With the administration of various nutrients, blood glucose concentrations showed a slight correlation with an improvement in performance ( $r = 0.507$ ). This improvement in performance was found at low (2.5 mMol) and middle high (4.0 mMol) concentrations of blood lactate. This suggests that high blood glucose concentrations lead to high blood lactate concentrations, but do not always lead to an improvement in performance.

All the compositional studies suggest that glycine contributes to the suppression of the decrease in blood glucose, but has little effect on the decrease in blood lactate. It might be expected that glycine is metabolized to two ways; one is threonine which is metabolized to propionyl CoA, thus finally might reduce the production of lactate and suppresses the decrease in glucose. Another way is serine which produces pyruvate, then also lactate. It was also found that serum NEFA induction by leucine (VAAM 8) and isoleucine (VAAM 9) is similar, so that these amino acids may be interchangeable. Detailed compositional analyses show that the induction of serum NEFA is not equivalent to the effect of VAAM, but rather represents only a partial effect. Synthetic nutrients are not better than either of VAAM 6, 8 or 9. However, compositional changes in VAAM show that at a minimum, the components of VAAM are required for serum NEFA induction and higher levels of plasma NA and A (Tables 3 and 4). The results show the importance of both the compositional ratio and components of VAAM for its effects. Effective components, such as VAAM 6 to 9, contain large amounts of branched chain amino acids, which are utilized by muscle cells. This suggests that the peculiar nutritional effect of VAAM, high levels of serum NEFA and blood glucose and low levels of blood lactate during exercise, is produced by a special balance of amino acid composition brought about exclusively by nature. The dietary function of the complicated amino acid composition of VAAM is not known completely at present. The complicated composition of VAAM may affect the transfer of some information to cells or organs as an amino acid language. Future studies should examine the importance of amino acid composition in certain specific food functions.

A comparative compositional study of

catecholeamine induction suggests that the induction of plasma A requires another special amino acid composition, as shown for plasma NA by VAAM 8. Further, plasma A probably has another effect besides the induction of serum NEFA (Table 4). These phenomena show the apparent response of both hormones to special amino acid nutrients, and suggest that these amino acid nutrients play a role in serum NEFA induction through plasma NA activation. Considering that high concentrations of serum NEFA lead to more fatty acid oxidation, it is possible that lipolysis during exercise is brought about by an increase in fat consumption. However, the inductions of serum NEFA and plasma NA do not always show a close relationship to one another or to improvements in blood biochemical indices during exercise as shown by, for example, the suppressive effect on the increase in blood lactate or decrease in blood glucose as shown in Tables 3 and 4, and in the previous study (2). The fact that both catecholeamines are hardly distinguishable from each other strongly suggests that the ratio of plasma NA to A must be nearly equal ( $NA/A = 1.1$ ) and the correlation coefficient between them high ( $r = 0.862$ ), in other words, their excretion in a nearly equal ratio in each individual is a minimum requirements for exercise improvement (Fig. 3). This suggests that plasma A plays an important role in the improvement of performance. The synchronous stimulation of organs controlled by both  $\alpha$ - and  $\beta$ -receptors is required for optimal exercise performance. In fact, plasma NA activates fatty acid hydrolysis in fat bodies (5, 35) and glycogen degradation in liver (18). Plasma A, in the meantime, induces the hydrolysis of muscle TG (3). Thus, VAAM is a multifunctional complex that probably controls complicated physiological functions of exercise.

The effect of VAAM as a metabolic controller during endurance exercise can be thought of as follows. VAAM absorbed from the intestines stimulates the adrenergic system, maybe the adrenal, leading to increases in NA and A. As shown in Table 4 and Fig. 3, the ratios and correlation coefficients between NA and A are obviously higher with VAAM than with CAAM ingestion. A large increase in NA has been found to interfere with the development of hypoglycaemia directly by stimulating the production of glucose through hepatic glycogenolysis, but A does not appear to be critical for the prevention of hypoglycaemia during exercise. The adrenergic system and the cyclic AMP cascade play crucial roles in the activation of hormone-sensitive TG lipase and the subsequent TG hydrolysis in adipose tissues (4, 5, 34). The intercellular lipoprotein lipase activity in each type of rat muscle is increased by A (28). The



pattern of plasma A is similarly and significantly correlated with that of serum NEFA and with glycerol concentration (29). There is other evidence that the adrenergic system also plays an important role in activating the lipolysis of muscle TG (12). Further studies of agonists and antagonists of  $\beta$ -adrenergic receptors strongly suggest that the process is probably controlled exclusively by the adrenergic system (3). The higher levels of serum NEFA and blood ketone bodies induced by plasma NA and A (Figs. 1 and 2, Tables 2 and 3) would produce excess amounts of acetyl CoA by  $\beta$ -oxidation. An activation of both lipolysis and oxidation is found in training adaptation of skeletal muscle (33). Endurance training in particular increases the capacity of muscle to oxidize fats derived from muscle TGs (19). For the control of energy in the fatty acid metabolism, this adaptation increases the uptake and oxidation of serum NEFA, and concomitantly brings about a decrease in glucose uptake. The activation of fat hydrolysis and fatty acid oxidation during endurance exercise after VAAM ingestion is likely, therefore, to be a kind of metabolic adaptation from the untrained to the trained condition. This might be important for the improvement of exercise performance. Glucose uptake and oxidation decrease because of the higher glucose level and lower plasma insulin level (Table 2) (2). In fact, this response is also observed in trained subjects, that is, glucose uptake by skeletal muscle is decreased late in the exercise period despite higher blood glucose concentrations (8, 33). The reason for this lower glucose uptake in trained subjects during exercise is not readily apparent. One possibility is that increased fat oxidation in trained subjects leads to a citrate-mediated inhibition of phosphofruktokinase (21). At the same time, glycerol, as a counterpart to fat hydrolysis, is probably metabolized mainly in the liver, which has a high activity of glycerol kinase (22). The rate of utilization by the liver is directly proportional to its concentration (31). This metabolic regulation, which is responsible for the activation of lipid hydrolysis following VAAM ingestion, finally brings about both the decrease in lactate production and the maintenance of glucose levels (Table 2). Certainly, the higher pyruvate/lactate ratios demonstrate aerobic metabolism (see Table 2). The suppressive production of lactate during endurance exercise following VAAM ingestion could lead to the increase in serum NEFA, because lactate, which lowers the pH (15), increases the re-esterification of serum NEFA in adipose tissue (11). The lactate concentration increases in contracting muscles, and muscle pH is further reduced as lactate accumulates. The effect would then be further potentiated by

the concomitant reduction in muscle pH. Lowering the pH also reduces the lipolysis stimulated by NA, ACTH, and glucagon (30). Lactate as the end-product of anaerobic glycolysis would be expected to inhibit NEFA supplementation into muscle cells. Under such circumstances, the lactate could be involved in the metabolism of fats and carbohydrates. These findings suggest that VAAM causes a shift from carbohydrate to fat combustion. These metabolic responses to VAAM ingestion during endurance exercise would prevent the occurrence of fatigue. It is thus considered that the complex effects of VAAM, its anti-fatigue effects, finally result in an improvement in exercise performance such as the elongation of swimming time (2).

#### Footnote

The data reported in this study were presented at the Annual Meetings of the Japanese Biochemical Society between 1990 and 1993.

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