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The Acute Effects of Beta-Alanine on Blood Gas of Athletes after Maximal Exercise.

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ABSTRACT

This study aimed to examine the effect of acute beta-alanine consumption after maximal exercise on blood gas responses. 18 volunteers, male athletes, participated into the study. Athletes were divided into two groups as experiment (beta-alanine) (n=9) and placebo (n=9). After maximal exercise, beta-alanine supplement was given to the experimental group while the placebo group received placebo. Blood was taken from the athletes three times as basal, post-exercise (PE) and 2 hours after ingestion supplement (PS); blood gas values have been analyzed. Statistically significant differences in many blood gas parameters were found when comparisons of in-group basal, PE and PS time courses of two groups were reviewed ($p < 0.05$). But, there was no significant difference between the basal and PS levels of any parameters in the beta-alanine group although there were significant differences among the PE, basal and PS values as a result of beta-alanine supplementation. When all inter-group comparisons of all findings obtained were considered, a significant difference was not detected at any time periods ($p > 0.05$). It can be said that the beta-alanine supplementation after maximal exercise had positive effects on the blood gas responses, body oxygenation status, which is important for performance and recovery, and acid-base balance of the athletes.

Keywords: Beta-alanine, pH, buffering, oxygenation, acid-base balance, recovery.

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INTRODUCTION

Acute high-intensity physical activity is known to cause a decrease in adenosine triphosphate (ATP) molecules, creatine phosphate stores, and glycolytic substrates which are necessary for the energy metabolism in the muscle cell. At the same time, high-intensity exercise causes an increase in intracellular metabolites such as adenosine diphosphate, inorganic phosphate, hydrogen ions, and lactate. The breakdown of energy stores in that way and increase in intracellular metabolites are among the factors causing muscle fatigue during short-term high-intensity exercise. Since early fatigue coming with decreased power and muscle capacity in the athletes decreases performance, it is an important performance output[1]. However, an acute accumulation of hydrogen ion (H^+) causes a decrease in intramuscular pH, which could lead to fatigue in some exercise methods and intense training may induce oxidative stress which leads to excessive H^+ and power loss[2].

A recent trend in the research of nutritional supplements for athletes is the evaluation of multi-component performance supplements. Beta-alanine, which is known to increase neuromuscular adaptation in training, is one of these supplements[3]. Beta-alanine is a non-proteogenic, non-essential amino acid which is in combination with histidine, results in the formation of carnosine within the muscle cell and is produced endogenously in the liver. Although the ergogenic characteristics of beta-alanine are limited, it is the precursor of carnosine synthesis and increases carnosine levels in the human muscle cell. Carnosine is defined as intramyocellular pH buffer due to its interest for H^+ produced during anaerobic glycolysis. Therefore, due to its H^+ buffering capability, carnosine concentration increasing in skeletal muscle improves aerobic and anaerobic performance[2,4,5]. The primary role of carnosine is to maintain acid-base homeostasis through increased intramuscular H^+ buffering capacity and to increase high-intensity exercise performance by increasing fatigue threshold[6,7]. Although training improves muscle buffering capacity, beta-alanine supplementation was shown to increase the intramuscular carnosine concentration in well-trained athletes beyond the level produced by training. For this reason, beta-alanine supplementation increases muscle buffering capacity and reduces the rate of fatigue by increasing carnosine levels[4]. Some of the studies on beta-alanine demonstrate that beta-alanine intake, alone or in combination with creatine, can upgrade aerobic and anaerobic athletic performance (stamina and strength/power training, repeated sprints)[6].

The present study aimed to examine the effect of acute beta-alanine consumption after maximal exercise on blood gas responses, body oxygenation status, which is important for pre-training/training performance and which is important for recovery after training, and acid-base balance in the metabolism. When the similar studies have been reviewed, no blood gas parameters have been encountered as comprehensive as presented research.

MATERIALS AND METHODS

Research Group

In this study, 18 volunteers, in shape, male athletes (elite soccer players) between the ages of 19-24, participated in this study (Table 1). Athletes were divided into two groups as experiment (beta-alanine) (n=9) and placebo (n=9). In athletes, requirements of being healthy, not having chronic or acute disease and not having any movement limitation depending on disability occurred for any reason were looked for. For this study, by Sinop University Human Research Ethics Committee it was decided that there was no inconvenience ethically and it was found appropriate (Number: 57452775-604.01.02-E.).

Table 1: Participant characteristics of study (M \pm SD)

	Beta-Alanine (n=9)	Placebo (n=9)
VO₂max	54.95 \pm 5.17	51.67 \pm 4.98
Total supplement consumed (g)	3	-
Total water consumed (ml)	250	250

Study Design

In the study firstly some blood gas parameters were analyzed by taking basal blood samples prior to shuttle run and supplementation. Later 20-metre shuttle run was applied to athletes and right after maximals exercise venous blood was again taken from the athletes for tests and then drink products were given to the beta-alanine and placebo groups. After athletes consumed the drinks given to them under the supervision of researchers and in allotted time venous blood was taken for the last time for blood gas analysis two hours after consumption and some blood gas parameters of athletes were analyzed after acute beta-alanine supplementation. Measurements and tests were made at the same physical conditions in both the beta-alanine and placebo groups.

Blood Measurements

Venous blood was taken from the athletes by expert nurses for blood gas tests and analysed by biochemistry specialists. Blood gas tests were studied from whole blood samples taken by the injector with heparin and analyses were carried out with Radiometer ABL800 FLEX blood gas analyzer. Blood was taken from the athletes three times as basal, post-exercise (PE) and 2 hours after supplement ingestion (PS); Power of Hydrogen (pH), Partial Pressure of Oxygen (PO₂), Partial Pressure of Carbon Dioxide (PCO₂), Oxygen Saturation (SO₂), Hematocrit (Hct), Actual Bicarbonate (HCO₃.act), Standard Bicarbonate (HCO₃.std), Base Excess (BE), Base Excess of Extracellular Fluid (BE(ecf)), Total Carbon Dioxide Content (tCO₂), Methemoglobin (MetHb) and Carboxyhemoglobin (COHb) values were analyzed.

Supplementation

Immediately after 20-metre shuttle run test, 18 athletes were randomly divided into two groups as beta-alanine (n=9) and placebo (n=9) group. The study was conducted as a single blind application. Beta-alanine supplement was given to the beta-alanine group in accordance with administration and daily dosage (with 250ml water), an equal amount of placebo (water) to the given nutritional supplement was given to the placebo group. The supplementation was prepared beforehand and as a single dose that includes beta-alanine 3 g. This usage dose is standard recommended amount of beta-alanine product. Besides, considering the usage amount in literature, it is understood that the product about 3 g is enough for athletes. The athletes were not informed about the substance given to them. So the psychological effects that may occur in athletes were removed and the study was conducted in more reliable conditions. In addition, the athletes were warned about not consuming any alcohol and stimulants one day before the test, caring the nutrition and resting.

Shuttle Run Test

In the study, 20-metre shuttle run test was applied to increase level of fatigue of athletes. This test that is used to measure aerobic capacity test is frequently preferred since it is a method that can be applied easily. In addition, 20-metre shuttle run test is a test that its validity and reliability to measure aerobic capacity is proven [8]. They do not need to warm up before starting to the test. Because the 20-metre shuttle run test is a multi-stage test, first stages are in warming tempo [9]. This test; is a test starting with 8.5 km/h and in every 1 minute running speed increases by 0.5 km/h, 20 m distance is run as round trips [10]. Running speed is controlled with a tape that beeps at regular intervals. The subject begins to run from the signal heard first and has to reach the other line until the second beep. And when the second beep is heard, returns to the starting line and these running signals continue. When the subject hears the signal, he sets the tempo himself to be on the other end of the runway at the second signal. If the subject misses a signal and catches the second, he continues to the test. The test is over when the subjects cannot catch the line 3 times before the beep or they quit running due to exhaustion [9,119].

Statistical Analyses

The research data obtained were given in the form of the mean \pm standard deviation (M \pm SD) and standard error of the mean (M \pm SEM). We assessed the distribution of the analyzed variables using a Shapiro-Wilk test. The Mann-Whitney U test was used to compare basal, PE, PS values between the two groups. A Friedman rank test was undertaken to evaluate the statistical differences in time for each parameter. When a significant F-value in Friedmans' analysis was found, a post-hoc test with a Bonferroni correction was used to

determine the between-means differences. Statistical significance was accepted as $p < 0.05$. In making of statistical analysis derived from the study and comparing the results SPSS v.22 package program was used.

RESULTS

The changes of pH, PO₂, PCO₂ and SO₂

Although there was a significant difference between the basal and PE pH level as well as between PE and PS pH level in the beta-alanine group ($p < 0.05$), there was no significant difference between basal and PS pH level ($p > 0.05$). In the placebo group, a statistical significance was found only between basal and PE pH level ($p < 0.05$). A significant difference was detected only between the basal and PE PO₂ level of the beta-alanine group when the PO₂ levels of the beta-alanine and placebo group were compared at all time periods ($p < 0.05$). All of the PCO₂ levels were similar for both groups ($p > 0.05$). A significant difference was detected only between the basal and PE SO₂ level of the beta-alanine group when the SO₂ levels of the beta-alanine and placebo groups were compared at all time periods ($p < 0.05$) (Figure 1).

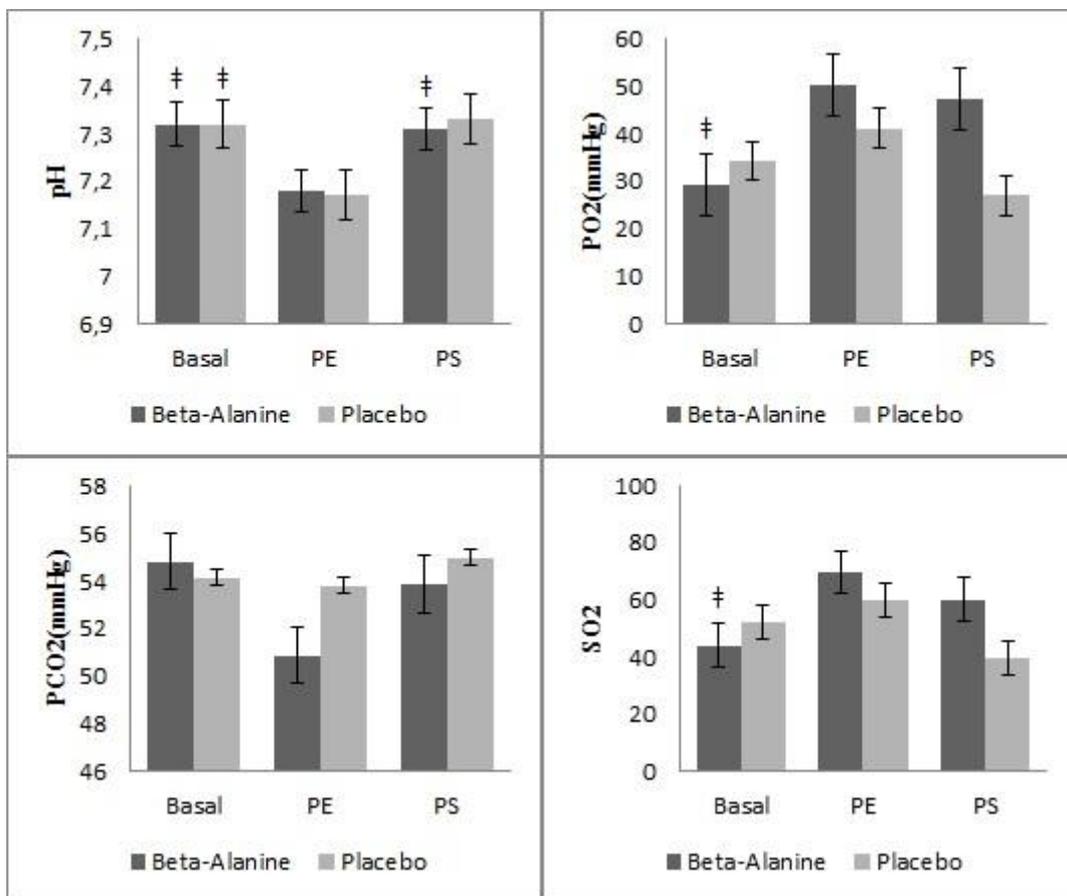


Figure 1: Changes in mean values of beta-alanine and placebo groups. *Significant Difference compared with basal. †Significant difference compared with post-exercise. #Significantly different between beta-alanine and placebo groups (M±SEM).

The changes of Hct, HcO₃.act, HcO₃.std and BE

All of the Hct levels were similar for both groups ($p > 0.05$). There was also a significant difference between the basal and PE HcO₃.act level and between PE and PS level of the beta-alanine group ($p < 0.05$). Considering the HcO₃.act levels of the placebo group, there was a significant difference between the basal and PE time periods ($p < 0.05$). In the beta-alanine group, a significant difference was found between the basal and PE HcO₃.std level as well as between PE and PS levels ($p < 0.05$). Whereas in the placebo group, a significant difference was found only between the basal and PE HcO₃.std level ($p < 0.05$). A statistical difference was

detected between the basal and PE BE levels as well as between PE and PS levels in the beta-alanine group ($p < 0.05$). Considering the BE levels in the placebo group, there was a statistical difference between the basal and PE time periods ($p < 0.05$) (Figure 2).

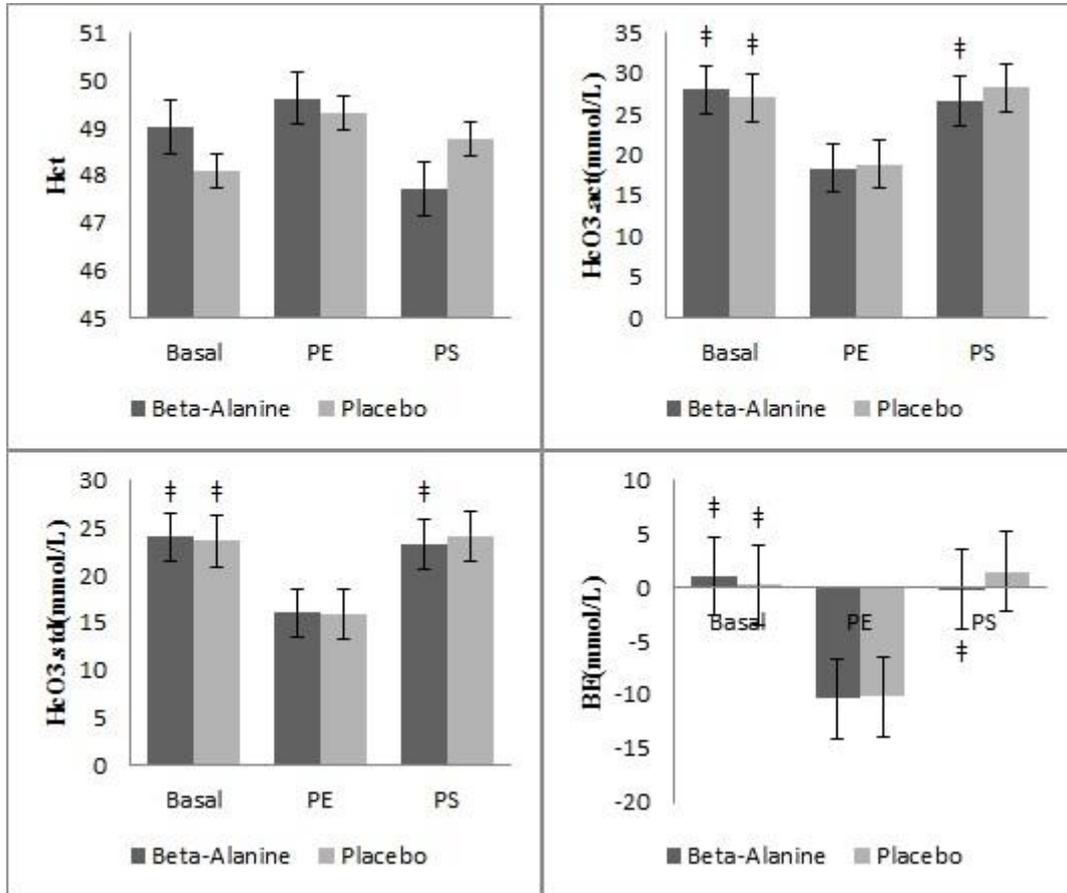


Figure 2: Changes in mean values of beta-alanine and placebo groups. *Significant Difference compared with basal. †Significant difference compared with post-exercise. #Significantly different between beta-alanine and placebo groups(M±SEM).

The changes of BE(ecf),tCO₂, MetHb and COHb

There was also a significant difference between the basal and PE BE(ecf) levels as well as between PE and PS levels of the beta-alanine group ($p < 0.05$). Regarding the placebo group, there was only a statistical significance between the basal and PE BE(ecf) levels ($p < 0.05$). Although there was a significant difference between the basal and PE tCO₂ levels as well as between PE and PS levels in the beta-alanine group ($p < 0.05$), there was no significant difference between the basal and PS tCO₂ levels ($p > 0.05$). Whereas in the placebo group, a statistically significant difference was found only between the basal and PE tCO₂ levels ($p < 0.05$). All of the MetHb levels were similar for both groups ($p > 0.05$). When the COHb levels of the beta-alanine and placebo groups at all time periods were compared, a statistically significant difference was found only between the PE and PS COHb level of the beta-alanine group ($p < 0.05$) (Figure 3).

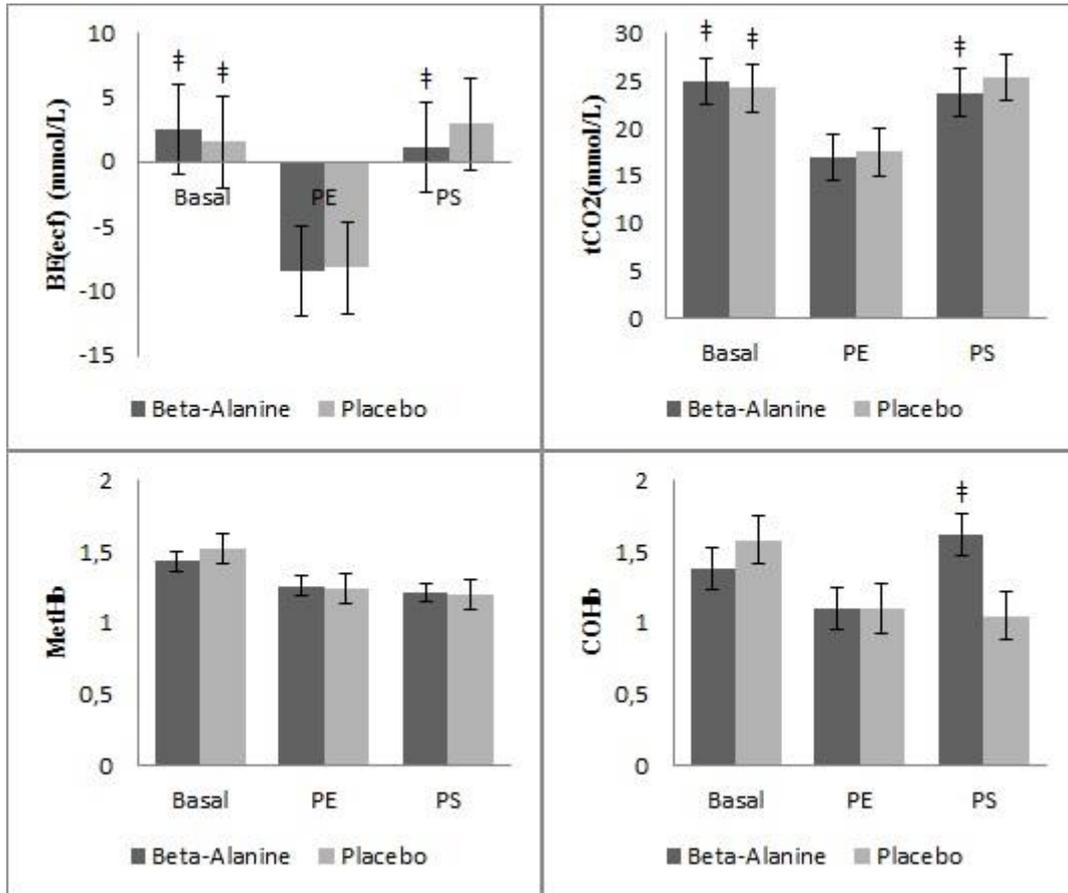


Figure 3: Changes in mean values of beta-alanine and placebo groups. *Significant Difference compared with basal. †Significant difference compared with post-exercise. ‡Significantly different between beta-alanine and placebo groups(M±SEM).

When all inter-group comparisons of all findings obtained were considered, a significant difference was not detected at any time periods ($p>0.05$) (Table 2).

Table 2: Changes in mean values of beta-alanine and placebo groups(M±SD)

	Beta-Alanine			Placebo		
	Basal	PE	PS	Basal	PE	PS
pH	7.32±0.02 †	7.18±0.05	7.31±0.04 †	7.32±0.03 †	7.17±0.05	7.33±0.01
PO2 (mmHg)	29.32±6.41 †	50.37±14.65	47.12±38.38	34.20±6.41	41.20±7.29	27.00±9.01
PCO2 (mmHg)	54.82±4.23	50.87±7.15	53.86±2.91	54.14±4.52	53.80±8.81	54.97±5.98
SO2	43.96±12.10 †	69.85±13.72	60.23±23.90	51.96±14.85	60.00±15.13	39.65±22.21
Hct	49.02±8.97	49.62±7.33	47.72±10.69	48.10±9.86	49.30±3.42	48.77±7.76
HcO3.act (mmol/L)	27.97±1.73 †	18.37±2.87	26.65±2.45 †	27.08±2.21 †	18.84±2.18	28.27±2.02
HcO3.std (mmol/L)	24.01±1.53 †	16.00±2.14	23.26±1.61 †	23.56±1.75 †	15.86±1.35	24.10±0.48
BE (mmol/L)	1.10±1.84 †	-	-0.16±2.46 †	0.26±2.22 †	-	1.47±0.94

BE(ecf) (mmol/L)	2.55±1.75 ‡	-8.53±3.10	1.12±2.80 ‡	1.54±2.22 ‡	-8.24±2.11	2.92±1.69
tCO₂ (mmol/L)	24.93±1.85 ‡	16.91±2.70	23.73±1.55 ‡	24.18±2.68 ‡	17.46±2.22	25.27±1.72
MetHb	1.43±0.47	1.26±0.10	1.21±0.25	1.52±0.26	1.24±0.08	1.20±0.14
COHb	1.38±0.80	1.10±0.46	1.62±1.00 ‡	1.58±2.05	1.10±0.84	1.05±0.68

*Significant difference compared with basal. ‡Significant difference compared with post-exercise. #Significantly different between beta-alanine and placebo groups.

DISCUSSION

During high-intensity exercise, acidosis is known to be the important reason for fatigue caused by high levels of hydrogen ions (H⁺) in the muscle fibers. Shortly, muscle acidosis is the main cause of fatigue during high-intensity exercise. This increase in hydrogen ion (H⁺) may cause a decline in the pH levels of muscle and blood and in conclusion, leading to the deterioration of performance[12,13]. Beta-alanine, an amino acid pioneering the increase in muscle carnosine, has positive effects on muscle function during exercise and is particularly important during maximal exercise. Because carnosine is a proton buffer within the physiological pH range, it has the ability to reduce exercise associated acidosis and fatigue secondary to acidosis[14,15].

Considering the literature, there are numerous different studies on the effect of beta-alanine supplementation on exercise performance. These studies using different supplementation methods include a vast majority of studies in which various performance analyses such as aerobic capacity[16,17], anaerobic capacity[18], strength[19], muscle performance[20], repeated sprint performance[21], blood lactate accumulation[22], neuromuscular fatigue[23,24], hormonal response[19,25], body composition[22,26] were associated with the use of beta-alanine.

When examining some of the research, Baguet *et al.* stated that the use of 4-week beta-alanine may attenuate the decline of blood pH during high-intensity exercise. Regarding the other data, blood lactate elevated at the end of an exercise, however, decreased slowly in 4 minutes during the recovery process. Bicarbonate and base excess were markedly decreased during high-intensity exercise. They interpreted the result of their study as the fact that the use of beta-alanine during high-intensity exercise can reduce acidosis[27]. Sale *et al.* examined the effect of beta-alanine consumption on high-intensity bicycle capacity and determined blood pH, lactate, bicarbonate, and base excess levels at the time periods of baseline, pre-exercise, immediately after exercise and 5 minutes after exercise. At the end of the study, they concluded that beta-alanine consumption improved the capacity of high-intensity cycling[28]. Saunders *et al.* investigated the effect of the use of beta-alanine on repeated sprint performance. As a result of the study examining blood lactate, pH, bicarbonate, and base excess levels, the researchers determined that beta-alanine consumption did not improve the repeated sprint performance[29]. In the study by Gross *et al.* measuring pre- and post-exercise muscular pH levels, they noted that the use of beta-alanine did not affect buffering significantly but, it had beneficial effects on exercise metabolism[14]. In the same way, Ducker *et al.* examined blood lactate and pH levels in their studies on the 28-day use of beta-alanine. Although they did not find a difference in terms of pre- and post-supplementation values in the comparison of the experimental and placebo groups, they detected an improvement in 800-meter run performance while they could not detect an improvement in 2000-meter rowing ergometer performance[30,31]. Considering the study presented, the pH, HCO₃⁻, HCO₃⁻ std, BE, BE (ecf), tCO₂ and COHb levels of the subjects were examined, the intra-group comparisons of the beta-alanine groups revealed a statistical significance between the PS and PE levels, unlike the placebo group. In respect to the PO₂ and SO₂ values, a statistically significant difference was found between the PE and basal levels in the beta-alanine group, unlike the placebo group. According to these data obtained as a result of our study, there was no significant difference between the basal and PS levels of any parameters in the beta-alanine group although there were significant differences among the PE, basal and PS values as a result of beta-alanine supplementation.

The beta-alanine supplement is known to improve exercise performance and reduce acidosis and acidosis associated fatigue during training[32,33]. In the studies measuring similar parameters, Painelli *et al.*, Hobson *et al.* and Tobias *et al.* stated that beta-alanine supplement had positive impact on performance[13,34-36] whereas Chung *et al.* stated that it did not create a significant change in blood parameters although it

affected performance positively[37]. Ducker *et al.* reported that it did not have ergogenic effect on some sport branches including repeated short sprints[38]while Mero *et al.*stated that they could not find a positive impact of it on performance in their study examining blood lactate, pH, bicarbonate, and base excess levels[12]. Considering other studies, Howe *et al.*did not observe a difference in terms of blood parameters examined including blood pH, lactate, and HcO₃ whereas they found that beta-alanine caused changes in performance[39]. Similarly, in the study by Bellinger *et al.*examining blood pH and HcO₃ levels, the beta-alanine supplement was determined to increase the performance of the trained cyclists[40]. In the present study, the PCO₂, Hct and MetHb levels of the subjects were studied and similar results were obtained in both beta-alanine and placebo groups and no significant difference was found. Therefore, the study conducted showed that the acute intake of beta-alanine supplement did not cause any change in PCO₂, Hct, and MetHb values.

Since this study was conducted with limited resources, it did not reveal whether there were any changes in the blood gas levels 24 hours after exercise of the subjects. Another limitation of the study can be said that the number of subjects were 18.

CONCLUSION

In conclusion, the decreases and increases in the data obtained showed us that when comparing the research data with the placebo group, it can be said that the acute use of beta-alanine supplement after maximal exercise had positive effects on the blood gas responses, body oxygenation status, which is important for pre-training/training performance and which is important for recovery after training, and acid-base balance of the athletes by considering especially the values of pH, PO₂, SO₂, HcO₃. act, HcO₃. std, BE, BE (ecf), tCO₂ and COHb at the basal, post-exercise and post-supplement period and also by evaluating the data all in all.

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The authors declare that they have no conflicts of interest.

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