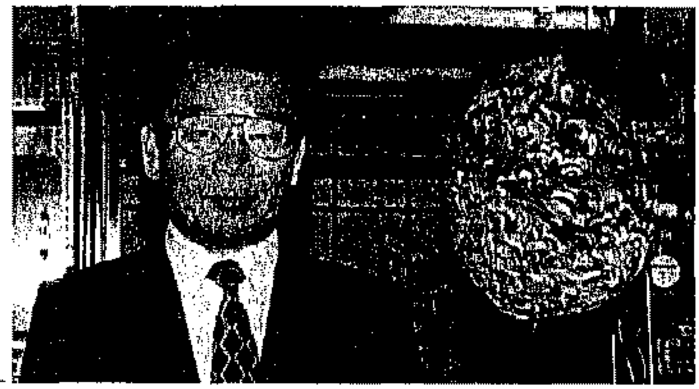
Special Feature I

Discovery of Vespa Amino Acid Mixture (VAAM) and It's Functions



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For many years amino acids have been popular with athletes, to grow and strengthen muscles, but as more and more beneficial effects are identified the popularity of amino acid drinks continues to grow exponentially. In 2002, the sale of amino acid products in Japan — including sports drinks, jellies and powders — totaled 30 billion yen, and is predicted to exceed 70 billion this year. One of the most popular is VAAM produced by Meiji Milk Products Co. Ltd. and promoted by Olympic Gold Medallist marathon runner, Naoko Takahashi.

VAAM is unique, because it is based on a mixture of 17 amino acids found in the saliva of hornet larvae. The mixture burns fat so effectively to produce energy that the adult hornet is able to fly up to 70 km per day. The fat-metabolism qualities of VAAM are equally effective for mice and humans during exercise and recently, have been linked to other health benefits, such as improved liver and kidney function, and improved autonomic nerve function control.

But how did I discover the fat-oxidation qualities of hornet larval saliva for exercising humans? What is the science behind the theories and how did I prove it is effective?

Hornet Behavior

The turning point in my research came when I had a brush with death after being stung by a hornet, and started to carry out field observations to find out how I could catch hornets safely. My studies focused on the unique behavior of *Vespa mandarinia* — more commonly known as the Japanese Giant Hornet — which not only has the most toxic venom but is also the most robust species of hornet, building the largest colonies and flying 70 km at 35 km/hr every day for hunting, carrying one third of it's body weight in food. For a long time burning body fat has been recognized as an abundant, efficient source of long term energy for endurance activity, producing few fatigue-inducing compounds, like lactate. From larva through to the adult stage, many insects store abdominal fat as an energy source.

During my observations I realized that adult hornets could not survive for more than a couple of days without contact with their larvae [1]. Closer study revealed the reason to be nutritional dependence. The peculiar body structure of the adult — a very narrow esophagus through a coarctate waist — makes it impossible to eat solid food; even the insects which they prey on. Instead they turn the flesh into meatballs to feed the larvae, who in turn excrete a special rich cocktail which is the main source of nutrition for the adult. Without a regular supply of larval saliva the adult is unable to continue it's normal hunting behavior, and will eventually die (Fig. 1). The larvae uses trophallaxis — food exchange — to effectively control the adult's behavior, manipulating the amino acid composition of the mixture to manage the social colony [1] (Fig. 2). Although larval saliva is mainly comprised of trehalose, glucose and 17 amino acids (predominantly proline and glycine) it is compositionally very different from other protein-rich foods, like eggs, meat or milk. However, the compositional similarity of the salival mixtures of different hornet species suggests common functional properties [2]. We dubbed this special mixture '*Vespa* Amino Acid Mixture' (VAAM) (Fig. 3).

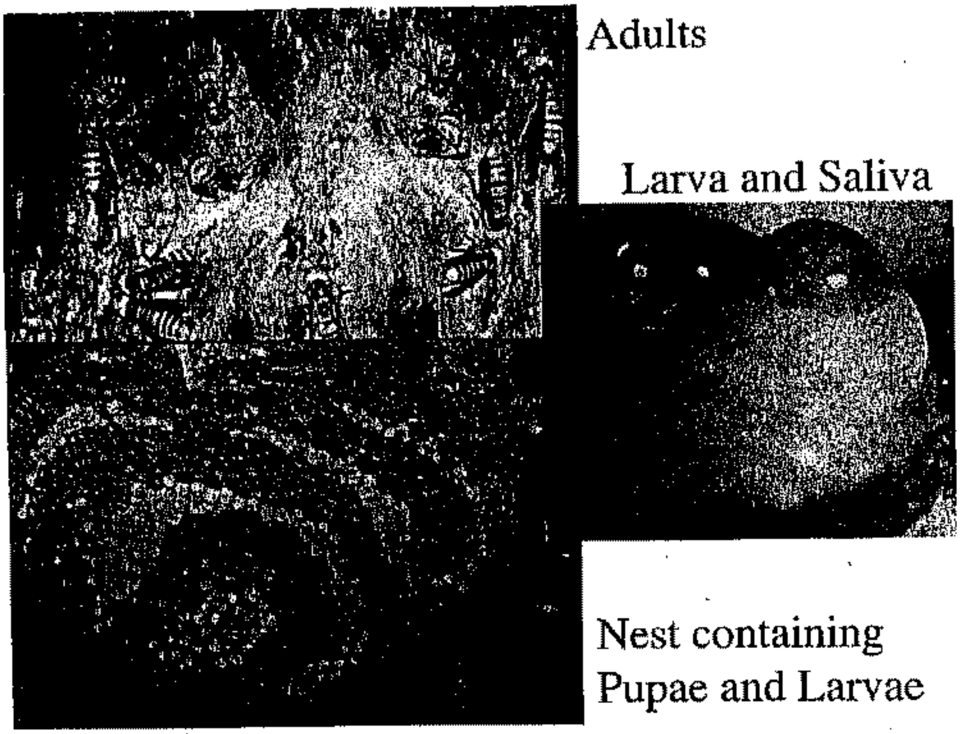


Fig. 1: Pictures of adults, larvae, saliva and a nest of the giant hornet, Vespa mandarinia

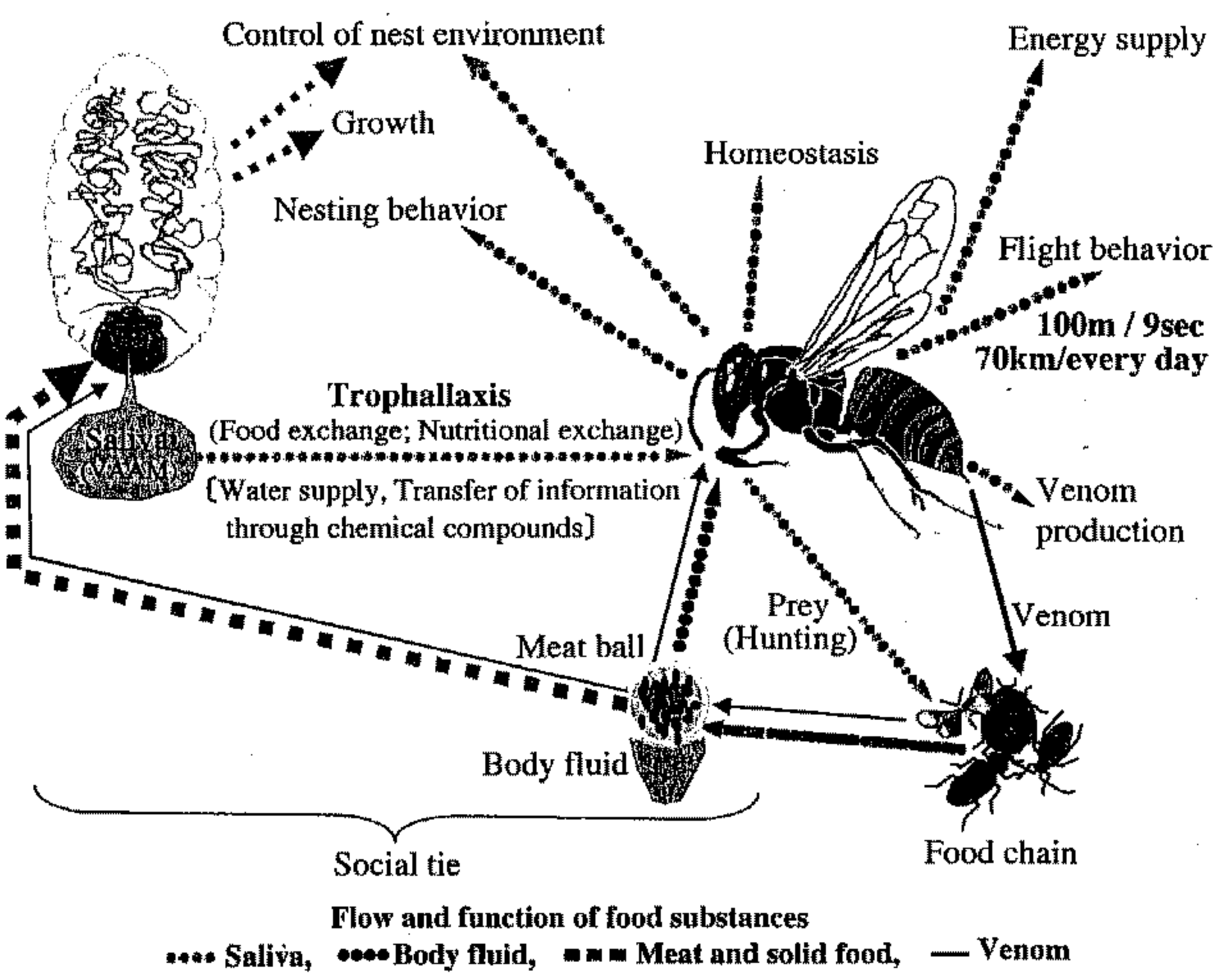


Fig. 2: Hornet behavior and food exchange

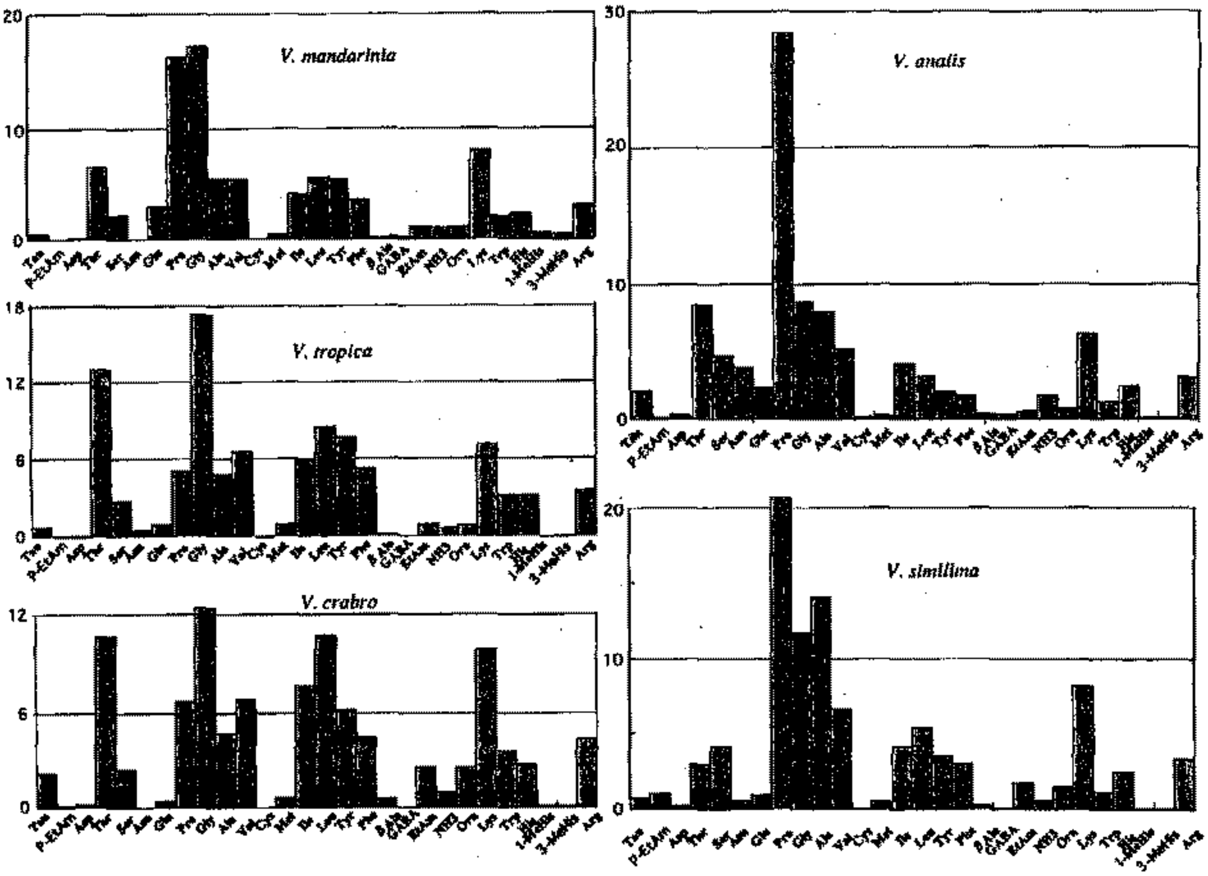


Fig. 3: Amino acid composition of hornet larval saliva.

VAAM function for energy metabolism in insect flight muscles

The flight muscle in the wings of flying insects is one of the most ingenious of all bio-organisms. Flight requires a large amount of instant energy, especially in the case of the giant hornet, whose 2 g body weight makes flight aerodynamically impossible in theory. Many insect flight muscles usually have more 10 to 100 times more outstanding activity, ability and power based on their metabolic system than any other animal's muscles [3,4,5,6]. The insect muscle reaches the maximum activity within a couple of seconds but take a couple minutes in other active animals. It is difficult to explain how a body can suddenly generate sufficient energy to fly [6], but basically it requires the short and fast activation of the tricarboxylic acid (TCA or Krebs) cycle to produce as much ATP energy as possible, for which the tracheal respiration of insects is certainly essential [7].

The importance of enzymes for controlling insect energy metabolism is widely recognized, so I examined the enzyme activity in the flight muscle of six popular flight insect species to try to elucidate the principal metabolic process [8]. Insects metabolize glucose and trehalose through glucolysis and the TCA cycle to produce energy. During glucolysis, the α-glycerophosphate shuttle producing ATP is very active in flight muscle. Although plasma contains a high concentration of amino acids, it is proline which is one of the major sources for flight energy. The proline cycle, which converts proline to pyruvate using a part of the TCA cycle, produces 13 ATPs [9].

The enzymes that deeply control flight energy metabolism were classified into five enzyme groups: glycolysis, α-glycerophosphate shuttle, proline cycle, TCA cycle and fat hydrolysis (Fig. 4). There was a significantly high correlation between the enzymes in the glycolysis and α-glycerophosphate shuttle, and glycolysis and the TCA cycle, suggesting this is where the major part of glycolysis takes place. The high correlation between the α-glycerophosphate shuttle and TCA cycle suggests they are also linked.

However, the enzymes involved in glycolysis and fat hydrolysis showed a marked negative correlation. From this we extrapolated that they work independently; when energy is mainly supplied by glycolysis, fat hydrolysis is reduced and vice versa. The presence of the two enzymes involved in the proline cycle, proline dehydrogenase (1.5.1.12) (PDH) and lactate dehydrogenase (1.1.1.27) (LDH) correlated closely with the production of pyruvate, and monoacyl glycerol lipase, suggesting a close relationship between pyruvate production and fat hydrolysis. In other words, there is a link between the metabolism of amino acids and fat as energy sources.

	HK	PFK	Cyt GPDH	Mit GPDH	[illegible]	[illegible]	CS*	SDH	IDH	TGL	DGL	MGL	CPT
GP	0.220	0.513	0.626	0.922	0.206	-0.065	-0.278	0.969	0.182	-0.472	-0.537	0.916	-0.505
HK		0.833	0.883	0.458	-0.429	-0.493	0.806	0.838	0.899	-0.393	-0.546	-0.697	-0.519
PFK (Glycolysis)			0.966	0.958	-0.066	-0.064	0.733	0.721	0.758	-0.706	-0.885	-0.189	-0.747
Cyt GPDH				0.812	-0.142	-0.325	0.547	0.917	0.816	-0.558	-0.735	-0.452	0.686
Mit GPDH (α-Glycerophosphate shuttle)					0.198	-0.107	0.043	0.897	0.467	-0.455	-0.605	0.120	-0.587
[illegible]						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]							[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CS								-0.055	0.698	-0.049	0.279	[illegible]	-0.160
SDH									0.958	-0.569	-0.610	1.000	0.677
IDH* (TCA cycle)										-0.481	-0.557	0.500	-0.686
TGL											0.928	-0.545	0.945
DGL												-0.327	0.903
MGL (Fat hydrolysis)													-0.352

(Glycolysis)
GP*: glycogen phosphorylase (2.4.1.1)
HK*: hexokinase (2.7.1.1)
PFK*: phosphofructokinase (2.7.1.11)

(α-Glycerophosphate shuttle)
GPDH: α–glycerophosphate dehydrogenase (1.1.1.8)
LDH: lactate dehydrogenase (1.1.1.27)

(Proline cycle)
GPDH and LDH are in the test

(TCA cycle)
CS*: citrate synthetase (4.1.3.7)
SDH: succinate dehydrogenase (1.3.99.1)
IDH*: isocitrate dehydrogenase (1.1.1.41)

(Fat hydrolysis)
TGL: triacylgrycerol lipase (3.1.1.3)
DGL: diacylgrycerol lipase
MGL: monoacylglycerol lipase
CPT: carnitine palmitoyl transferase (2.3.1.21)

*: rate limiting enzyme.

Fig. 4: Correlations of enzyme activities related to energy metabolism in insect flight muscles

From the origin of life, there is a strong and deep relationship between life and amino acids. Each amino acid is changeable metabolically, and has multiple functions that work independently of each other, but can have synergistic effects when combined in certain situations. When I realized the complex and peculiar combination of the amino acids in VAAM, I knew the effect and function would not simply be the sum of the individual amino acids. I was also confident that the large concentrations of proline and glycine in hornet larval saliva, (especially in *Vespa mandarinia*) could not be a coincidence. I needed a working hypothesis to progress the experiments.

The amino acid engine working hypothesis

We know how hard a diet to reduce body fat is, because of the difficulty to initiate fat oxidation metabolically. In β-oxidation fat hydrolysis only produces acetyl CoA, while, sugar metabolism produces pyruvate, a precursor of acetyl CoA and oxaloacetate. The TCA cycle is started by acetyl-CoA working in conjunction with oxaloacetate and citrate synthetase, the most active rate limiting enzyme among the TCA cycle enzymes. The TCA cycle activation is therefore started more easily by sugar than by fat. In other words, sugar will be burned faster than fat.

Insects usually have very high amino acid concentrations in their body fluids. The proline cycle, like a short cut TCA cycle, is the most common form of energy metabolism in insect flight muscle. The cycle reaction finally produces ATP and alanine, not pyruvate. Therefore, proline cycle alone is not able to complete the cycle reaction to attempt exceptional activity of the flight muscle. The question arises how is the destiny of alanine being accumulated and why VAAM has a high content of glycine. It is possible to make pyruvate from alanine by high activities of transamination and dehydrogenation in an insect flight muscle environment [10]. Alanine glyoxylate transaminase known to be present in

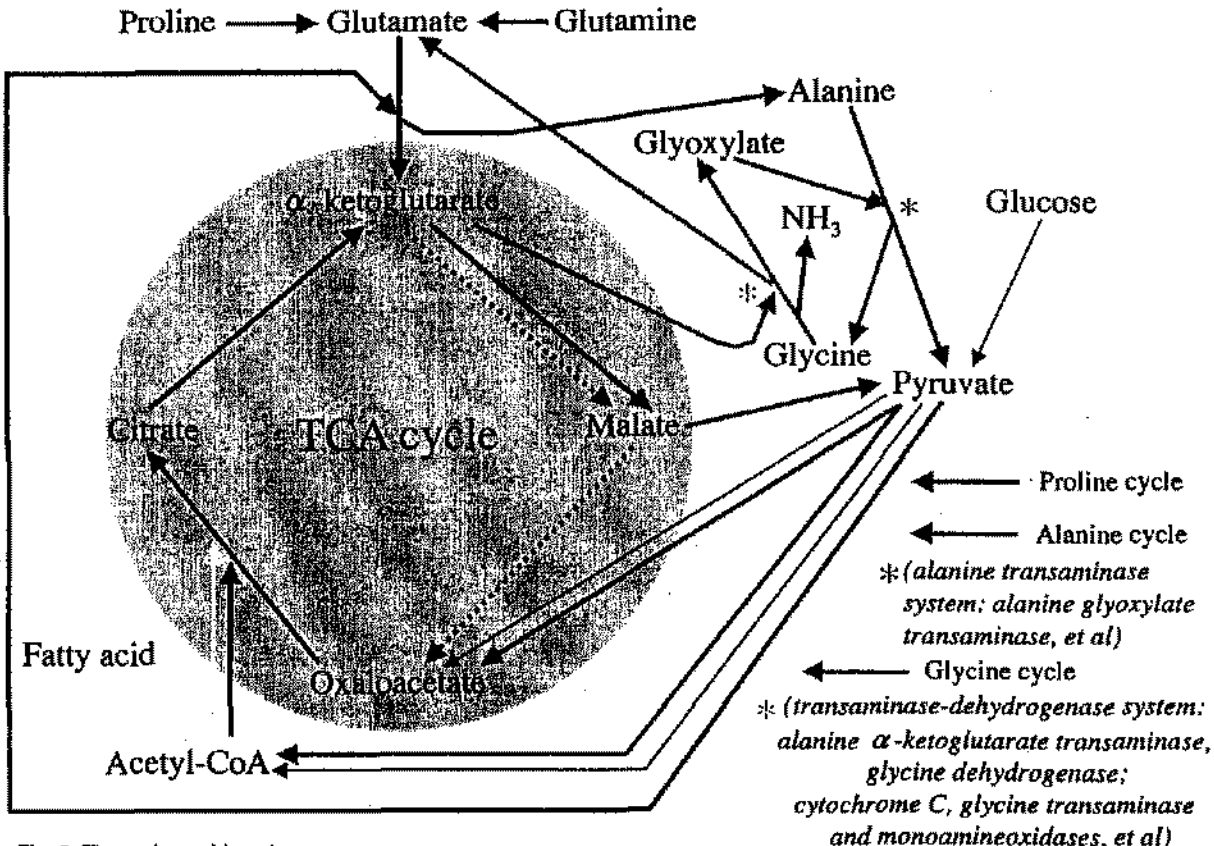


Fig. 5: The amino acid engine

insects is an enzyme that produces pyruvate and glycine, but glyoxylate is also needed. Glyoxylate is commonly produced from glycine by the transaminase-dehydrogenase system in the flight muscle. Glycine α-ketoglutarate transaminase is especially highly active in the flight muscle [11,12,13]. So, a large amount of glycine cyclically makes glyoxylate, then glyoxylate is converted by alanine to glycine and produces pyruvate. Pyruvate changes to oxaloacetate then is incorporated into the TCA cycle with acetyl CoA. The reaction of alanine to pyruvate is coupled with the proline cycle. The enzyme reactions of ther proline cycle, alanine cycle and glycine cycle stimulate and work together in a system named 'the Amino Acid Engine' (Fig.5). This amino acid engine seemed to be a likely trigger for the TCA cycle and was my working hypothesis. It would not only explain the immediate, fast activation in the early period of flight muscle work in flying insects, but also explain the strong correlation between amino acids and fat oxidation. These results explained the large amounts of proline and glycine in VAAM, and indicated potential effects and uses [14].

Swimming mice experiment

This hypothesis would probably be equally beneficial for vertebrates and man, because the energy metabolism systems of insects and man are fundamentally similar. Amino acids, like those contained in VAAM, are basic substances that support all forms of life. A series of swimming experiments were carried out using mice administered with VAAM, distilled water, 10% glucose, 18% casein amino acid mixture (CAAM) or other amino acid compositional mixtures. The mice administered with VAAM consistently swam significantly longer [1] (Fig. 6). Post-exercise tests showed lower concentrations of blood lactate and negligible decreases in blood glucose levels in the mice administered with VAAM or native hornet saliva, compared to all other nutrients [15] (Fig. 7).

Changes in blood non-esterified fatty acid (NEFA) during the swimming exercise were observed with the VAAM mice showing significantly higher levels [16] (Fig. 8). This suggested increased fat oxidation, and lipolysis was confirmed when measurements of the changes of blood ketone bodies, lipase, adrenaline and noradrenaline were also greater in the VAAM mice (Fig. 9).

In the progressive exercise experiments, blood insulin, pyruvate and lactate were also analysed. In the VAAM-administered mice, blood insulin levels were lower and the ratio of pyruvate to lactate was higher (Table 1). Again, this supported the activation of fat oxidation and the amino acid engine, namely activation of the TCA cycle.

VAAM effectiveness for humans

The effectiveness of VAAM for exercising humans was tested in a similar way to the mice, but with a greater variety of exercise activities. In the case of soccer athletes, each subject

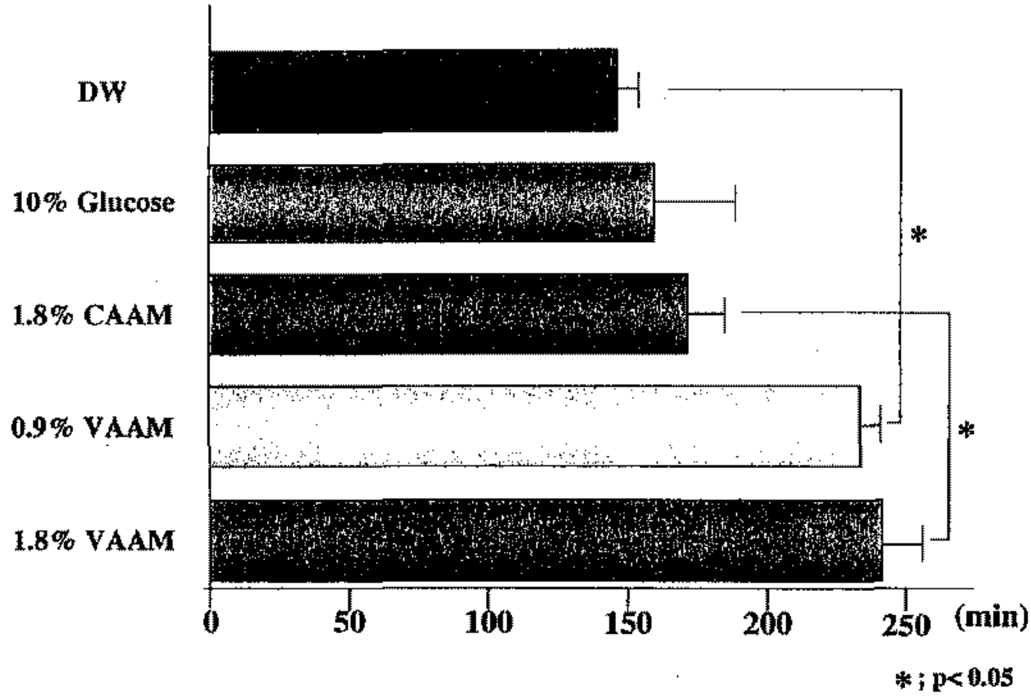


Fig. 6: Swimming time of mice administered VAAM, CAAM, Glucose and Distilled water.

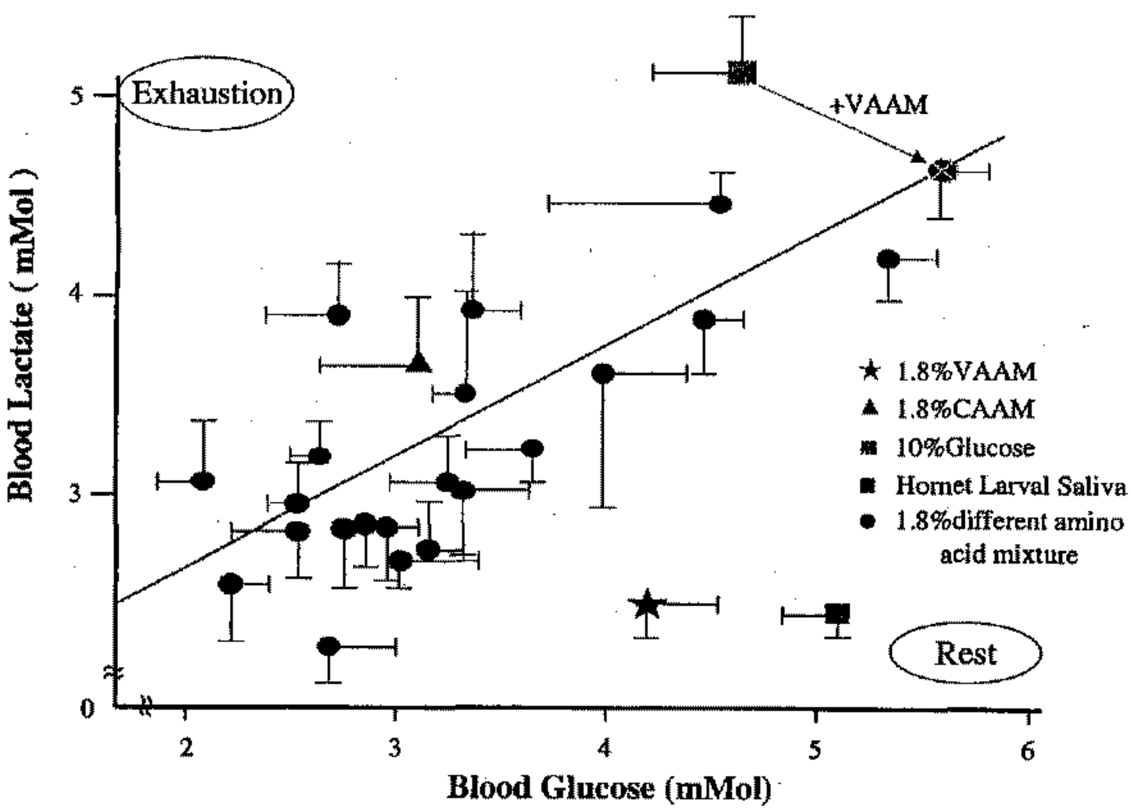


Fig. 7: Correlation between blood lactate and glucose of swimming mice after 30-minute exercise

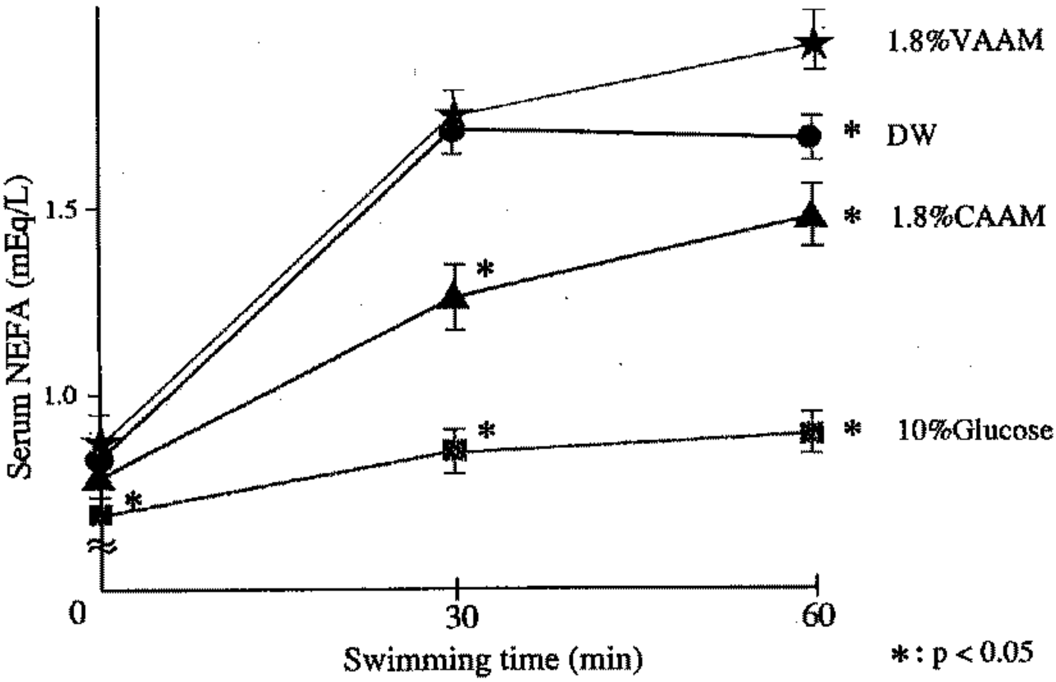


Fig. 8: Changes of serum NEFA in mice during swimming exercise

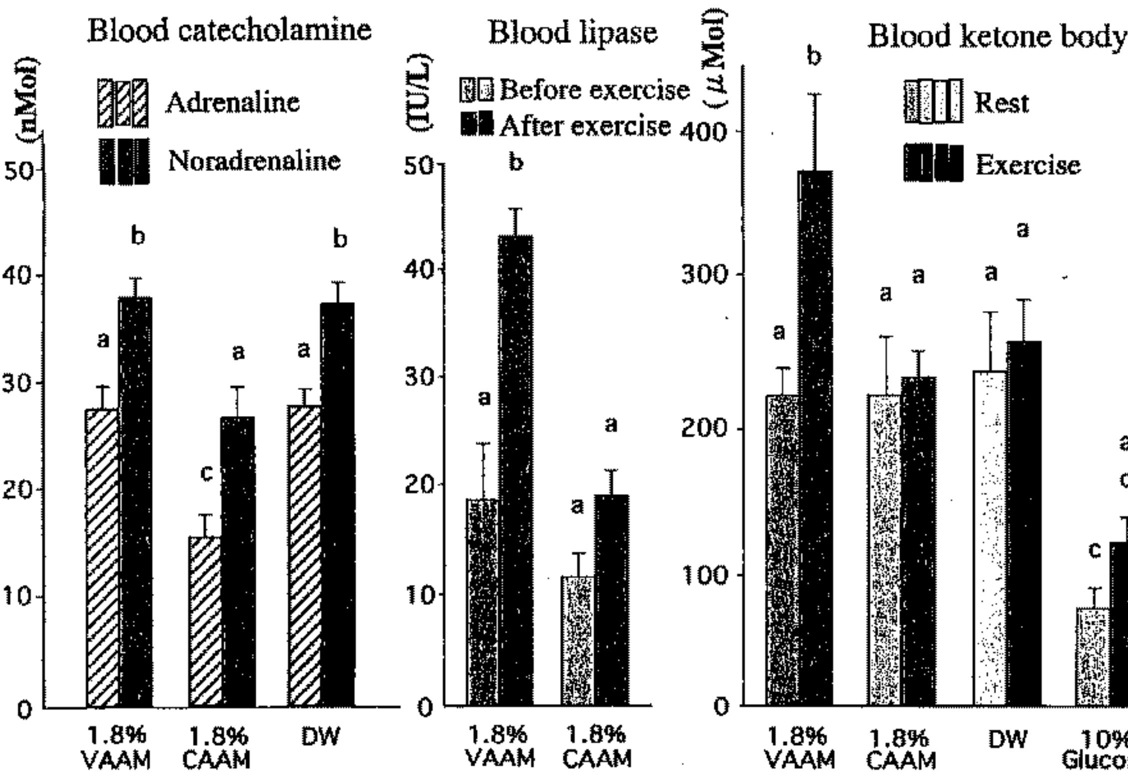


Fig. 9: Changes of catecholamine, lipase and ketone bodies in blood of mice after swimming for 30 minutes

Table 1. Comparison of serum NEFA and insulin, and blood glucose, lactate and pyruvate levels of mice ingesting 1.8% VAAM, 1.8% CAAM, 20% Glucose and DW after 60 min of swimming exercise .

Nutrients	1.8% VAAM	1.8% CAAM	20% Glucose	DW
Serum NEFA (mEq/L)	**1.74±0.17** *a (n=9)	**1.35±0.22** a (n=10)	**1.04±0.09** (n=9)	**1.57±0.18** (n=15)
Blood Glucose (mMol)	**3.98±0.46** *b (n=6)	**2.33±0.48** b (n=8)	**4.81±0.48** (n=6)	**2.76±0.10** b (n=6)
Serum Insulin (μU)	**4.51±0.86** (n=8)	**6.00±1.93** (n=8)	**5.01±0.83** f (n=8)	**3.64±1.35** (n=8)
Blood Lactate (mMol)	**1.02±0.07** *c (n=6)	**1.37±0.13** c (n=8)	**2.12±0.13** c (n=6)	**1.28±0.07** (n=6)
Serum Pyruvate (mMol)	**0.170±0.012** **c (n=11)	**0.135±0.018** (n=10)	**0.256±0.016** e, f (n=8)	**0.142±0.010** (n=9)
Ratio of means Pyruvate/Lactate	**0.167**	**0.099**	**0.121**	**0.111**

n = number of mice
Date of significant differences were compared with reference to 1.8% VAAM.
*a and a, *b and b, *c and c show significant differences ($p<0.05$).
d indicates values after 30min of swimming.
**e and e show a very significant difference ($p<0.01$).
f = 10% glucose.

worked at 1/2 AT loaded ergometric exercise.All the results were similar to the data collected from the animal experiments [17]. Compared to the athletes who were given CAAM, those who were given VAAM exhibited lower respiration and heart rates. Their higher levels of NEFA, ketone bodies [18], blood glucose and blood amino acids indicated similar results to the animal experiments. These results also showed that VAAM prevents an imbalance of amino acid concentration in body fluids (Fig. 10), suggesting VAAM has homeostasis maintenance properties.

Recent studies have shown VAAM has many additional effects, such as improving liver and kidney functions; improved central nervous system response during exercise; helping to retain body heat; improving autonomic nerve function control and so on. These functions probably stem from the synergistic interaction of the complicated amino acid composition.

Lipolytic amino acid mixture was isolated from VAAM composition following the animal experiments. Human subjects administered 3 g of lipolytic amino acid mxture every day for one month exhibited decreases in both body weight and fat at the end of the trial period, without any changes in lifestyle (Fig. 11). Pre- and post-trial blood tests indicated that numerous blood lipid indexes had decreased (Fig. 12), particularly, total cholesterol, high density choresterol and phospholipids. However, partially metabolized fatty acids, NEFA and lipid peroxide, showed a marked increase. These would also have fallen if the subject had exercised. These results suggest that lipolytic amino acid mixture refines fat metabolism, with emphasis on accelerating lipolysis and fat burning.

Manufactured products: VAAM and Diet Amino Acid

For the last 8 years VAAM, the same name as its scientific name, has been marketed by Meiji Milk Products Co. Ltd. as a sports drinks, popular with professional and amateur sports enthusiasts. It is available in many forms, including powder and jelly.

The lipolytic amino acid mixture is sold by Hoshi Chemical Co. Ltd. under

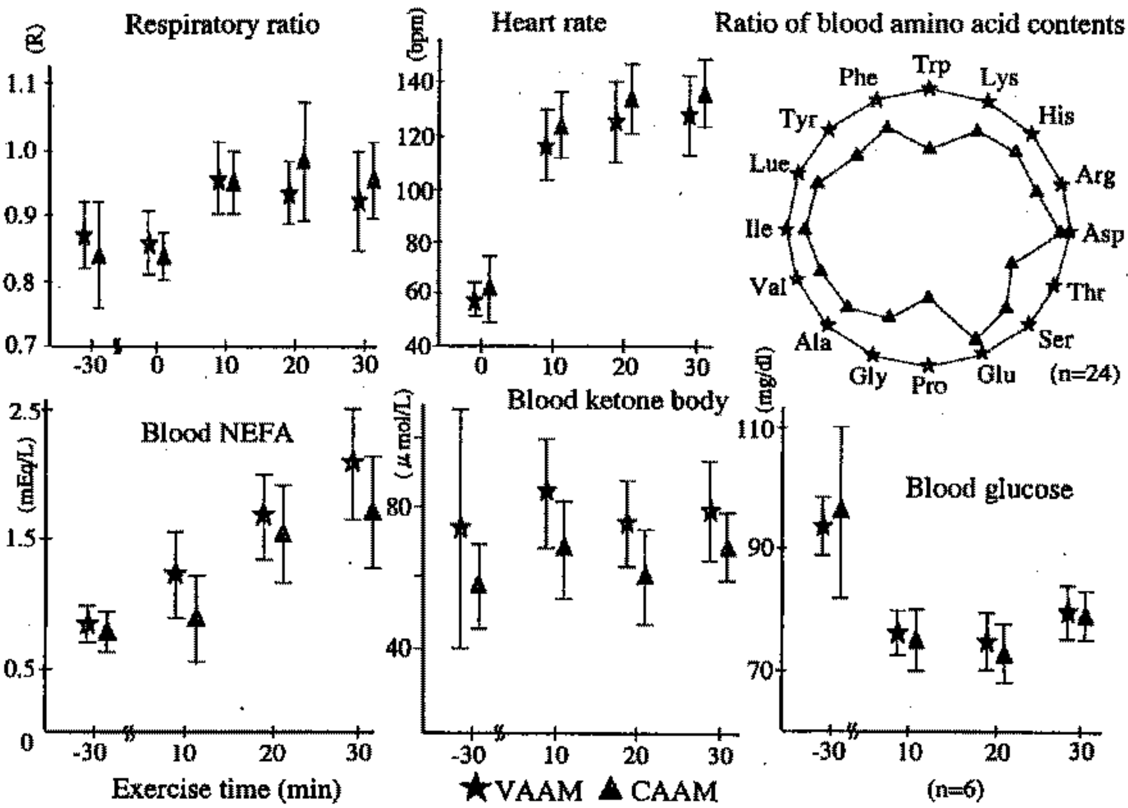


Fig. 10: Changes of respiratory ratio, heart rate, blood NEFA, blood ketone body, blood glucose and ratio of blood amino acid contents of humans during ergometric exercise

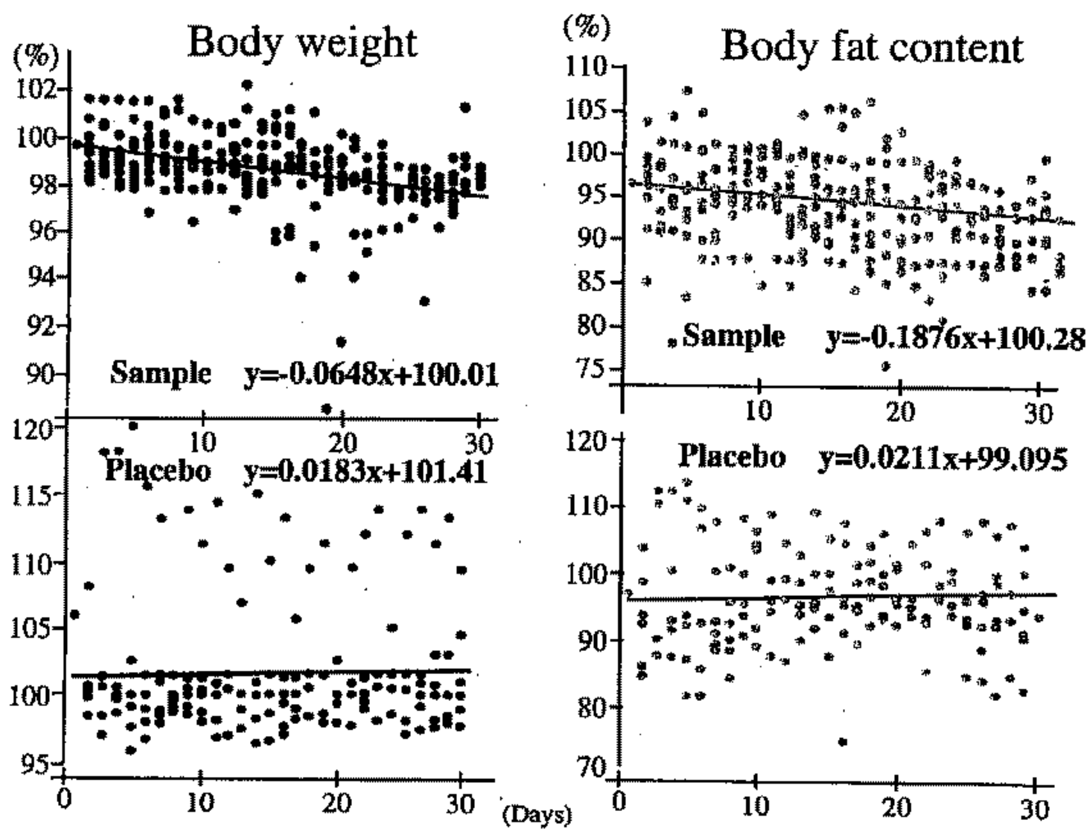
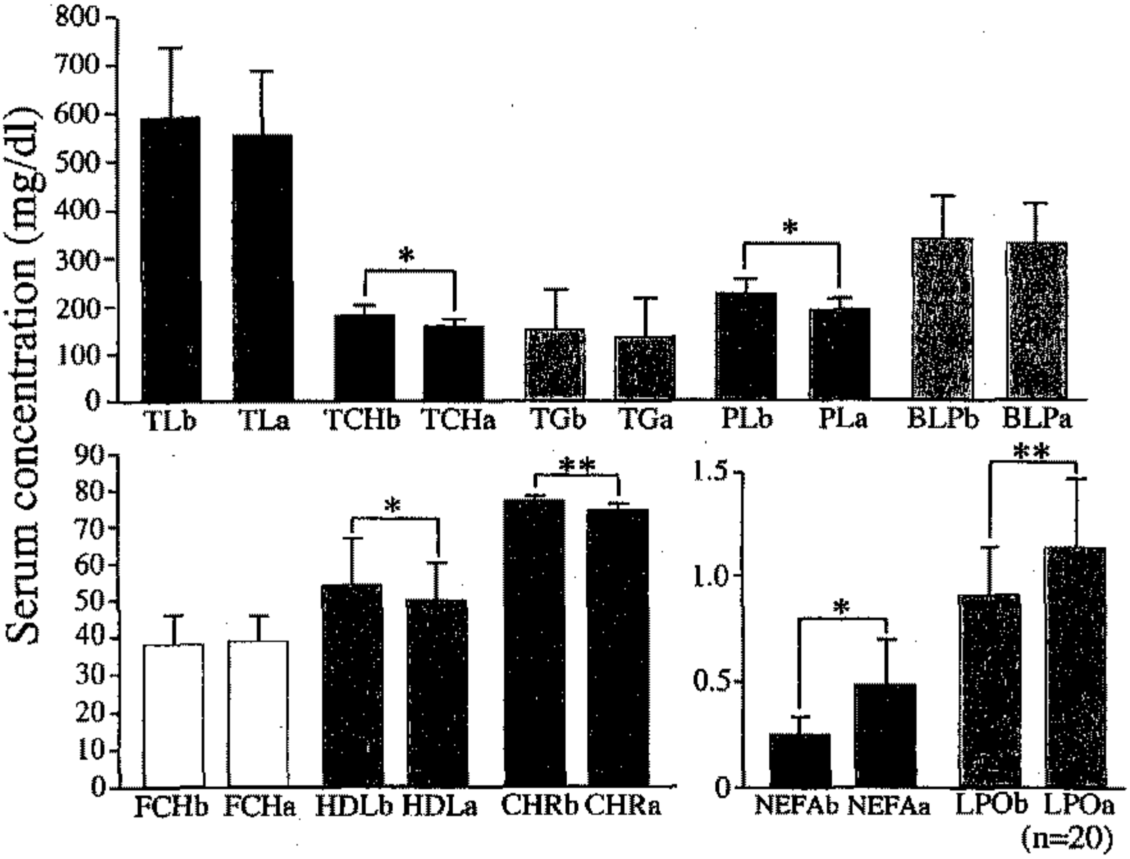


Fig. 11: Changes of body weight and body fat content in humans after continuous administration of Diet Amino Acid for 1 month



TL: total lipid
TCH: total cholesterol
TG: triglyceride
PL: phospholipid
BLP: β-lipoprotein
FCH: free cholesterol
HDL: high density cholesterol
CHR: cholesterol ratio
NEFA & LPO: in the text
b: pre-trial,
a: post-trial
*: $p < 0.05$,
**: $p < 0.01$

Fig. 12: The difference between pre- and post-trials of blood lipid metabolite levels during Diet Amino Acid administration for 1 month

the commercial name, Diet Amino Acid. Together these two products are promoting the popularity of amino acids as multifunctional health foods in Japan.

References

1) Abe, T.: Honey bee science, 16, 1-8 (1995)
2) Abe, T.: Comp. Biochem. Physiol., 99C, 79-84 (1991)
3) Wei-Fogh, T.: Phil.Trans. Roy. Soc. B., 237, 1-36 (1952)
4) Beenakkers, A.M.T.: J. Insect Physiol., 11, 879-888 (1965)
5) Pearson, O.P.: Condor, 52, 145-152 (1950)
6) Zebe,E.: Z.Vergl.Physiol., 36, 290-317 (1954)
7) Weis-Fogh,T.: J.Expt.Biol., 41, 290-317 (1954)
8) Crabtree, B. & Newsholme, E.A.: Insect Muscle, Ed. P.N.R. Usherwood, Academic Press (1975)
9) Sacktor, B. & Wormser-Shavit, E.: J. Biol. Chem., 241, 624-631 (1966)
10) Bursell, E., An Introduction to Insect Physiology, Academic Press (1970)
11) Hansford, R.G.: Biochem. J., 127, 271-283 (1972)
12) Johnson, R.N. & Hansford, R.G.: Biochem. J., 146, 527-535 (1975)
13) Hansford, R.G. & Johnson, R.N.: Biochem. J., 148, 389-401 (1975)
14) Abe, T.: Japanese J. of Mountain Medicine, 23, in press.
15) Abe,T., Takiguchi, Y.,Tamura, M., Shimura, J . & Yamazaki, K.: Japanese J. Physical Fitness Sports Medicine, 44, 225-238 (1995)
16) Abe, T., Inamori, M., Iida, K., Tamura, M., Takiguchi, Y., Yasuda, K.: Adv. Exerc.Sports Physiol., 3, 35-44 (1997)
17) Mizuno, K., Asano, K., Abe, T. & Morishita, K.: Hiro-Kyuyo, 12, 31-41 (1997)
18) Saito, S., Tsuchita, H., Mukai, M. & Abe, T.: Bull. Inst. Health & Sports Sciences Univ.Tsukuba, 24, 71-78 (2001)