


# White Paper on the Effect of an Immune-Supporting Supplement, ASEA, on Athletic Performance based on a Pilot VO<sub>2</sub>max Test

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## Description:

This is a technical paper describing the pilot study used to measure the possible effects of an immune-supporting supplement on athletic performance as measured by a standard VO<sub>2</sub>max and Ventilatory Threshold (VT) athletic endurance test.

## Objectives of Study:

The objectives of the pilot study are to (1) confirm the general observation that an immune-supporting supplement, ASEA™, has an effect on athletic performance and (2) determine the specific physiological parameters: Heart Rates (HR), volume of O<sub>2</sub> inspired (VO<sub>2</sub>), volume of CO<sub>2</sub> expired (VCO<sub>2</sub>), volume of expired gas (VE), Respiration Rate (RR), Respiratory Exchange Ratio (RER), Aerobic Threshold (AeT), Anaerobic Threshold (AT), VO<sub>2</sub>max and Ventilatory Threshold (VT) that are affected by oral ingestion of this supplement during both the aerobic and anaerobic phases of exercise.

## Theory Overview

The immune-supporting supplement, ASEA™, contains a balanced mixture of Redox Signaling molecules that purportedly increases the efficiency of the communication channels between cells, enabling faster response of the immune system and cellular healing activities. Enzymes in the body also break down these Redox Signaling molecules into salt water and nascent oxygen.

There are two proposed mechanisms involving Redox Signaling that can affect athletic performance, (1) increased efficiencies in cellular absorption or use of oxygen, prolonging aerobic metabolism, and (2) more efficient processing of lactate energy stores and tissue repair mechanisms, prolonging anaerobic metabolism.

During physical activity, the increased power requirements from muscle tissues require increased metabolism of available energy stores. Sustainable aerobic metabolism of sugars can supply this energy demand as long as there is an adequate supply of oxygen and sugars in the blood. As energy demands exceed the ability of the respiratory and

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cardiovascular system to deliver sufficient oxygen to the muscle tissue, methods involving the anaerobic metabolism of carbohydrates, creatines, pyruvates, etc. start to become prevalent.

Anaerobic metabolism supplies the excessive demand for energy but is accompanied by the production of CO<sub>2</sub> and lactates. Prolonged or excessive anaerobic metabolism depletes the available energy stores faster than they can be renewed; the buildup of CO<sub>2</sub> and lactates can also interfere with aerobic metabolism and thus, when the energy stores are spent, exhaustion will result.

Since anaerobic metabolism is marked by an excess in CO<sub>2</sub> and lactate production, it can be monitored by measuring the excess CO<sub>2</sub> exhaled during exercise or the buildup of lactates in the blood. The Ventilatory Threshold (VT) is the point where the excess CO<sub>2</sub> is first detected in the expired breath; it is related to the point at which anaerobic metabolism is starting to become prevalent.

In this pilot study, VT was determined graphically from the VCO<sub>2</sub> vs. VO<sub>2</sub> graph. VCO<sub>2</sub> is the volume of CO<sub>2</sub> expired per minute and VO<sub>2</sub> is the volume of O<sub>2</sub> inspired per minute. VO<sub>2</sub>max is simply the maximum volume of O<sub>2</sub> inspired per minute possible for any given individual. VO<sub>2</sub>max is measured in ml/kg/min (milliliters of O<sub>2</sub> per kilogram of body weight per minute). VO<sub>2</sub>max is measured at the peak of the VO<sub>2</sub> curve. The Aerobic Threshold (AeT) was determined by the software and indicates when fat-burning metabolic activities start to be dominated by aerobic metabolism. The Anaerobic Threshold (AT) was also software-determined and marks the point where the anaerobic metabolism starts to completely dominate (usually close to exhaustion).

## Recruitment Methods:

A standard VO<sub>2</sub>max test was run on 18 athletes who responded to recruitment flyers posted in athletic clubs and to invitations extended to a local competitive Triathlon team. The participants were selected based on answers from qualification questionnaire which affirmed that they:

1. Perform a rigorous physical workout at least five hours per week on average.
2. Have no medical conditions that might prevent participation
3. Agree to follow diet and hydration instructions.
4. Will perform only normal daily routines during the study.
5. Have no history of heart problems in the family.

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The final selections were athletes of a caliber much higher than the expectations reflected in the recruitment flyers, a majority being athletes involved in regular athletic competitions. All of the participants had never taken the supplement prior to the study.

The participants did not receive any monetary compensation, but did receive a case of product and results from the VO2max tests.

## Experimental Methods:

The VO2max testing was done at an athletic club by accredited professionals holding degrees in exercise physiology and with more than 10 years daily experience in administering VO2 tests. The participants were given a choice of performing the test on either a treadmill or a stationary cycle. A CardioCoach® metabolic cart measured heart rate (HR), inspired and expired gases (VO2, VCO2, VE) and recorded weight, height, age, and body mass indexes (BMI). Power settings on the treadmill or cycle were recorded every minute.

Each participant was scheduled to take two VO2max tests, (1) a baseline test and (2) a final test. The baseline test was performed before any supplement ingestion. The participants drank 4 oz. of the supplement per day between the baseline test and the final test (7 to 10 days later) and drank 8 oz. of the supplement ten minutes before starting the final test. For the baseline test, the power settings on the cycle or treadmill were determined by the test administrator. The power settings for the final test were matched exactly to the power settings of the baseline test for each participant. Participants were encouraged to strictly maintain their regular diet and exercise routine and to come to each test well hydrated (at least 8 oz. of water in the last 2 hours before each test).

Each participant was fitted with a breathing mask and heart monitor. Each VO2max test consisted of a 10 min. warm up period where participants walked or cycled at a low power setting determined by the administrator. This was followed by a ramp up period, where the administrators increased the power settings every minute, according to their evaluation of the physical condition of the participant, and termination when the administrators started seeing the indications of a maximum VO2 reading when RER ( $VCO_2/VO_2$ ) > 1.0 or at the administrator's discretion. The administrators had ample experience in obtaining consistent VO2max results on this equipment, estimated at about 6% test to test variation over the last 5 years.

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## Data Analysis Methods:

The raw data (HR, VO<sub>2</sub>, VCO<sub>2</sub>, VE, Power Settings) were collected from the CardioCoach® software for analysis. Data points were automatically averaged over 15 to 25 second breath intervals by the software, VO<sub>2</sub>max is also determined by the software with an averaged VO<sub>2</sub> peak method. VT was determined graphically from the slope of the VCO<sub>2</sub> vs. VO<sub>2</sub> graph.

Linear regression methods were used to determine the slope, change in VCO<sub>2</sub> over change in VO<sub>2</sub>. In theory, when aerobic metabolism switches to anaerobic metabolism, the volume of CO<sub>2</sub> expelled (VCO<sub>2</sub>) is increased in proportion to the Volume of O<sub>2</sub> inhaled (VO<sub>2</sub>). This is reflected as an increase of slope on the VCO<sub>2</sub> vs. VO<sub>2</sub> graph, seen as a clear kink on the graph around the VT point. Linear regression was used to determine the slope both before VT and after. Slopes were determined by linear regression on the linear region of data points before and after VT point, excluding points surrounding the VT and near VO<sub>2</sub>max. The intersection of the before and after lines was used to determine the reported VT point.

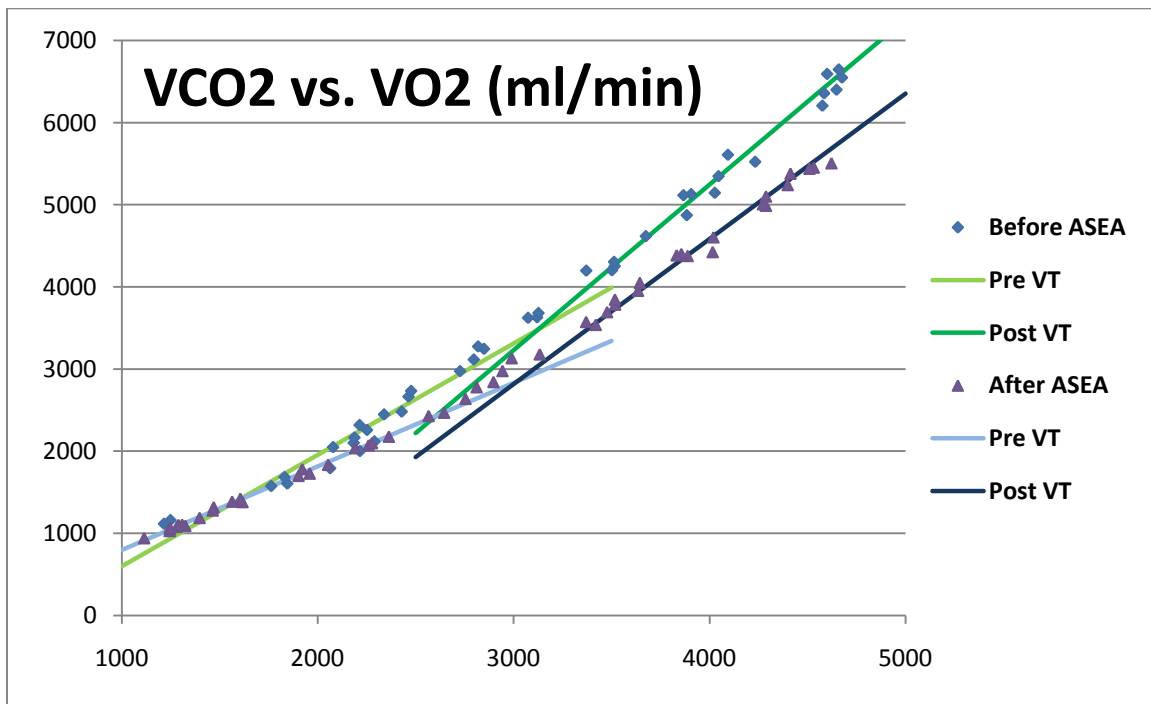


Fig. 1 - Typical data set displaying graphical analysis methods used to determine Ventilatory Threshold (VT)

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Methods for determining the VT point on any individual participant were kept consistent from the baseline test to the final test. Average HR was averaged over the linear range of HR increase during the power ramp, excluding points a few minutes into the beginning and before the end of the data set. In every case, the same data analysis methods were used for the final test as were used for the baseline test for each participant.

## Study Results:

Compliance to protocol was very high by both participants and administrators, based on answers to compliance questions. One data set was discarded for low VCO2 values, probably due to a loose mask. The ventilatory data for this one participant was rejected, leaving 17 valid data ventilatory data sets. The Heart Rates (HR), however, were compared for all 18 participants.

Total Participants	Average Age	Male/ Female	Average Weight (Kg)	Cycle/ Treadmill	Average BMI	Data Sets Selected
18	41±9	16/2	76±11	7/11	24.4±3.4	17

Table 1 - Average Profile of Participants

The average VO2max reading over all participants (N=17) was measured at the relatively high value of 62.5 ml/kg/min, indicative of the quality of athletes in the sample. Only four participants had VO2max readings below 55 ml/kg/min; these four were not involved in competitive training programs.

The data shows that two significant changes in physiological parameters could be attributed to ingestion of the supplement, as determined by a statistical paired t-test analysis. The average time taken to arrive at VO2max was increased by 10% with very high confidence (P = 0.006) and the average time taken to arrive at Ventilatory Threshold (VT) was increased by 12% with a marginal level of confidence (P = 0.08).

Given that the power ramp-up-points between the baseline and final test for each participant were identical, an increase in the amount of time to obtain VO2max and VT on the final test also indicates a higher average power outputs at such thresholds. Calibrated power output measurements were not available, however, the test administrator for the final test, upon reaching the maximum power recorded for the baseline test, regularly surpassed this maximum power before the participant reached VO2max on the final test.

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All other physiological parameters (VO2max, VT, AeT, AT, Start HR, HR at AeT, HR at AT, HR at VO2max, and overall average HR) were not significantly changed by supplement ingestion. The high level of consistency between the baseline and final test for these parameters, however, supports the repeatability of the tests. The test to test repeatability has an estimated standard deviation of less than 5% for all parameters.

Averages (N=17)	Baseline	Final	Change	%Change	P-Value
VO2max (ml/kg/min)	62.5	63.6	+1.1	+2%	-
VT (ml/kg/min)	36.4	38.7	+2.3	+6%	0.34
Aerobic Thresh. (AeT)	43.6	43.8	+0.2	+0%	-
Anaerobic Thresh. (AT)	55.5	56.5	+1.0	+2%	-
Pre VT Slope of VCO2/VO2	1.030	1.030	0.0	0%	-
Post VT slope of VCO2/VO2	1.997	1.944	-0.053	-2.7%	-
Start Heart Rate (bpm)	87.4	85.9	-1.5	-1%	-
Heart Rate at AeT	147	145	-2	-2%	-
Heart Rate at AT	165	165	0	0%	-
Heart Rate at VO2max	174	175	+1	+1%	-
Heart Rate Overall	137	134	-3	-2%	-
Time to VT (secs)	306	344	38	+12%	0.08
Time to VO2max (secs)	639	703	64	+10%	0.006

Table 2 - Table of Results (P < 0.05 is deemed significant)

Of the 17 participants in the study, 70% of them experienced a significant increase in time to VO2max, 18% of the participants showing more than a 25% increase, 41% showing more than a 10% increase, 18% of the participants exhibiting no significant change and 12% showing a mild decrease (under 10%).

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There was a moderate but significant correlation between the increases in "time to VO<sub>2</sub>max" and "time to VT" (correlation coefficient 0.35), meaning that an increase in time to reach VO<sub>2</sub>max was moderately but not always proportional to the increase in the time it took to get to VT. There is a strong correlation between increase in time to VO<sub>2</sub>max and decrease in the average overall heart rate (correlation coefficient -0.67), meaning that an increase in time to VO<sub>2</sub>max would most often be accompanied by a decrease in average overall heart rate.

## Study Conclusions

Ingestion of the test supplement, ASEA™, for 7-10 days prior to and immediately before a VO<sub>2</sub>max test, was shown to significantly increase the time it took for 70% of the participants to reach VO<sub>2</sub>max under equivalent carefully regulated power ramp-up conditions. Time to VT likewise was significantly extended.

The extension of time to reach VT, under similar increasing demands for energy, is a direct indication that the aerobic phase of metabolism is being extended and/or the anaerobic phases somehow are being delayed as the demand for energy increases.

The lack of any other changes in the physiological parameters (VO<sub>2</sub>max, VT, AeT, AT and associated heart rates) suggests that cardiovascular capacity, lung capacity and blood oxygen capacity and regulation are not affected. This assumption is reasonable, given that the short duration of this study excluded the possibility of training effects.

One feasible explanation for the results lies in the enhancement of aerobic efficiencies, meaning that more aerobic energy can be extracted at the same physiological state, or that the clearance of lactates or CO<sub>2</sub> becomes more efficient, again allowing greater aerobic efficiency. Note that "time to AeT" and "time to AT" were not compiled in this study, however changes in these parameters would be expected and might offer clues to determine the underlying mechanisms.

The results of this pilot test indicate that there is a strong case for athletic performance enhancement and further investigation is warranted. A placebo-based double-blind test, measuring the more subtle effects in ventilation and heart rates along with increases in blood lactate levels during a controlled, calibrated power ramp would provide defensible evidence for this effect and better support for some specific underlying mechanisms of action.