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Ingesting Vespa Amino Acid Mixture Improves Plasma Lactic Acid Clearance During Anaerobic Exercise in Juniors

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Abstract: Certain kinds of amino acid may have special physiological function, such as improvement of muscle protein synthesis. While in the present study, a mixture composed of 17 kinds of amino acids in special ratio is confirmed to have benefits on exercise tolerance. This special amino acids composition is identical to the larvae's larval saliva of *Vespa Mandarinia* (Vespa amino acid mixture, VAAM). Adult hornet flies velocities over 30 km/hour all day long taking the larvae's larval saliva as the only food. The VAAM beverage was provided to young athletes with 6000mg amino acids per day for 10 weeks. Oral ingestion of VAAM beverage improved the blood lactate clearance rate, decreased the injury of red blood cell and maintained lower heart rate during exercise, shortened the running time for athletes, increased 6-km jog sweat volume and decreased body fat percentage for athletes. These results by this special amino acids composition is leading to a decreased incidence of sports injuries, which associated to improving the running times and exercise tolerance of young athletes in middle-distance running exercises.

Keywords: Unique Composition of Amino Acids, Anaerobic Metabolism, Lactate Clearance, Self-Controlled Study in Juniors, Red Blood Cell Injury

1. Introduction

The giant hornet, *Vespa 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/hour all day long. Adult hornet are not able to consume solid foods because of their constricted trunks. The meat ball of insects to be preyed on by adult hornets is brought back to the larvae, and as an exchange larvae give a special amino acids mixture to adult hornets as food. This amino acids mixture is analysis to contain 17 kinds of amino acids. The Vespa amino acids mixture (VAAM), which is composed of 17 amino acids (Table 1) that are identical to those found in the larvae's larval saliva, has been reported to increase swimming duration and to accelerate lipolysis and fat oxidation in mice [1, 2]. In clinical experiments, VAAM has been reported to improve exertion ability and aerobic fitness in older women [3, 4].

Table 1. *Amino acids compositions of VAAM.*

	(%)		(%)		(%)
Proline	16.8	Valine	5.6	Tryptophan	3.6
Lysine	12.8	Phenylalanine	5.1	Histidine	3.2
Glycine	11.6	Argnine	5	Serine	2.1
Threonine	6.9	Isoleucine	4.8	Methionine	0.6
Tyrosine	6.9	Alanine	4.4	Asparagine	0.2
Leucine	6.5	Glutamine	3.8	Total	100

Athletes need efficient energy supply during exercise, which is mainly generated by rapidly anaerobic metabolism of muscle glycogen in the early stage; this would followed by the accumulation of lactic acid in muscles and affect muscle performance. Athletes who endure heavy loads of physical training have been reported to suffer from adverse effects, including protein catabolism [5], muscle tissue damage, soreness [6], temporary immune depression and a high risk of upper respiratory tract infections, leads to poor performance in competitive events [7]. Nutritional intervention is a widely used method to counter the negative impacts induced by excessive exercise [8, 9].

The use of amino acids, which play an important role in maintaining a variety of physiological functions including protein synthesis, antioxidant activity, immune responsiveness and body recovery, has rapidly increased over the past few years [10]. In a randomized, double-blind study, Amino Impact®, a commercially marketed energy drink containing branched-chain amino acids (BCAAs), glutamine and other ingredients, increased time to exhaustion and improved subject feelings of focus, energy and fatigue during a moderate-intensity endurance run [11]. In addition to BCAAs, studies using either single or varied mixtures of amino acids have also been conducted. For example, L-glutamine (a nonessential, neutral amino acid) can be used as an oxidizable fuel for muscle and as a precursor for nucleotide synthesis, and it is also a component of GSH, which has been suggested to play a role in enhancing immunity and reducing fatigue [12, 13]. Ohtani et al. reported that when 13 college athletes were asked to maintain a constant training level for 6 months and were supplemented with 6.6 g/day of an amino acid mixture (mainly consisting of 12 AAs) during the 30-day test period, they presented with a significantly improved physical condition and an increase in red blood cell count, hemoglobin, hematocrit, serum albumin, and fasting glucose and a decrease in creatine phosphokinase ($p<0.05$), suggesting that the amino acid mixture increased hematopoiesis and glycogenesis and rapidly alleviated muscle inflammation [14]. This study is the first to evaluate the exercise tolerance effect of VAAM (a mixture of 17 amino acids) on intake on middle-distance runners.

2. Materials and Methods

2.1. Subjects and Preparations

We conducted a self-controlled study of ten juvenile athletes (six males and four females) who were recruited from Shandong Experimental High School (Jinan City, Shandong Province, China). None of the subjects smoked; used ergogenic aids; had any metabolic, cardiovascular, renal, or liver diseases; or suffered from psychiatric disorders. They were required to refrain from any nutritional supplements for 3 weeks preceding and during the course of the study. Additionally, subjects refrained from any other physical exercise during the course of the study, and none of the subjects showed signs of illness or malaise (Trial registration: NCT03121040; retrospectively registered 18th April, 2017, in clinicaltrials.gov.).

2.2. Ethics, Consent and Permissions

After receiving written and verbal explanations of the requirements, each of the 10 subjects provided written consent to participate in the study. The study and its consent form were conducted according to the guidelines detailed in the Declaration of Helsinki, and all procedures involving human subjects were approved by the human subjects ethical committee of the Shanghai University of Traditional Chinese Medicine affiliated Shanghai Shuguang Hospital (reference number 2008-N074-02).

2.3. Design and Procedures

The subjects of this study were instructed to maintain their usual dietary habits in addition to drinking VAAM (two cans every day, 188 ml/can, 3000mg amino acids in one can) for 10 weeks. On the 6 days/week of supervised running tests, participants were instructed to consume one can before their warm-up exercise and the other can while running. During their sessions and without supervision (1 day/week), participants were also instructed to consume one can in the morning after breakfast and the other can in the evening after dinner. The subjects were divided into three groups for the running tests of 1500-meter, 800-meter and 400-meter race. The running exercises began with a 45-min warm-up (leg stretching, etc.), followed by running the specific, designated race.

2.4. Measures

Blood draws via venipuncture were performed by a trained phlebotomist immediately after and 5 minutes following the running tests at three specified time points: the first day of the test session without supplementation (week 0); after 6 weeks (week 6) and 10 weeks (week 10) of consecutive VAAM ingestion. The blood samples were analyzed by an external laboratory (Jinan Yingfeng Medical Examine Center, Jinan, China). In addition, basal body function indices were also collected for observation at week 0, 6 and 10. Data were analyzed using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Anthropometric and Physiological Characteristics of the Subjects

Measurements and statistics were recorded for all ten subjects (6 males and 4 females, 17.1±0.88 yr, 174.9±6.69 cm height, 62.08±1.86 kg weight) before study (Table 2). The calculated BMI, BMR and BFP values were in the normal range. The body weight, BMR (1556.50±54.89 Kcal/kg/min versus 1506.30±54.59 Kcal/kg/min) and BFP (13.72±1.47% versus 10.56±1.21%) of the subjects were substantially decreased after 6 or 10 weeks of ingesting VAAM compared to these parameters at week 0 (Table 2). The amount of 6-km jog sweat volume produced by the subjects increased after the ingestion of VAAM at both week 6 and week 10.

Table 2. *Anthropometric and basal body function indices at the different tested time points. Values are the means±SD; n=10. BMI: Body Mass Index, BMR: Basal Metabolic Rate, BFP: Body Fat Percentage. * Significantly different from 0 week. # Significantly different from 6 week.*

Items	week 0	week 6	week 10
Age (yr)	17.1±0.88		
Height (cm)	174.9±6.69		
BMI (kg/m^2)	20.3±1.21		
Body weight (kg)	62.08±1.86	60.29±1.76*	58.02±2.04*$^{\#}$
BMR (Kcal/kg/min)	1556.50±54.89	1533.50±51.82*	1506.30±54.59*$^{\#}$
BFP (%)	13.72±1.47	12.36±1.45*	10.56±1.21*$^{\#}$
6-km Jog Sweat (g)	480±35.90	535±21.15*	565±15.00*

3.2. Updated Running Time Records

Athletes' running times were recorded at three time points (week 0, week 6 and week 10). There was a downtrend in running time after taking VAAM (124.26±27.45s versus 121.12±27.43s), especially in the 800-meter race (Figure 1).

3.3. Accelerated Blood Lactate Metabolism

As shown in Figure 2 the blood chemistry indices change at different test points. At week 10, the blood lactate levels at 5 minutes after running were significantly lower than those at weeks 0 and 6, and the blood lactate levels 0 minute immediately after running were significantly higher than those at weeks 0 and 6, indicating the improvement of blood lactate clearance rate (1.58±0.64 mmol/L versus -1.31±0.34 mmol/L).

3.4. Decreased RBC Injury During Exercise

The RBC concentration of the athletes was significantly higher at weeks 6 and 10 after the ingestion of VAAM than week 0 (4.49±1.42 $\times 10^{12}$/L versus 4.99±1.58 $\times 10^{12}$/L), indicating a decreased injury in RBC cells by running. Heart rate after running was significantly lower at week 10 than week 0 (155.30±4.02/min versus 138.50±4.05/min), indicating improved heart and pulmonary function. In addition, the results in Table 3 show that the serum levels of CK and LDH at week 6 were substantially increased compared with the levels at week 0.

Table 3. *Blood chemistry and heart rate measured immediately after the running races in weeks 0, 6 and 10. Values are the means±SD; n=10. * Significantly different from 0 week. # Significantly different from 6 week. CK: Creatine kinase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin, CORT: Cortisol, TEST: Testosterone, Glu: Blood glucose, RBC: Red blood cell.*

Items	week 0	week 6	week 10
CK (U/L)	365.94±44.15	576.77±56.44*	333.40±45.88$^{\#}$
LDH (U/L)	234.4±7.57	256.6±10.46*	237.89±9.59$^{\#}$
BUN (mmol/L)	6.07±0.94	5.93±1.11	5.95±1.02
IgG (g/L)	14.81±0.57	13.00±0.46*	13.10±0.43*
IgM (g/L)	1.29±0.13	1.12±0.10	1.22±0.10
IgA (g/L)	2.88±0.34	2.54±0.30*	2.78±0.34$^{\#}$
MCH (pg)	31.34±9.91	28.10±8.89*	28.50±9.01*
MCHC (g/L)	344.90±109.07	313.90±99.26*	319.60±101.07*$^{\#}$
CORT (nmol/L)	732.61±31.61	780.12±30.54*	750.53±43.88
TEST (ng/mL)	2.32±0.64	3.14±1.08	2.77±0.82
Glu (mmol/L)	8.04±0.20	8.16±0.31	8.33±0.26
RBC (10^{12}/L)	4.49±1.42	4.81±1.52*	4.99±1.58*$^{\#}$
Heart rate	155.30±4.02	146.50±4.93	138.50±4.05*

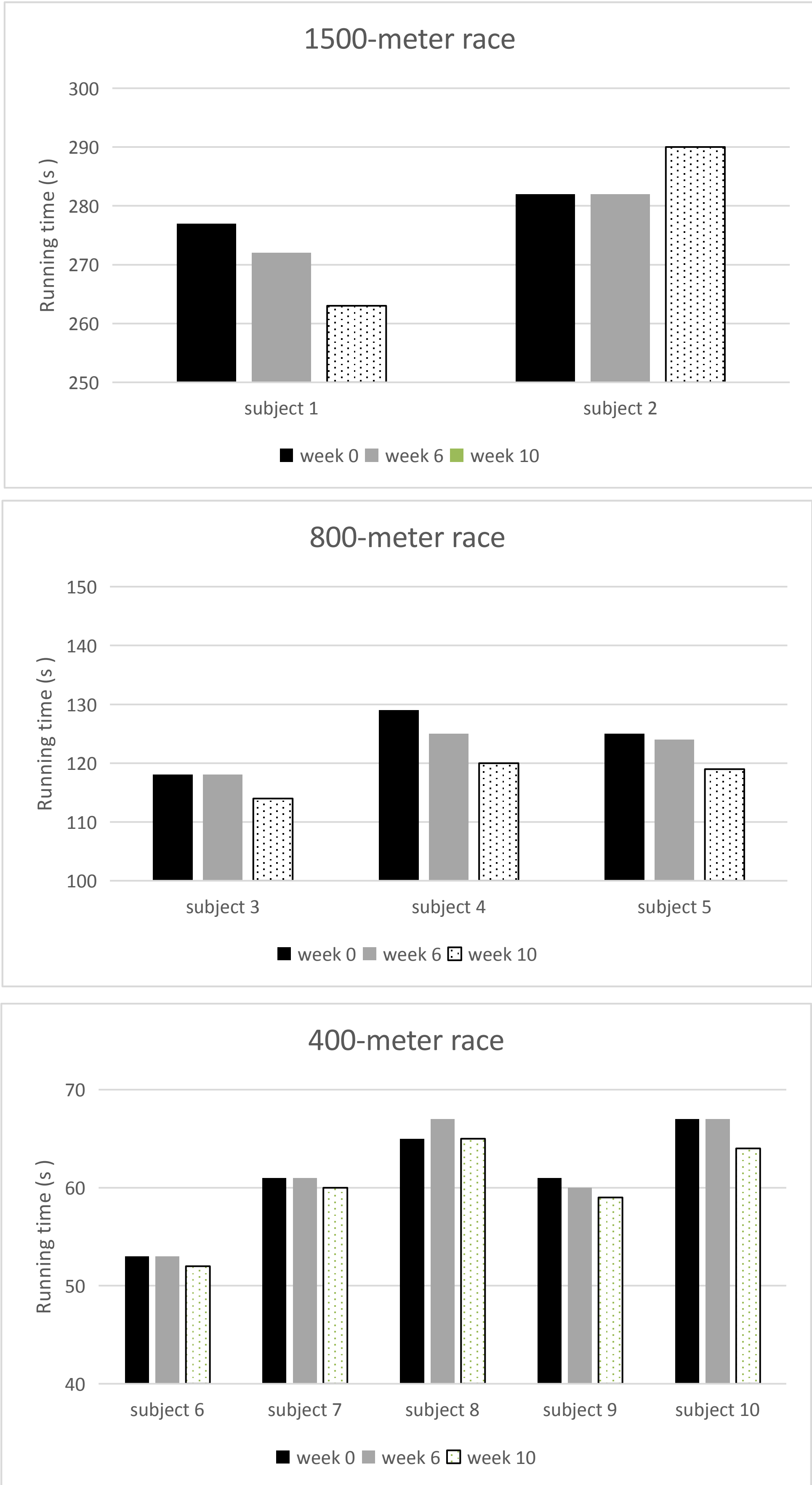

Figure 1. *Running times of each subject measured at different tested points.*

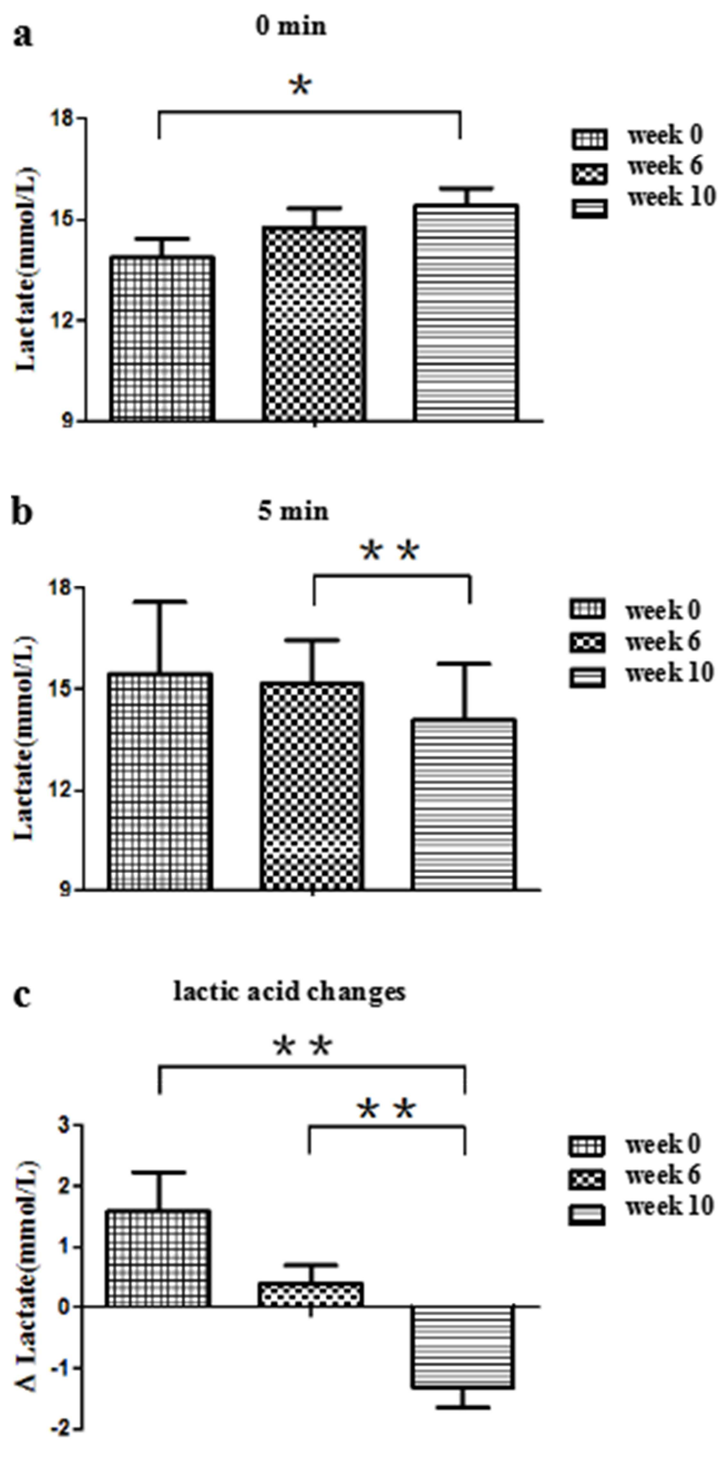

Figure 2. *Plasma lactate values of the ten athletes after exercise. a) Plasma lactate values of the ten athletes immediately (0 min) after exercise. b) Plasma lactate values of the 10 runners 5 min after exercise. c) Changes in lactate. p < 0.05 and < 0.01 are indicated by * and **, respectively.*

4. Discussion

There are two types of muscle in the human body. White muscle has few mitochondria and is rich in glycogen for glycolysis, which produces lactate; in contrast, red muscle is rich in mitochondria and generates energy from lactate (Figure 3). During low-intensity exercise, energy is mainly produced by red muscle through the TCA cycle, which uses the lactate generated in the white muscle. In the TCA cycle, one glucose molecule provides 38 molecules of ATP, but in glycolysis, only 2 molecules of ATP are produced for each molecule of glucose. However, glycolysis generates ATP 100-fold faster than the TCA cycle.

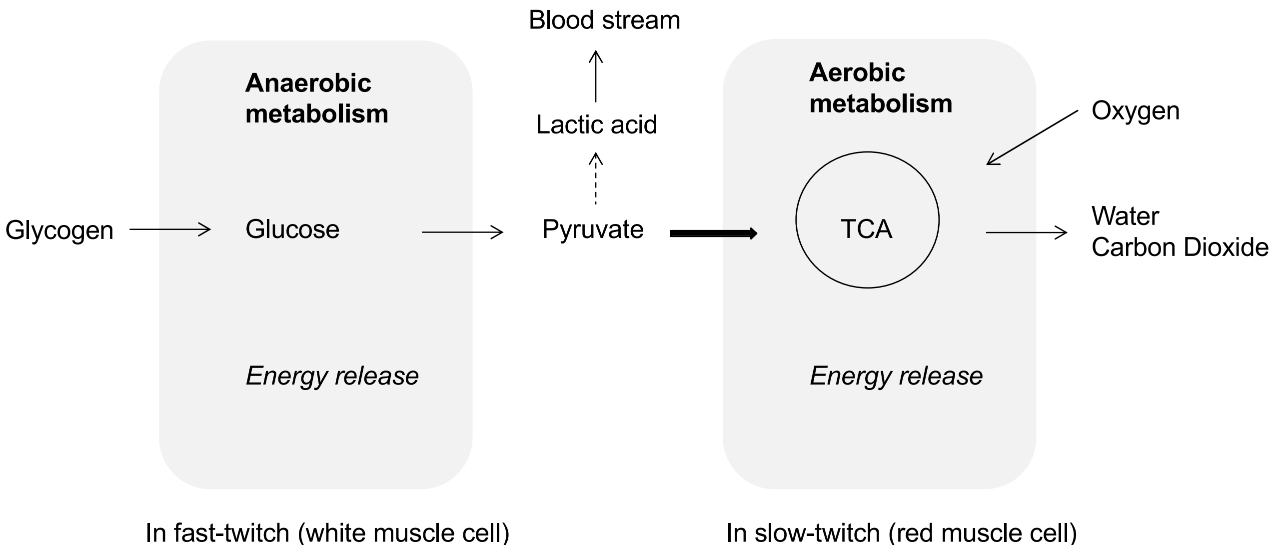

Figure 3. *Energy source of middle-distance athlete. Black bold arrow indicates the significantly accelerated reaction for athletes during exercise after ten weeks intake of VAAM, dotted arrow indicates the significantly decelerated reaction for athletes during exercise after ten weeks intake of VAAM.*

In this study, the blood lactate concentration in athletes after racing exceeded 10.0 mM, confirming that the 400-, 800- and 1500-meter races were anaerobic sports. The difference between lactate levels immediately after and 5 min post exercise was significantly bigger at 10 weeks than at weeks 0 and 6. Lactate accumulation during exercise is thought to facilitate acidosis, suppressing the key enzyme of glycolysis (fructose phosphokinase), impeding the formation of adenosine triphosphate (ATP), and contributing to delayed onset muscle soreness (DOMS) [15]. Therefore, lactate clearance can be used as a parameter to evaluate the efficacy of supplements. The results revealed that the oral ingestion of VAAM enhanced athletes' blood lactate clearance ability, allowing them to more rapidly recover from the fatigue induced by anaerobic metabolism.

A significant increase in RBC count at weeks 6 and 10 and a significant decrease in heart rate at week 10 were observed immediately after running the races, indicating decreased RBC injury and improved heart/pulmonary function. This might indicate that aerobic metabolism was activated by long-term VAAM intake, which might also explain the improvement in lactate clearance by the TCA cycle in red muscle cells. Stored glycogen can be used to rapidly generate ATP, which is useful during high-intensity exercise. In the resting state or during low-intensity exercise, energy is provided by free fatty acids, free amino acids and glycogen, and the proportion of energy from glycogen increases with exercise intensity. In this study, glycogen from the glycolytic pathway was the main source of energy, as discussed above. Free fatty acids may also provide part of the energy and may increase the utilization coefficient of the VAAM beverage. Compared to the week 0 level, BFP was significantly decreased at weeks 6 and 10. Sweat volume after the 6-km running exercise was significantly increased at weeks 6 and 10 compared to that at week 0. Together, the decreased BFP and increased sweat volume might indicate that fat metabolism by aerobic metabolism was improved by VAAM intake.

The serum levels of CK and LDH as well as lactate generally increase after prolonged physical exercise, and they are commonly used as crucial indices of the extent of muscle fatigue and damage caused by the anaerobic metabolism of glucose. Kim et al. showed that compared with the water placebo group, providing BCAAs to subjects resulted in lower levels of CK and LDH [16]. Additional studies have used other AA mixtures during exercise and have observed certain benefits such as immune function [17]. In the present study, VAAM (a mixture of 17 amino acids) was administered to subjects for 10 weeks in combination with consistent exercise 6 days/week, and their CK and LDH levels at week 6 were significantly higher than those detected at weeks 0 and 10; however, no significant difference was observed between weeks 0 and 10. It is assumed that the high levels of CK and LDH at week 6 were due to the continuous, repeated training, which potentially created a "window of opportunity" for the accumulation of fatigue and delay of recovery triggered by VAAM. Totsuka et al. demonstrated that in subjects participating in three consecutive days of exercise consisting of 90 min of bicycling, serum CK activity peaked immediately after the third exercise session compared with the level on the first day [18]. The return of both enzymes to basal levels by week 10 may be due to the extended supplementation of VAAM exerting beneficial effects on the subjects' adaption to exercise, as the improved recovery was partly due to the ATP supplied by aerobic metabolism.

Gleeson et al. reported that with the control group, elite swimmers participating in long-term intensive training showed lower serum levels of IgA, IgM and IgG [19]. However, the results by McKune et al. showed that the levels of IgD and IgM decreased significantly immediately after a race, but the concentration of IgG significantly increased; changes in IgA and IgE were not detected [20]. In the present study, serum levels of IgG were significantly lower at weeks 6 and 10 than those at week 0. IgA was significantly decreased at week 6 compared to that at weeks 0 but recovered completely at week 10. The change in IgM levels was not significant throughout 10 weeks. As mentioned above, it is believed that week 6 was a "window of opportunity" for the accumulation of exercise-induced damage; however, longer-term VAAM supplementation counteracted the negative results caused by exercise by reversing the physiology back to the baseline or even better, as evidenced by the changes in the abovementioned parameters.

5. Conclusion

To our knowledge, this study is the first to evaluate the effect of VAAM® (a mixture of 17 AAs) intake on middle-distance runners. The results revealed that the oral ingestion of VAAM® enhanced the ability of juniors to clear blood lactate, allowing them to more rapidly recover from fatigue induced by anaerobic metabolism, which both contributed to the improved time scores and decreased sports injuries observed in the juniors.

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Authorships

ZSJ and HT designed the study and manuscript preparation. XW and HD carried out subject screening and subject recruitment. HZ assisted in data collection. LY assisted in sample analysis. MY, TK and MO assisted in data analysis. EL, MG and TS wrote the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interests

The authors declare that they have no competing interests.

List of Abbreviations

VAAM: Vespa amino acid mixture, CK: Creatine kinase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen, BCAA: Branched-chain amino acids, ROS: Reactive oxygen species, RNS: Reactive nitrogen species, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, CORT: Cortisol, TEST: Testosterone, Glu: Blood glucose, RBC: Red blood cell, BMI: Body mass index, BMR: Basal metabolic rate, BFP: Body fat percentage, ATP: Adenosine triphosphate, DOMS: Delayed onset muscle soreness.

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