

β-alanine supplementation and high-intensity interval training improves aerobic performance in  
recreationally active men

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## ABSTRACT

This study evaluated the effects of combining  $\beta$ -alanine supplementation with high-intensity interval training (HIIT) on endurance performance. Forty-six men (Age:  $22.2 \pm 3.3$  yrs,  $VO_{2peak}$ :  $3.3 \pm 0.6$  l·min<sup>-1</sup>) were assessed pre-, mid- and post-training for peak O<sub>2</sub> utilization ( $VO_{2peak}$ ), time to fatigue ( $VO_{2TTE}$ ), ventilatory threshold (VT), and total work done at 110% of pre-training  $VO_{2peak}$  (TWD). Following initial testing, all subjects were randomly assigned into one of three groups: placebo (PL – 16.5g dextrose powder per packet; n=18),  $\beta$ -alanine (BA – 1.5 g  $\beta$ -alanine plus 15 g dextrose powder per packet; n=18) or control (n=10) groups. Each treatment group supplemented 4 times per day (total of 6g/day) for the first 21-day adaptation phase, followed by 2 times per day (3g/day) for the subsequent 21 days. All participants in the BA and PL groups engaged in a 3 week supplementing and training adaptation phase, consisting of 5 bouts of a 2:1 minute cycling work to rest ratio, introduced in an undulating progression starting at 90%  $VO_{2peak}$  power output and reaching 110%. The second 3 week training phase progressed, reaching intensities up to 115% of  $VO_{2peak}$ . Both training groups demonstrated significant improvements in post-test  $VO_{2peak}$ ,  $VO_{2TTE}$ , VT, and TWD. Increases in TWD were greater for the BA group, compared to the CON, with no differences between training groups. Individual responses demonstrated greater improvements in the BA group for  $VO_{2peak}$  (83%) and  $VO_{2TTE}$  (72%) performance over the PL group (61% and 56%, respectively). These findings suggest that chronic BA supplementation may enhance HIIT and improve endurance performance.

aerobic capacity; time to fatigue; muscle buffer capacity

High-intensity exercise results in diminished stores of adenosine tri-phosphate (ATP), creatine phosphate (CP) and glycogenic substrates, and is accompanied by an accumulation of metabolites (adenosine di-phosphate (ADP), inorganic phosphate ( $P_i$ ), hydrogen ions ( $H^+$ ), and magnesium ( $Mg^{2+}$ ))(6, 9, 57, 65, 67). The accumulation of  $H^+$  results in a decrease in intramuscular pH leading to fatigue during high-intensity exercise(65, 84, 85). The majority of protons that are produced during exercise are buffered by the bicarbonate ( $HCO_3^-$ ) buffering system (5), which functions by binding to  $H^+$  to inhibit the accumulation of intracellular protons (67). Enhancing an individual's ability to buffer protons may delay fatigue by improving the use of energy substrates and maintaining muscular contraction (13, 55, 62, 73). A decrease in pH as a result of the  $H^+$  build up, may inhibit the activity of phosphofructokinase (PFK) and ultimately decrease energy production from glycolysis (84). Furthermore,  $H^+$  accumulation has been thought to influence fatigue by inhibiting  $Ca^{2+}$  release from the sarcoplasmic reticulum (SR). Low pH has also been associated with a reduction in  $Ca^{2+}$  sensitivity, due to the competitive binding between  $H^+$  and  $Ca^{2+}$  and troponin C. The consequences of a drop in pH, as a result of the suggested intramyocellular phenomena, are related to a decrease in force per cross-bridge(28), and alterations to the actin-myosin kinetics, due to a reduced  $Ca^{2+}$  sensitivity, so that fewer cross-bridges are engaged resulting in a decrease in force(15, 29, 57).

In addition to the body's natural bicarbonate buffering system, carnosine ( $\beta$ -alanyl-L-histidine), a cytoplasmic dipeptide, has been shown to be an important non-bicarbonate buffering element(80). It has been suggested that carnosine may have a higher proton sequestering capacity (pKa 6.83) than the bicarbonate buffering systems (4, 39, 40, 80). This suggests that carnosine can more quickly accept protons during high-intensity exercise, compared with  $HCO_3^-$  and the other bicarbonate buffers (80).

Mechanisms for increasing muscle carnosine concentration have been somewhat disputed. While carnosine may be increased in chronically trained athletes, the effects of acute training are less clear. Earlier research suggests that 10 days to 8 weeks of intensive training may increase intramuscular carnosine content (50, 79). However, more recently, both invasive and non-invasive techniques have shown that intense training has been unable to promote a rise in skeletal muscle carnosine levels(35, 36, 51). Harris et al. (35) reported that 5 weeks of high-intensity interval training (HIIT) had no significant effect on muscle carnosine levels in females. In addition, using a 4 week unilateral training program for the vastus lateralis resulted in no change in carnosine content for either the trained or untrained legs. However, carnosine content in skeletal muscle has been shown to increase by 60% or more with  $\beta$ -alanine supplementation (35, 39). Furthermore, the combination of training and supplementation may stimulate a greater increase in carnosine concentration than supplementation alone(36). Harris et al. (35, 36) reported that  $\beta$ -alanine supplementation combined with intense training may double the increase in intramuscular carnosine content. Kim et al.(49) also showed an enhanced effect of combining supplementation and training. In a group of elite cyclists, muscle carnosine was not altered after 12 weeks of training in the absence of  $\beta$ -alanine, but increased 46% with  $\beta$ -alanine supplementation (49).

While carnosine is synthesized in the muscle from its two constituents,  $\beta$ -alanine and histidine (2), according to Dunnett and Harris (23, 39) muscle carnosine synthesis is limited by the availability of  $\beta$ -alanine. Furthermore,  $\beta$ -alanine supplementation, not histidine, has been shown to significantly increase the intramuscular carnosine content (37, 51). More interestingly, enhancing intramuscular carnosine content via  $\beta$ -alanine supplementation, has been shown to improve performance (35, 37, 44, 51, 76-78, 82, 93). Recently, Hill and colleagues (44) demonstrated a 13% improvement in total work done (TWD) following four weeks of  $\beta$ -alanine supplementation, and an additional 3.2% increase after 10 weeks. Zoeller et al. (93) also reported significant increases in ventilatory threshold (VT) in a sample

of untrained men after supplementing with  $\beta$ -alanine ( $3.2\text{g}\cdot\text{d}^{-1}$ ) for 28 days. In agreement, Kim et al. (51) also reported significant increases in VT and time to exhaustion (TTE) in highly trained male cyclists after 12 weeks of  $\beta$ -alanine ( $4.8\text{g}\cdot\text{d}^{-1}$ ) supplementation and training. Furthermore, Stout et al. (76, 77) reported a significant delay in neuromuscular fatigue, measured by physical working capacity at the fatigue threshold ( $\text{PWC}_{\text{FT}}$ ), in both men and women after 28 days of  $\beta$ -alanine supplementation ( $3.2\text{g}\cdot\text{d}^{-1}$ - $6.4\text{g}\cdot\text{d}^{-1}$ ). Despite the improvements in VT, TTE, TWD, and  $\text{PWC}_{\text{FT}}$  after supplementation, there have been no observed increases in aerobic power, measured by  $\text{VO}_{2\text{peak}}$  (76, 77, 93).

Although HIIT has not been shown to increase skeletal muscle carnosine levels (35, 36), it does appear to improve muscle buffering capacity (26, 54, 91). Maximal exercise generates  $\text{H}^+$  and lactate at a higher rate than can be metabolized or buffered inside the cell. As a result, pH can decrease to approximately 6.3 in muscle and 6.94 in blood after intermittent maximal exercises (43, 59, 60, 72-74). When repeated bouts of high-intensity intervals are interspersed with short rest periods, subsequent trials are initiated at a much lower pH (16). Training in such a manner subjects the body to an acidic environment, forcing several physiological adaptations. HIIT has been shown to improve  $\text{VO}_{2\text{peak}}$  and whole body fat oxidation in only 2 weeks (7 sessions at 90%  $\text{VO}_{2\text{peak}}$ ) (81). Furthermore, over a longer period of time (4-6 weeks), HIIT has been reported to increase high-intensity exercise performance (6-21%), muscle buffering capacity, whole body exercise fat oxidation, and aerobic power ( $\text{VO}_{2\text{peak}}$ ) (26, 54, 91). Edge et al. (26) also reported that 5 weeks of HIIT (2 min. cycle, 1 min. rest; 3 x/week) resulted in an increase in muscle buffering capacity, which is consistent with previous literature (7, 91, 92). Muscle buffering capacity is an important factor in performance during high-intensity exercise. An increased muscle buffering capacity after training may improve performance by preventing a considerable drop in pH during intense muscle contraction (25, 26), and ultimately delay the onset of neuromuscular fatigue.

In theory, increasing intramuscular carnosine levels with β-alanine supplementation may enhance the quality of HIIT and lead to greater physiological adaptations. Therefore, the purpose of this study was to determine the effects of chronic (6 weeks) β-alanine supplementation in combination with HIIT on endurance performance measures in recreationally trained individuals.

## METHODS

Forty-six college-aged men, who were recreationally active one to five hours per week, volunteered to participate in this study (Table 1). Subjects were informed of the potential risks, benefits, and time requirements prior to enrolling and giving written consent. All study procedures were approved by the University's Institutional Review Board.

*Experimental overview.* The present study involved two three-week periods of high-intensity interval training (HIIT), with assessments completed at zero, four, and eight weeks. All participants completed a series of baseline testing, including maximal oxygen consumption ( $VO_{2peak}$ ), time to exhaustion (TTE) and body composition using air displacement plethysmography (BodPod®). The  $VO_{2peak}$ , TTE and body composition tests were completed prior to-, mid-way, and after the training and supplementing intervention. Participant's initial  $VO_{2peak}$  values were used to establish the TTE intensity and the training intensity for the six week duration, with no modification to intensity following mid-testing. The first three-week period of training and supplementing was established as an adaptation phase for both training and supplementing. Supplementing with 6 g per day of  $\beta$ -alanine, for 28 days has demonstrated a 60% increase in carnosine concentration (39, 44), supporting the 21 day adaptation phase to allow for an adequate loading period for  $\beta$ -alanine to elicit increases in intramuscular carnosine concentration. Furthermore, several HIIT studies have reported large increases in aerobic performance and metabolism in a similar untrained population, following one and two weeks of training. Following the three-week adaptation phase, mid-training and post-training tests were completed in the same order as the pre-testing, allowing at least 48 hours between each testing session. All subjects were instructed to maintain their current diet throughout the duration of the study and were asked to refrain from caffeine and vigorous activity prior to any testing session. Food logs were distributed to all participants and

completed (two non-consecutive weekdays and one weekend day) at baseline-testing, mid-testing and post-testing, to evaluate any changes in total kcal and/or protein intake. Following baseline testing subjects were randomly assigned, in a double-blind fashion, to one of two supplementing groups,  $\beta$ -alanine or placebo, both with HIIT. Furthermore, the final 10 to enroll were assigned to a control group (no training or supplementing).

*Determination of  $VO_{2peak}$ .* At pre-, mid-, and post-training, all participants performed a continuous graded exercise test (GXT) on an electronically braked cycle ergometer (Corival 400, Goningen, The Netherlands) to determine  $VO_{2peak}$  and ventilatory threshold (VT). Pedal cadence was maintained at 70 rpm, while the power output was initially set at 50 W for a five minute warm-up, and increased by 25 W every two minutes, until the participant could no longer maintain the required power output (cadence dropped below 60 rpm). Respiratory gases were monitored breath by breath and analyzed with open-circuit spirometry (True One 2400<sup>®</sup> Metabolic Measurement System, Parvo-Medics Inc., Provo UT) to determine  $VO_{2peak}$  and VT. The data was averaged over 15-s intervals. The highest 15-s  $VO_2$  value during the GXT was recorded as the maximal oxygen uptake ( $VO_{2peak}$ ) if it coincided with at least two of the following criteria: (a) a plateau in heart rate (HR) or HR values within 10% of the age-predicted HRmax, (b) a plateau in  $VO_2$  (defined by an increase of note more than  $150 \text{ ml}\cdot\text{min}^{-1}$ ), and/or (c) an RER value greater than 1.15 (17). Heart rate was also monitored continuously during exercise by using a heart rate monitor (Polar FS1, Polar Electro Inc. Lake Success, NY). The amount of time to reach exhaustion ( $VO_{2TTE}$ ) during the  $VO_{2peak}$  was also recorded in seconds. Ventilatory threshold (VT) was determined from a plot of ventilation ( $V_E$ ) against  $VO_2$  as described previously (63). Two linear regression lines were fit to the lower and upper portions of the  $V_E$  vs.  $VO_2$  curve, before and after the break points, respectively. The intersection of these two lines was defined as VT, and was recorded with respect to the corresponding power output (W).

*Time to Exhaustion cycling test.* Each subject performed a constant-load TTE test on an electronically braked cycle ergometer, at a cadence of ~70 rpm. Participants performed a five minute warm-up at 50 W, followed by a cycle to exhaustion at their individual pre-determined workload, established at 110% of the maximum  $\text{VO}_2$ peak workload (W). The subject's TTE was defined by the time (in seconds), that could be maintained without dropping below a cadence of 60 rpm. Total work done was further calculated as the primary variable of interest, using the product of time (in seconds) and the power output (W), divided by 1,000, and presented in kilojoules (kJ).

*Training intervention and  $\beta$ -alanine supplementation.* Training was performed on an electronically braked cycle ergometer (Corival 400, Groningen, The Netherlands) to maintain testing specificity. Participants began the supervised training session within two to four days following testing. Following the baseline-testing and group randomization, subjects began the first of two, three-week training periods. Training followed a fractal periodized plan to allow for adequate progression and to prevent overtraining (10). The training intensity began at 90% of the maximum power output achieved during the baseline  $\text{VO}_2$ peak test and progressed in an undulating manner, reaching a maximum of 115% by the end of the second, three-week training period. The first three-week adaptation period consisted of five sets of two-minute intervals with one-minute rest periods. The second three-week session followed a similar protocol, modifying the progression by alternating the repetitions during weeks six and seven (Figure 1).

In addition to training, during the first three-week period, the participants also supplemented with 6 g per day  $\beta$ -alanine (1.5 g  $\beta$ -alanine, 15 g dextrose) or placebo (16.5 g dextrose). Supplements were mixed with water in an orange flavored dextrose powder and were consumed four times throughout the day. On the three days that subjects visited the lab for training, they consumed 2 pre-mixed doses, one 30 minutes before, and one immediately after completion of the training session. The remaining two doses were taken that day, *ad libidum*. For the remaining 4 days of the week,

participants were instructed to mix and consume the four doses (6 g per day) of their respective supplement. Throughout the second three-week training period, participants supplemented in a similar manner for on- and off-training days, for an additional 21 days, at a dose of 3 g per day, taken in two, 16.5 g doses (1.5 g  $\beta$ -alanine, 15 g dextrose). The participants in the placebo group consumed an isovolumetric flavored powder (16.5 g dextrose), identical in appearance and taste to the  $\beta$ -alanine. Participants were asked to record each dose on a designated dosing log for each day and they were asked to bring in the supplement to allow investigators to monitor compliance.

*Determination of body composition.* Body composition was assessed prior-to, mid-way, and following training and supplementing by using air displacement plethysmography (Bod Pod®). The subjects' weight (kg) and body volume were measured and used to determine percent body fat, fat mass (kg), and lean body mass (kg) using the revised formula of Brozek et al. (11).

*Statistical analysis.* Separate one-way analyses of covariance (ANCOVA) were used to analyze the data based on the recommendations of Huck and McLean(46). Preliminary least squares regression analyses were conducted to examine the linearity of the relationships between the covariate and the dependent variable within all groups, and the interaction between the covariate and group was used to test for homogeneity of slopes (ref). Performance variables ( $VO_{2peak}$ ,  $VO_{2TTE}$ , VT, and TWD) were compared between treatment groups ( $\beta$ -alanine vs. Placebo vs. Control) using the one-way repeated measures ANCOVA. Mid-test values were used as the constant covariates in the analyses. When appropriate, Bonferroni-corrected post hoc pair-wise comparisons were used to examine the differences among groups. Partial eta squared ( $\eta^2$ ) was calculated to determine effect size, and according to Green et al.(33), an  $\eta^2$  of 0.01, 0.06, and 0.14 represents small, medium, and large effect sizes, respectively. Body composition measurements were compared using a mixed factorial repeated measures analysis of variance (ANOVA) (group x time). An alpha of  $p \leq 0.05$  was used to determine statistical significant. The analyses were performed using SPSS v. 14.0 (SPSS Inc., Chicago, IL).



## RESULTS

Table 2 contains the mean and standard error of the mean (SEM) values for the mid-testing and post-testing performance variables. Due to the changes resulting from the training and supplementing adaptation phase, the post-test means ( $VO_{2peak}$ ,  $VO_{2TTE}$ , VT and TWD) were adjusted during the ANCOVA procedure based on the mid-test differences between groups. There were significant linear relationships between all of the mid-test variables and treatment groups. Furthermore, there was no interaction between the mid-test values and group, which supported the homogeneity-of-slopes assumption and use of ANCOVA.

### *$VO_{2peak}$ , $VO_{2TTE}$ , VT during GXT*

The ANCOVA indicated a significant difference ( $p=0.000$ ,  $\eta^2 = 0.82$ ) among the group means for the post-test  $VO_{2peak}$  values after adjusting for the mid-test differences. The strength of the association (i.e., effect size,  $\eta^2$ ) indicated that the treatment groups ( $\beta$ -alanine, placebo, control) accounted for 82% of the variance of the post-test  $VO_{2peak}$  values. Follow-up, Bonferroni corrected pairwise comparisons indicated no significant differences between groups for the change in  $VO_{2peak}$  from mid- to post-testing, although there were significant improvements over time ( $p=0.043$ ). (Figure 1A) Furthermore, the individual responses for  $VO_{2peak}$  indicated that 83% of the subjects in the  $\beta$ -alanine group improved from mid- to post-testing, compared to 61% in the placebo group. (Figure 2).

Time to exhaustion during the GXT ( $VO_{2TTE}$ , sec) at the post-test was significantly different among groups, when adjusting for the mid-test differences ( $p=0.000$   $\eta^2 = 0.63$ ), with the mid-test  $VO_{2TTE}$  values accounting for 63% of the variance. (Figure 1B). Post-hoc analyses indicated no significant differences between groups from mid- to post- $VO_{2TTE}$  performance. However, although not significant, 72% of the subjects in the  $\beta$ -alanine group improved over time, while only 56% of the placebo subjects demonstrated improvements. (Figure 3). The power-output at which VT occurred, resulted in similar

results as reported for the other GXT variables. There was a significant difference ( $p=0.015$ ,  $\eta^2=0.134$ ) in the post-test scores, when holding constant the mid-test values, with no reported differences between treatment groups following post-hoc analyses. (Figure 1C) Similarly, individual responses between groups were not different, with 44% of the participants improving in each group. (Figure 4).

#### *Time to exhaustion test-TWD*

The ANCOVA indicated a significant difference ( $p=0.000$ ,  $\eta^2=0.389$ ) among post-test mean values for TWD, when accounting for differences in mid-test scores. Furthermore, the partial  $\eta^2$  indicates that the mid-test TWD values account for 39% of the variance in post-TWD values for a treatment group. Bonferroni-corrected, post-hoc pairwise comparisons indicated that the increase in TWD from mid-testing to post-testing was greater for the  $\beta$ -alanine group than for the control group ( $p=0.029$ ). (Figure 1D). There were no differences between the  $\beta$ -alanine and placebo groups ( $p=0.164$ ) or placebo and control groups ( $p=0.268$ ). The individual performance values demonstrated an improvement in 78% of the subjects in the  $\beta$ -alanine group and 72% of the participants in the placebo group. (Figure 5).

#### *Body Composition*

The physical characteristics of the subjects determined at mid-testing and after six-week of HIIT and supplementing are presented in Table 3. Body mass did not change significantly with supplementing or training. In addition, the determination of body composition with the use of air displacement plethysmography (Bod Pod<sup>®</sup>) did not reveal any significant changes in percent body fat ( $p=0.794$ ), fat free mass ( $p=0.165$ ) or fatmass ( $p=0.976$ ) between treatment groups after six weeks of HIIT and supplementation.

#### *Dietary Analysis*

There was no significant difference between groups for their supplement or training compliance rate, representing a 6.4 -3.2 g per day intake for the  $\beta$ -alanine group, for the 3 and 6 weeks, respectively and 3 sessions of training each week. Analyses of the dietary recalls demonstrated no significant

differences in caloric intake ( $p > 0.05$ ) between the β-alanine ( $3120 \pm 244$ ) and placebo ( $2775 \pm 209$ ) groups. Furthermore, there were no differences in macronutrient daily intake, with both groups consuming 47% of their daily calories from carbohydrates, 34% from fat and 16% from protein.

## DISCUSSION

The current study is the first to examine the effects of concurrent high-intensity interval training (HIIT) and  $\beta$ -alanine supplementation on a series of endurance performance measures. The primary findings suggest significant improvements in endurance performance after six-weeks of HIIT (Table 2). The maximal oxygen uptake and time to reach maximum oxygen consumption ( $VO_{2peak}$ ,  $VO_{2TTE}$ ) increased significantly in both training groups ( $\beta$ -alanine and placebo). Although not significant,  $\beta$ -alanine supplementation had a greater influence on  $VO_{2peak}$  and  $VO_{2TTE}$ , with 80% and 72% of the subjects demonstrating improvements, compared to 61% and 56% of participants in the placebo group, respectively (Figures 2 and 3). Improvements in  $V_T$  were also reported, with no differences between groups. Furthermore, the present study also identified a significant influence of  $\beta$ -alanine supplementation on enhanced TWD. While there was no significant difference between both training groups, the  $\beta$ -alanine group demonstrated significantly greater improvements than the control group (Figure 4).

### *Study Design*

The experimental group, consisting of recreationally trained healthy men, were required to consume a large dose (6.4 g per day) of  $\beta$ -alanine or placebo, before and after each training session on 3 days per week for the first three-week training period. This adaptation period was established according to previous investigations, recommending at least 28 days of  $\beta$ -alanine supplementation to allow for an adequate increase in intramuscular carnosine (2, 23, 39). Harris et al. reported a ~60% increase in carnosine concentration after four weeks of  $\beta$ -alanine supplementation (39). In addition, intramuscular carnosine concentration may be further amplified when combining high-intensity training and  $\beta$ -alanine supplementation, eliciting even greater improvements in muscle buffering capacity (35, 36, 49). Furthermore, due to the initial cardiovascular state of the participants (Table 1) and the intensity of the

training program, the adaptation phase was implemented in order to offset the substantial improvements reported in endurance performance and metabolic efficiency following acute (~2 weeks) HIIT programs. Talanian and colleagues, employing a similar 2:1 work to rest ratio protocol in a group of recreationally active participants, demonstrated a 13% improvement in  $VO_{2peak}$  and a subsequent increase in metabolic efficiency, by increasing whole body fat oxidation and mitochondrial enzyme activity, after just 2 weeks (81). Additional studies report that untrained/recreationally trained individuals primarily rely on anaerobic glycolysis and Type II fibers during intense activities, creating an environment with large amounts of circulating ammonia, hypoxanthine, hydrogen ions, and lactate, leading to rapid physiological adaptations (3, 20, 21, 45, 58). To ensure an environment with an accumulation of  $H^+$ , lactate and substrate depletion, the training sessions were designed with short rest periods and demanding work intervals (90-115%). Although blood samples were not taken to quantify blood lactate and pH, HIIT has been accepted as a sufficient stimulus for metabolic acidosis. Implementing a three-week phase to allow for an increase in intramuscular carnosine levels and for initial fitness improvements was hypothesized to maximize the influence of  $\beta$ -alanine on training and performance.

#### *Enhanced $VO_{2peak}$ , $VO_{2TTE}$ , and VT after training*

A series of HIIT interventions have suggested that interval exercise (>80%  $VO_{2max}$ ) elicits greater gains in aerobic capacity than moderate-intensity exercise (41, 69, 83). Consequently, the improvements reported in cardiorespiratory fitness in the current study were similar to most studies that have employed short-term (2-9 weeks) endurance interval training programs in untrained and recreationally active individuals (1, 8, 12, 22, 24, 26, 27, 41, 81, 87, 90). In particular, the average reported increases in  $VO_{2peak}$  have ranged from 6-20% in male and female populations. Although the training regimens utilized have varied slightly, all supporting studies applied a similar protocol. The use of a 1:1 (8, 12, 24) and a 2:1 (22, 41, 71, 81) work-to- rest design (1-4 minutes) has been the most effective for promoting

an increase in aerobic capacity. Our data supports previous literature, suggesting a 7-9% increase in  $VO_{2peak}$  during the adaptation phase and a 3-4% increase following the second 3 week session, with both the  $\beta$ -alanine and placebo groups demonstrating significant improvements, and no differences between groups. Similarly, commonly reported increases in  $VO_{2peak}$  utilizing a 2:1 work:rest design have been between 10-13%. Edge et al. showed a 12% increase in recreationally trained females after 5 weeks of training, and Talanian et al. reported a 13% increase, after just 2 weeks (26, 81). Additionally, a few studies (12, 41, 87) have reported smaller increases with 5%, 7.2%, and 7.5%, respectively, in recreationally active men and women after 7 and 8 weeks of HIIT. Along with the increase in  $VO_{2peak}$ , concomitant improvements in the time to exhaustion were observed for both treatment groups after the initial adaptation phase (11.5%) and the second 3 weeks (6.0% for  $\beta$ -alanine and 3.3% for placebo), with no significant differences between groups (Table 2). Although there are no significant between-group differences, particular attention should be paid to individual within-group responses for both  $VO_{2peak}$  and  $VO_{2TTE}$ . While 83% of the participants in the  $\beta$ -alanine group demonstrated improvements in  $VO_{2peak}$ , only 61% of the placebo participants increased (Figure 2). Similar trends were seen for  $VO_{2TTE}$ , with 72% of the  $\beta$ -alanine participants improving compared to 56% of the placebo group (Figure 3).

The use of high-intensity exercise as a training modality has been shown to stimulate acute and chronic physiological adaptations (cardiovascular, metabolic, respiratory and neural), which ultimately lead to improved performance (8, 41, 71). The increases in  $VO_{2peak}$ , and  $VO_{2TTE}$  reported in the current study are in line with other studies, which have suggested that the improvements in aerobic performance are attributable to a reduction in anaerobic ATP production, resulting from an increased contribution of aerobic energy production at higher intensity workloads (31, 34). The greater reliance on aerobic metabolism for energy has been further linked to an up-regulation of various glycolytic enzymes (phosphofructokinase, hexokinase, citrate synthetase, and sodium potassium ATPase) (31, 42,

52, 61, 68, 70), as well as with increased mitochondrial density and improved blood flow due to increased capillarization (22, 42, 52, 88). While a series of physiological adaptations are apparent, improvements in aerobic performance are also manifested in respiratory gas exchange (quantified by  $\dot{V}T$ ). When oxygen stores become inadequate to supply energy, the availability of ATP becomes a source of anaerobic glycolysis. Anaerobic glycolysis consequently forms lactate, which is primarily buffered by the bicarbonate system(86). This buffering mechanism further results in an accumulation of  $\text{CO}_2$ , which is expired, resulting in a non-linear increase in  $\dot{V}O_2$  during an incremental exercise test. This non-linear increase is better known as  $\dot{V}T$ . As a result of the improvements in the  $\dot{V}O_2$  kinetics, HIIT has been associated with a reduced accumulation of lactate and decreased  $\text{H}^+$  production,(8, 47) which can amount to an improvement in  $\dot{V}T$ . As hypothesized, the current study demonstrated improvements in  $\dot{V}T$  for both the  $\beta$ -alanine (23%) and placebo (48%) groups in the second three weeks of training, with no significant difference between groups.

While HIIT has been shown to enhance performance after as a little as two weeks, the actual training recruits a large level of muscle fibers resulting in several contraction-induced metabolic disturbances(32), causing a significant reduction in muscle energy substrates (adenosine triphosphate (ATP), phosphocreatine (PCr), and glycogen)(31, 34, 42) along with increasing intramuscular metabolites (adenosine diphosphate (ADP), inorganic phosphate ( $\text{P}_i$ ), and  $\text{H}^+$ )(34, 48). The acute metabolic response is believed to be the stimuli that results in chronic adaptations, leading to an increase in the muscle's efficiency for fuel utilization and the ability to delay the onset of acidosis, ultimately leading to improved exercise performance(56, 75, 91).

Scientists have suggested the use of  $\beta$ -alanine to enhance training adaptations, in conjunction with HIIT(39, 44, 77). In particular, Harris et al.(39) and Hill et al.(44) have posited that increasing skeletal muscle carnosine concentration with  $\beta$ -alanine supplementation may improve the ability to stabilize the intramuscular pH during intense exercise by buffering accumulating  $\text{H}^+$ . Offsetting the

indirect effect of proton accumulation on contractile function with the use of  $\beta$ -alanine, has been shown to be effective in delaying neuromuscular fatigue, improving VT and time to exhaustion in both trained and untrained individuals(44, 51, 77, 93). While the use of  $\beta$ -alanine alone as a method to improve  $VO_2$ peak has not been reported to be effective,(51, 77, 93) the combined effects of  $\beta$ -alanine supplementation and training on aerobic performance are equivocal. The current study demonstrated no additional significant improvements in  $VO_2$ peak or  $VO_{2TTE}$  when adding  $\beta$ -alanine supplementation to HIIT, which is in agreement with Harris et al.(35) who demonstrated a 9-10% improvement in  $VO_2$ peak after 5 weeks of interval training, but no difference between the  $\beta$ -alanine supplementing and non-supplementing groups. A study by Kim et al.(51) also reported a non-significant improvement in  $VO_2$ peak after 12 weeks of combined endurance and resistance training while supplementing  $\beta$ -alanine in highly trained cyclists. However, Kim et al. did show significant improvements in VT for the  $\beta$ -alanine supplementing group. While our results are not in agreement with Kim et al., 44% of the individuals in both training groups did demonstrate significant improvements in VT (Figure 4). The differences in training status (elite vs. recreationally trained) may have resulted in the conflicting results between the current study and Kim and colleagues. Additional research examining the effects of concurrent  $\beta$ -alanine supplementation and HIIT in trained and untrained men and women on aerobic performance is warranted.

#### *Improvements in TWD*

In addition to augmenting  $VO_2$ peak,  $VO_{2TTE}$  and VT, the HIIT program utilized in the current study demonstrated significant improvements in TWD (Figures 1D and 5). Interestingly, the increases in total work performed in the current study were greater than in previously reported improvements in TWD following HIIT alone(14, 18, 30), with both groups demonstrating a 50-53% improvement during the adaptation phase and the  $\beta$ -alanine group showing a 48% increase compared to the 22% increase in the placebo group, after the second three-week training phase. While neither training group was significantly different from each other, the  $\beta$ -alanine group resulted in a significantly greater increase

than the control group (Figure 1D). The gains reported in this study are in agreement with other studies supplementing with  $\beta$ -alanine(77) and with those combining  $\beta$ -alanine and training(44, 51). Stout et al. (77) reported a significant increase in time to exhaustion after 28 days of  $\beta$ -alanine supplementation, whereas Zoeller et al. (93) showed no significant change. While the data for  $\beta$ -alanine supplementation alone is inconclusive for TWD, the data reported for concurrent training and  $\beta$ -alanine supplementation support the improvements observed for TWD. Kim et al. (51) demonstrated significantly greater increases in TWD in highly trained cyclists after a 12 week  $\beta$ -alanine supplementation and training program, compared to training only. Hill et al. (44) also demonstrated significant improvements in TWD (13%) following four weeks of  $\beta$ -alanine supplementation and high intensity cycling. The combination of training and  $\beta$ -alanine supplementation has been shown to significantly enhance intramuscular carnosine levels more than supplementing alone and further influence performance more than training alone (19, 35, 44). The increase in carnosine concentration, as a result of  $\beta$ -alanine supplementation (39), may have had a strong influence in the improvements in TWD and the trend for improvements in endurance performance in the current study. A few investigations have demonstrated that an increase in intramuscular carnosine is followed by a subsequent increase in intramyocellular buffering capacity(19, 38), which may lead to improved performance (7, 9, 44, 59). Although recent findings question the role of accumulating  $P_i$ , ADP, and  $H^+$  on a decrease in performance (53, 64, 89), most researchers agree that they all are contributing factors in fatigue. Furthermore, carnosine has been recognized as an effective physiochemical buffer by alleviating the detrimental effects of accumulating  $H^+$  and, thereby, attenuating fatigue (56, 66). Although there was no significant difference between the two training groups for TWD, the  $\beta$ -alanine supplementing group demonstrated significantly greater improvements than the control, while there was no difference between placebo and control. In accordance with Kim et al. (51), the increase in intramuscular carnosine, as a result of  $\beta$ -alanine

supplementation, was the most likely cause for the greater performance improvements observed over the training only group, in the current study.

### *Summary*

In summary, our findings indicate three-weeks of combined  $\beta$ -alanine supplementation and HIIT, following a 21-day  $\beta$ -alanine loading and HIIT adaptation phase, significantly improves aerobic performance. The improvements in cycling performance ( $VO_{2peak}$ ,  $VO_{2TTE}$ , and TWD) may be attributed to a greater reliance on aerobic metabolism and an improved muscle buffering capacity at high-intensity workloads. While  $\beta$ -alanine supplementation did not statistically improve aerobic performance over HIIT alone, the individual data (Figures 2, 3) however, did suggest a trend for a greater improvement in  $VO_{2peak}$  and  $VO_{2TTE}$ . Furthermore,  $\beta$ -alanine supplementation demonstrated a significantly greater effect on TWD when combined with HIIT, than training alone. These improvements may have been a result of an increase in intramuscular carnosine levels, providing for a greater capacity to buffer  $H^+$ . While this study is original in its approach to combine HIIT and  $\beta$ -alanine supplementation, the results provide a foundation for future research examining the influence of  $\beta$ -alanine supplementation on intense exercise and recovery.

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### DISCLOSURES

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#### FIGURE LEGENDS

FIGURES 1A-1D. The change from mid- to post-testing for  $VO_{2\text{peak}}$  (1A),  $VO_{2\text{TTE}}$  (1B), VT (1C) and TWD (1D) adjusted for the differences in mid-test values. Values are presented as mean  $\pm$  SEM. \* indicates a significant change from mid- to post-testing. † The increase in TWD from mid- to post-testing was greater for the β-alanine group than for the control group.

FIGURE 2: Individual responses for each subject, within the β-alanine, placebo and control groups for maximal oxygen consumption ( $VO_{2\text{peak}}$ ,  $\text{l}\cdot\text{min}^{-1}$ ). Values are reported as percent change from mid-test assessments. The mean is denoted by the dashed line.

FIGURE 3: The percent change for time to fatigue during the maximal consumption test ( $VO_{2\text{TTE}}$ ), for each individual from mid- to post-testing. Mean values are denoted with a dashed line.

FIGURE 4: Individual responses for the change in ventilatory threshold (VT) from mid- to post-testing. Mean values are denoted with a dashed line.

FIGURE 5: The change from mid- to post-testing for total work done, for each individual. Mean values are illustrated by the dashed line.

Figure 1.

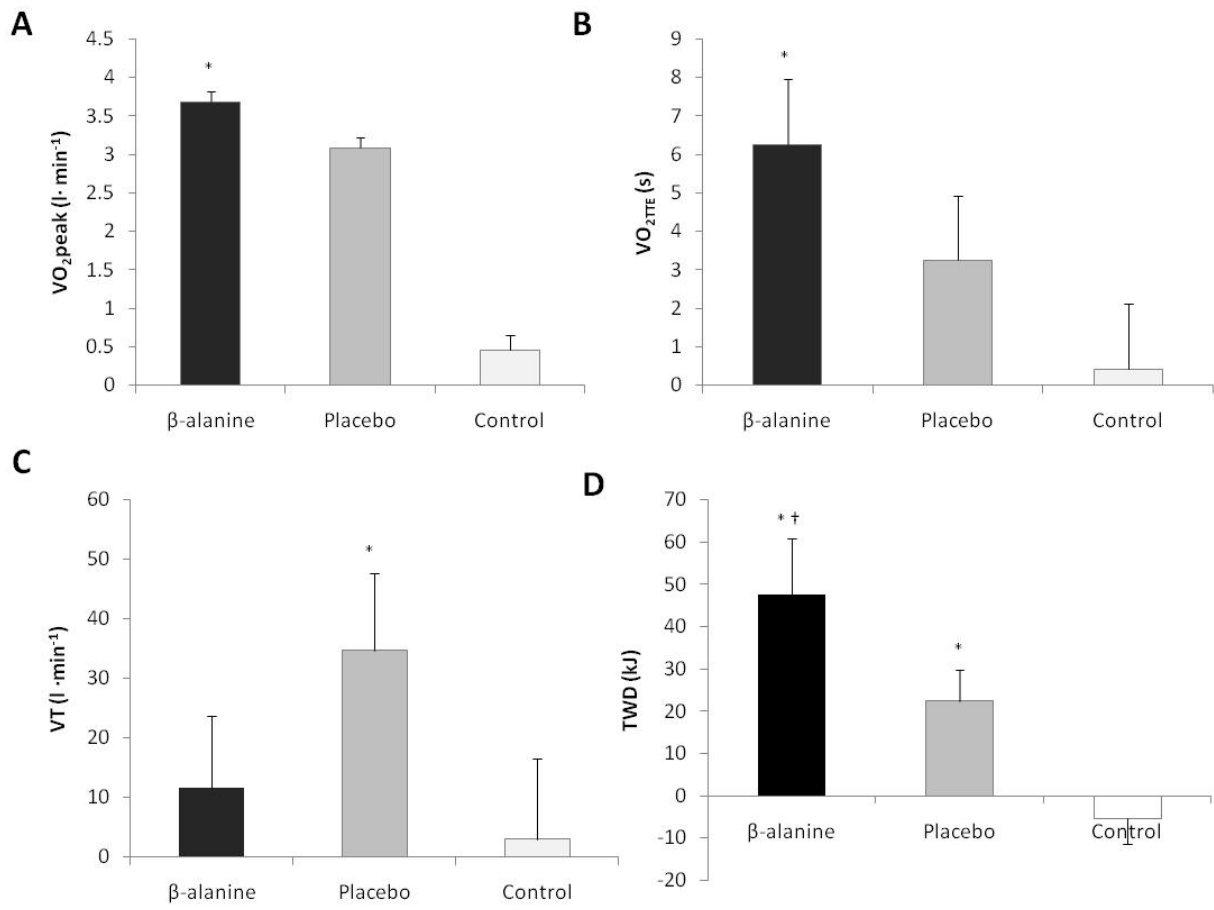


Figure 2.

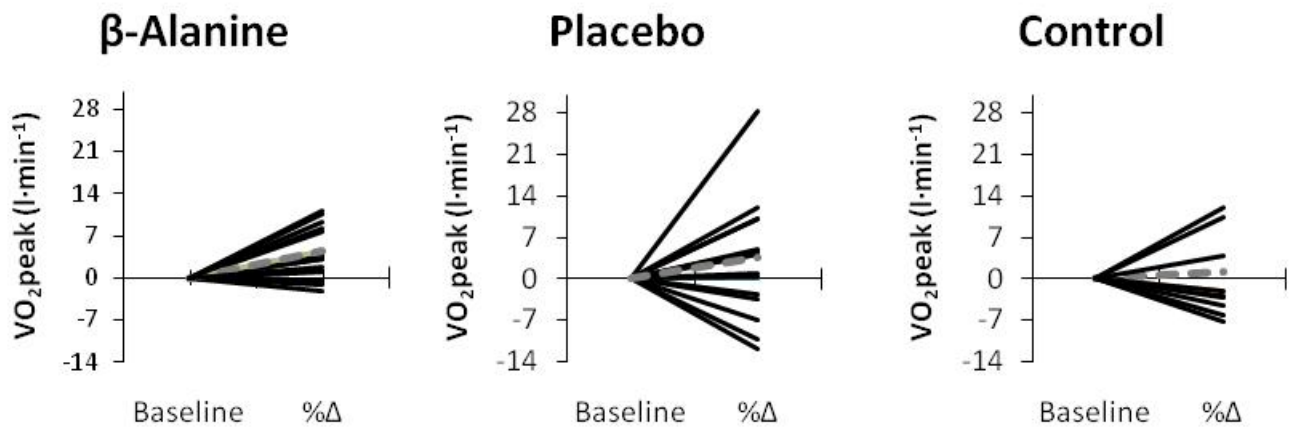


Figure 3.

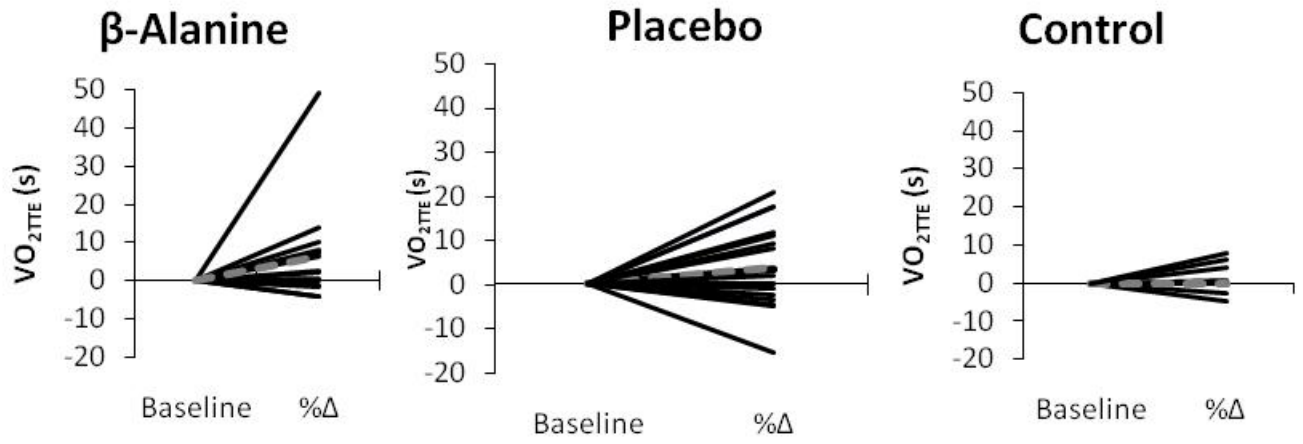


Figure 4.

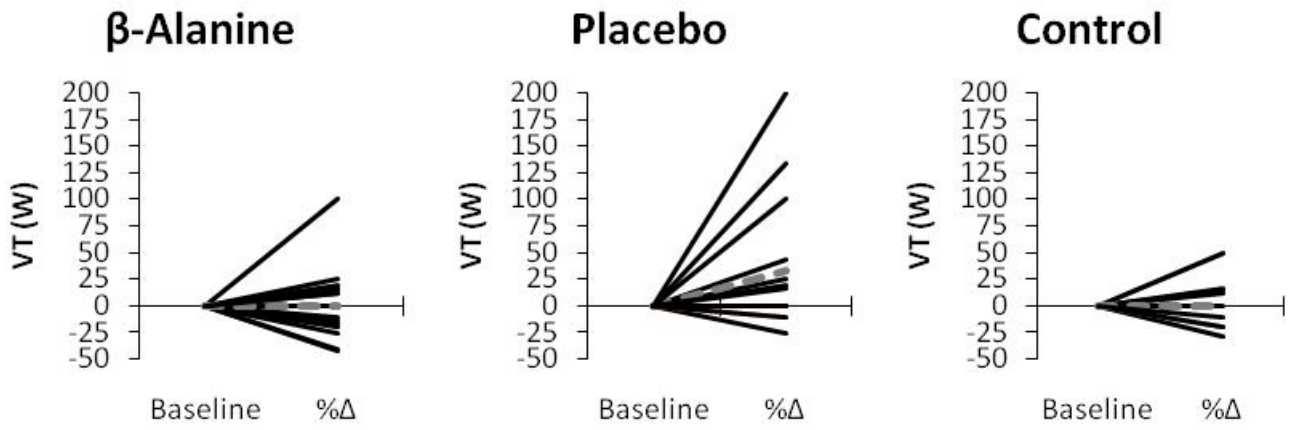


Figure 5.

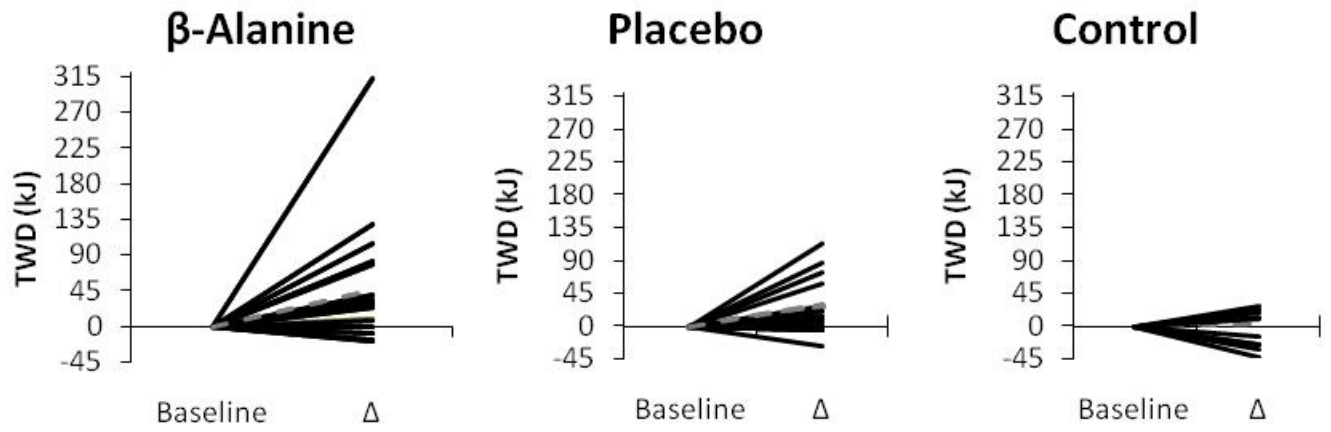


TABLE 1. Age, height, weight and VO<sub>2</sub>peak of participants at baseline.

		Age (yrs)	Height (cm)	Weight (kg)	VO <sub>2</sub> peak (l·min <sup>-1</sup> )
β-Alanine n=18	Mean	22.0	177.8	78.8	3.3
	SD	2.3	8.6	12.8	0.6
Placebo n=18	Mean	22.4	178.4	78.5	3.2
	SD	3.2	6.2	11.3	0.6
Control n=10	Mean	22.0	175.8	75.1	3.5
	SD	5.1	7	9.2	0.6

Values expressed as mean ± SEM

TABLE 2. Mean ± SEM values for VO<sub>2</sub>peak (l·min<sup>-1</sup>), VO<sub>2TTE</sub> (s), VT (W) and TWD (kJ) at mid-testing and post-testing. \* indicates significant difference over time(p<0.05)

		Maximal Oxygen Consumption (l·min <sup>-1</sup> )			Time to Exhaustion (s)			Ventilatory Threshold (W)			Total Work Done (kJ)		
		β-alanine	Placebo	Control	β-alanine	Placebo	Control	β-alanine	Placebo	Control	β-alanine	Placebo	Control
Mid-test	Mean	3.52	3.56	3.66	1304.91	1258.72	1262.70	154.17	140.28	167.50	88.98	83.32	69.68
	SEM	0.12	0.13	0.19	36.23	48.21	68.22	8.63	12.31	11.81	7.09	6.07	8.48
Post-test	Mean	3.65*	3.67	3.66	1386.67*	1299.61	1268.10	172.22	188.89*	172.50	131.3*	101.96*	65.94
	SEM	0.14	0.13	0.19	55.37	38.86	69.37	15.38	13.74	15.12	19.26	8.66	3.76

TABLE 3. Mean and SEM values for body weight (kg), body fat (%), lean body mass (kg), and fat mass (kg) from mid- to post-testing.

	β-alanine (n=18)			Placebo (n=18)			Control (n=10)		
	Mid-Testing	Post-testing	Change	Mid-Testing	Post-testing	Change	Mid-Testing	Post-testing	Change
Weight (kg)	75.72 ± 2.99	76.00 ± 2.96	0.37 ± 2.98	79.36 ± 2.89	78.85 ± 2.80	-0.64 ± 2.85	75.72 ± 2.99	76.00 ± 2.96	0.37 ± 2.98
Body Fat (%)	14.19 ± 1.68	13.72 ± 1.52	-3.31 ± 1.6	15.90 ± 1.95	15.96 ± 1.87	0.37 ± 1.91	14.19 ± 1.68	13.72 ± 1.52	-3.31 ± 1.60
Lean Body Mass (kg)	64.92 ± 2.53	65.46 ± 2.35	0.83 ± 2.44	66.17 ± 2.00	65.80 ± 1.98	-0.54 ± 1.99	64.92 ± 2.53	65.46 ± 2.35	0.83 ± 1.93
Fat Mass (kg)	10.79 ± 3.35	10.56 ± 1.35	-2.13 ± 2.35	13.13 ± 1.89	13.05 ± 1.83	-0.61 ± 1.86	10.79 ± 3.35	10.56 ± 1.35	-2.13 ± 2.35