Do aspartate and asparagine acute supplementation influence the onset of fatigue in intense exercise?

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Aim. Oxaloacetic acid represents a fundamental intermediary in the metabolism of energy substrate. Asparagine and aspartate constitute precursor compounds of this substance. Therefore, they could affect tricarbossilic acids cycle. Besides, it was suggested that supplementation with aspartate and asparagine determines a muscular glycogen sparing during strenuous physical exercise, even if the real effectiveness remain controversial. The aim of the present pilot study was to evaluate the hypothesis that a supplementation with oxaloacetate precursors, precisely aspartate and asparagine, could improve sport performance during high intensity endurance exercise.

Methods. We recruited 15 male trained athletes, aged from 20 to 30 years (mean age: 24.13±3.87 years), practicing triathlon. We administered them placebo or aspartate (7 g) and asparagine (7 g) mixture, using a double blind technique, before performing an exhaustion stress test on cycloergometer carried out to 90% of each athlete's maximum oxygen consumption, previously determined.

Results. We evaluated lactatemia through earlobe punctures at the end of warming up, at the maximum effort and at recovery time (3 min, 5 min, 10 min, 15 min, 30 min). Furthermore, subjects were submitted to three blood samples from brachial artery in order to assess the glycemia (before the exercise, at the end of the exercise, and 30 min after the end of the exercise).

Conclusion. The analysis of these parameters and the results of the ergometric tests after amino acids assumption indicate that acute supplementation with aspartate and asparagine do not significantly affect physical performance in athletes practicing high intensity exercises, and that acute administration of aspartate does not cause a sparing of muscle glycogen concentration.

KEY WORDS: Aspartatic acid - Asparagine - Physical fitness - Glycogen synthase.

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Asparagine and aspartate represent the precursors of oxaloacetic acid,[1] fundamental intermediary in the energy substrate metabolism. It was postulated by several authors[2] that an increased availability of these amino acids could be able to implement the oxaloacetic acid concentration and then to affect the cycle of the tricarbossilic acids (Figure 1), leading to a better utilization of fatty acids with consequent glycogen sparing effect.

Aspartic acid is transformed into fumarate in the urea cycle, which in turn, enters Krebs cycle and is converted into malate and later oxidized in oxaloacetate, whereas asparagine is synthesized through a reaction involving the transfer of the amidic nitrogen belonging to glutamine towards aspartate. This route of synthesis, catalyzed by asparagine-synthetase, is present in many mammals and is adenosine triphosphate (ATP)-dependent.

Oxaloacetic acid synthesis represents a funda-

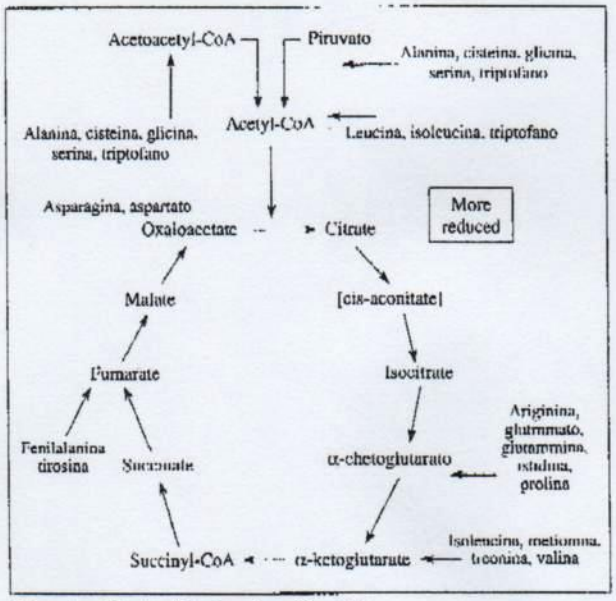

Figure 1.—Cycle of the tricarbossilic acids.

mental step for the regulation of fatty acids oxidation.[3] To confirm the importance of its role, it is worth to remark that in the skeletal muscle high pyruvate carboxylase[3] concentrations have been observed. Such enzyme, stimulated by acetyl coenzyme A (CoA) and ATP, regulates the oxaloacetate synthesis from piruvate.[4]

In addition, pyruvate carboxylase reaches very high concentrations in the muscle during physical activity,[5, 6] playing a key role in the control of Krebs cycle. The condensation of oxaloacetate and acetyl CoA, catalyzed by synthetase citrate, leads to the formation of citric acid, the start point of the Krebs cycle. Therefore, this reaction is essential for oxidation of acetyl CoA, either from pyruvate through pyruvate dehydrogenase or oxidation of fatty acids.

These mechanisms have been observed since the years 60s, when the first studies were carried out, showing an anti-fatigue effect of magnesium (Mg) and potassium (K) aspartate, considered as an oxidizable substratum in the citric acid cycle able to give rise to the oxaloacetate formation.[7, 8]

Other authors proposed that anti-fatigue effect depended on ATP and creatine phosphate sparing role in skeletal muscles, treated with aspartate, through sodium pump regulation.[9]

In the first half of years 90s, some studies confirmed that supplementation with asparagine and aspartate is able to determine a muscular glycogen sparing, supporting the utilization of the free fatty acids during strenuous physical exercise, even if the real results remain controversial.[10]

In a recent study carried out on experimental animals, supplementation with asparagine and aspartate appeared to be able to induce an increase of performance capacity during high intensity exercise led above the anaerobic threshold, with a final less important blood lactate accumulation.[11]

However, some other scientists did not find any significant change in the lipidic and glucidic metabolism and endurance performance in athletes chronically administered with aspartate and asparagine.[12, 13]

At present, there is no definitive evidence that a supplementation with precursors of oxaloacetate, specifically asparagine and aspartate, could affect the performance of athletes practicing endurance high intensity sport disciplines.

The aim of our study was to observe the possible positive effect of acute aspartate and asparagine administration in a selected group of triathletes, comparing performance (test duration) and some physiological (heart rate) and blood (lattatemia, glycemia) parameters before and after the supplementation.

Materials and methods

Subjects

We recruited 15 male trained athletes (age: 24.13±3.87 years), practicing triathlon (training routine: 4 times per week, 2 h session), that previously obtained eligibility to competitive sports. The subjects did not show any noteworthy discrepancy of the anthropometric characteristics (mean height: 171.3±2.58 cm; mean weight: 63.13±2.92 kg) and did not present statistically significant differences of age and maximum oxygen uptake (mean: 63.65 mL/kg/min; standard deviation [SD]: 2.75).

All athletes were previously informed about the purpose of the research and the possible risks before giving their informed consent. They were suggested not to drink coffee or alcoholics and not to eat at least 3 h before the test. They were also recommended not to practice strenuous physical activity and not to smoke in the 12 h preceding the test.

Protocols

MAXIMUM OXYGEN UPTAKE EVALUATION

The selected athletes undersigned the informed consent for maximal ergometric test. Before the beginning of experimentation, all the subjects underwent a maximal stress test to evaluate maximum oxygen uptake, carried out by a triangular incremental protocol utilizing an integrated system with an interfaced electrocardiography and a commercial cycloergometer (CardiO$_2$ Med Graphics).

Maximum oxygen uptake during test was performed with Cosmed K4b^2 metabolimeter that was accurately calibrated before starting the tests.

SUPPLEMENTATION

The athletes were divided into two groups, according to a double blind crossover design. In the first group, 7 g of aspartate and 7 g of asparagine melted in 100 mL of water were administered, while the second group assumed 15 g of placebo in 100 mL of water 1 h before the performance test. After a week, all the 15 subjects repeated the test according to the protocol. The ones that in the first test assumed placebo were administered with 7 g of asparagine and 7 g of aspartate mixture, and those who were administered with amino acids assumed placebo. Thus, each athlete underwent two performance tests.

PERFORMANCE TEST

The performance test consisted in a short phase (5 min) of warming up and a maximal stress test until exhaustion on cycloergometer carried out to 90% of the maximum oxygen consumption of each athlete, previously determined.

BLOOD MEASURAMENTS

Blood sampling was carried out from earlobe puncture in order to determine lactatemia using the lactate analyzer Miniphotometer 8 Dr. Lange during the following phases: 1) before starting; 2) at the end of warming up; 3) at the maximum effort; 4) in the recovery (3 min, 5 min, 10 min, 15 min, 30 min).

Furthermore, three blood samples were taken from the subjects' brachial vein to evaluate the glycemia: 1) before the exercise; 2) at the end of the exercise; 3) 30 min after the end of the exercise.

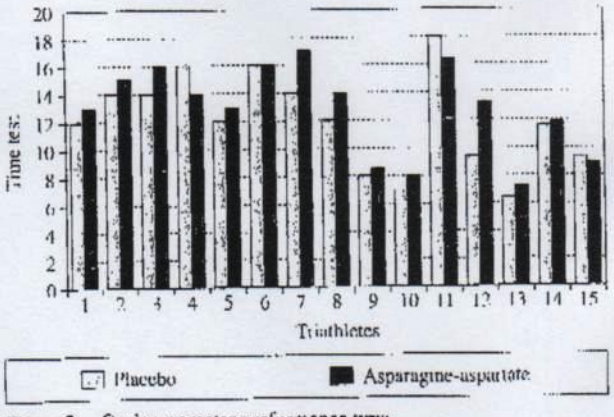

Figure 2.—Cycloergometer performance time.

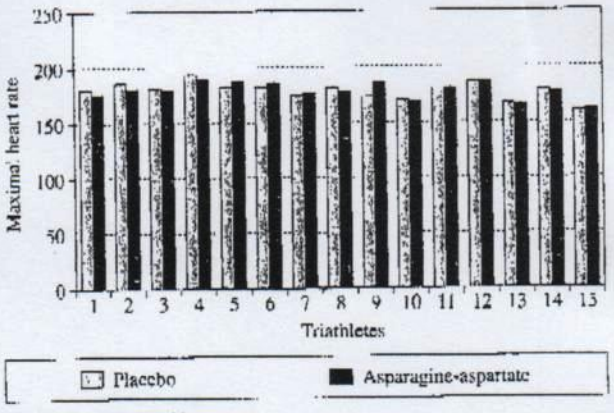

Figure 3.—Maximal heart rate

Statistical analysis

All data were evaluated as mean and SD. SPSS v14 software was utilized for statistical analysis. The differences of the experimental condition values were tested by ANOVA for repeated measure. Differences with a P value <0.05 were considered statistically significant.

Results

Test duration

In test duration, all athletes did not show statistically significant changes. In particular, the mean value in the subjects that assumed placebo was 11.9±3.4 min and it remained almost the same after supplementation (12.8±3.2) (Figure 2).

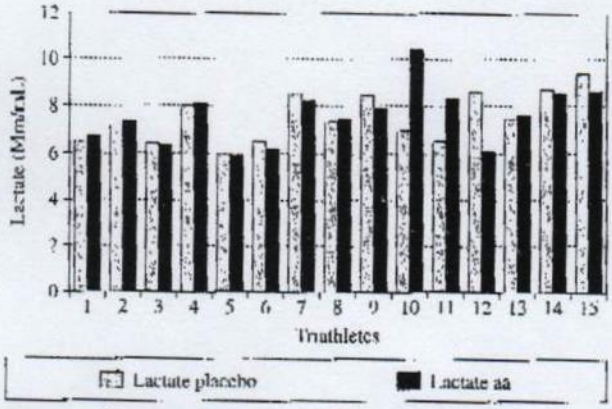

Figure 4.—Lactate peak.

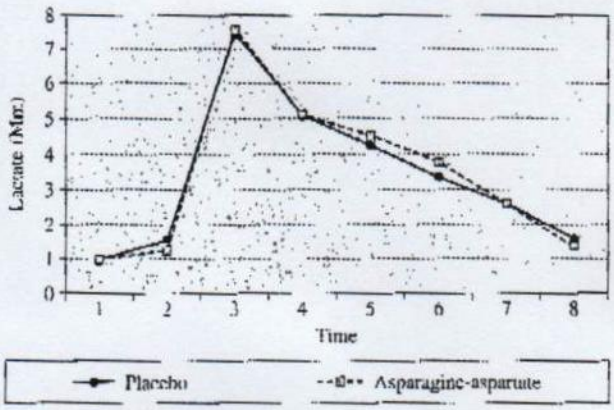

Figure 5.—Lactate trend.

Heart rate

At rest and after exercise, heart rate did not evidence a statistically significant change. In particular, mean basal cardiac frequency was 64.2±8.4 bpm in absence of asparagine and aspartate, and 63.4±9.8 bpm after the administration of amino acid. Even exercise peak heart rate did not vary significantly (178.9±6.6 bpm before supplementation and 179.4±6.5 bpm after supplementation) (Figure 3).

Lactate

Triathletes did not have a statistically significant variation of lactate concentration. In particular, after supplementation, it was detected a blood lactate concentration of 7.5±1.2 mM, while after placebo assumption it was 7.4±1 mM (Figures 4 and 5).

Glycemia

No statistically significant change was observed in athletes' glycemia both postexercise and during recovery time. Glucose concentration had a physiological trend during the test with a progressive drop of values and a gradual return to basal values after its end. In particular, average basal glycemia was 74.46±5.2 mg/dL after placebo administration and 75±5.3 mg/dL after asparagine and aspartate. The average glycemia at the end of ergometric test was respectively 68.3±4.2 mg/dL and 70.06±4.8 mg/dL. Finally, in the recovery time, we measured 71.06±4.2 mg/dL in the placebo group and 72.8±4.5 mg/dL in the supplementation group (Figure 6).

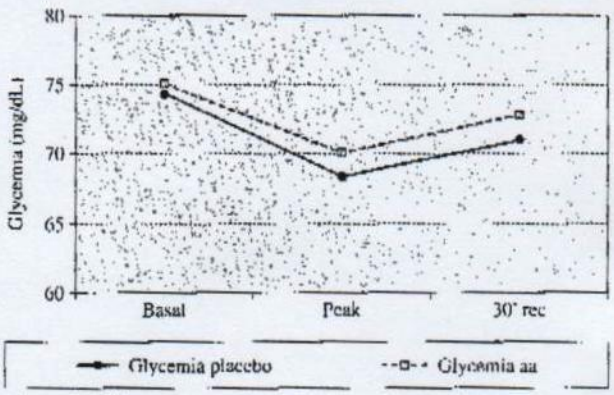

Figure 6.—Glycemia.

Discussion and conclusions

Recently, the possible effects of two amino acids, aspartate and asparagine, on athletic performance attracted the interest of researchers. In particular, early studies suggested that aspartate and asparagine may delay, in some way, glycogen depletion in muscles, prolonging the athlete's ability to exercise.[14] It was also proposed that aspartate and asparagine may help to maintain blood levels of tryptophan under control during prolonged exercise, also reducing mental fatigue.[3]

L-aspartate, especially the potassium magnesium aspartate salt, leads to a sparing of muscle glycogen stores increasing fatty acids oxidation and/or promoting a faster rate of glycogen resynthesis during exercise. In fact, aspartate and asparagine are rapidly

transaminated into muscle fibers, leading to the formation of oxaloacetic acid, which is a key component of the Krebs cycle, and represent a limiting factor in the control of fatty acids oxidation. With regard to this, De Haan *et al.* observed that L-aspartate can enhance short intensive exercise serving as a substrate for energy production in the Krebs cycle and stimulating the purine nucleotide cycle.[15]

Moreover, it was suggested that an increased concentration of aspartate and asparagine would enhance the activity of the malate-aspartate shuttle, whose purpose is to transport hydrogen ions from the cytoplasm of muscle cells into their mitochondria, where the hydrogens become involved in the aerobic production of ATP. This could explain the supposed performance improvement associated with aspartate-asparagine supplementation.

Conversely, the data we analyzed demonstrate the absence of statistically significant differences between the athletes who received supplementation of aspartate and asparagine and those of the control group. Therefore, we confirmed the hypothesis that acute supplementation with aspartate and asparagine do not significantly affect physical performance in athletes practicing high intensity exercises, and that acute administration of aspartate does not lead to a sparing of muscle or liver glycogen.

In early studies, Trudeau *et al.* did not observe any variation, after supplementation, in the content of muscle and liver glycogen of control rats after a 60-min swim to exhaustion.[10] In addition, some recent studies[12, 13] that evaluated chronic intake of aspartate and asparagine on endurance performance, did not show neither any increase on performance nor any influence on metabolic or endocrine parameters. Thus, we can state that the data at our disposal do not allow to draw definitive conclusions about the role of asparagine and aspartate on the athletes' performance.

A limit of our study could be represented by the small number of subjects evaluated. So, in order to obtain definite results about the possible effectiveness of amino acids supplementation, it could be advisable to enlarge the number of subjects, and to also further investigate on chronic administration of aspartate and asparagine to completely focus on their metabolic impact during strenuous exercise.

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