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COACHING & EXERCISE PHYSIOLOGY

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Beetroot extract improves specific performance and oxygen uptake in taekwondo athletes: A double-blind crossover study

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Key words: martial arts, aerobic exercise, sports performance, dietary supplement

Abstract

Background. Nitrate-rich supplements such as beetroot extract could improve aerobic performance by increasing neuromuscular functioning. However, little is known about the effects of beetroot extract on combat sports, such as taekwondo.

Problem and aim. This study investigated the acute effect of beetroot extract supplementation (1g) on taekwondo athletes undergoing a maximal aerobic test.

Methods. Twelve athletes (26.8±8.8 yrs.; 77.8±11.7 kg; 1.8±0.1 m; 25.3±3.2 kg/m²; 10.0±5.3 Fat%) were submitted to a specific Taekwondo aerobic protocol. All participants performed an alternated kick (*bandal-chagi*) maximal protocol under two randomized conditions: a) beetroot extract; and b) Placebo.

Results. Beetroot condition showed a higher absolute VO_{2Peak} (4.0±0.6 vs. 3.6±0.5 L/min; p=0.048), absolute VO_{2max} at anaerobic threshold (3.7±0.6 vs. 3.2±0.6 L/min; p=0.044), and complete stages (10.9±2.6 vs. 8.3±1.7 L/min; p=0.009). Lactate concentration increased significantly between measurements taken before, after and 3 min after (p≤0.001) the test, without differences between conditions (p=0.46).

Conclusion. Our results suggest an ergogenic effect of beetroot extract supplementation $(1 \mathrm{\,g})$ on aerobic capacity and specific test performance in taekwondo athletes.

Introduction

Similar to other combat sports, Olympic taekwondo is characterized by intermittent effort [Miarka *et al.* 2018] where high-intensity actions are those which punctuate or define combat [Miarka *et al.* 2018; Tornello *et al.* 2013]. However, creatine phosphate resynthesis occurs during low-intensity actions in which aerobic metabolism predominates [Kons *et al.* 2019] in order to be used for the next high-intensity action. Thus, it is essen-

tial that the athlete presents high aerobic capacity for high performance [Sant'Ana et al. 2018]. In a review, Ouergui et al. [2015] indicated that most studies point to the predominance of aerobic metabolism during taekwondo combat, However, few studies have been conducted regarding energy demand in this combat sport. According to Campos et al. [2012], approximately 66% of energy comes from aerobic metabolism.

A high aerobic capacity (VO_{2max}) enables an athlete to recover better in the intervals between rounds and dur-

ing low-intensity actions [Lopes-Silva *et al.* 2015]. In this sense, an increase in endurance is essential when aiming to improve athletic performance. Taekwondo athletes' endurance can be enhanced by a nutritional ergogenic supplement [Stebbins 2016]. However, few studies have been conducted in this combat sport with this focus. Lopes-Silva *et al.* [2015] observed that caffeine supplementation (5 mg/kg) did not increase the VO₂, oxidative capacity, or performance in a combat simulation (3 rounds, 2 min X 1 min interval). Thus, it is relevant to carry out new studies focused on ergogenic supplements which can improve aerobic performance in taekwondo.

Nitrate (NO₃⁻) is one of the possible supplements which can improve endurance in taekwondo athletes [Stebbins 2016]. A meta-analysis performed by McMahon et al. [2017] indicated that supplementation of NO₃ rich nutrients resulted in improved aerobic performance, mainly in time-to-exhaustion. The ergogenic effect is associated with lower O₂ spent during exercise [Christensen et al. 2013], increased muscle deoxyhemoglobin [Breese et al. 2013], higher muscle efficiency for phosphocreatine resynthesis [Bailey et al. 2010] and vasodilation from nitric oxide production [Zafeiridis 2014]. Beetroot is among the richest foods with the highest NO₃ concentration [Breese et al. 2013]. Beetroot juice has shown an ergogenic effect in some studies but failed in others [McMahon et al. 2017; Zafeiridis 2014]. Using a single dosage (140 ml, \sim 8.7 mmol NO $_{3}^{-}$) of beetroot juice Cermak et al. [2012] observed an increase in plasma nitrate concentration in cyclists, however, there was no difference compared to placebo for the 1h-timetrial and power output. However, the same dosage when ingested for six consecutive days resulted in an ergogenic effect in cyclists who exercised until failure [Lansley et al. 2011]. In general, studies have focused on beetroot juice supplementation [McMahon et al. 2017; Zafeiridis 2014], little is known about the effect of concentrated beetroot extract on sports performance. After an extensive search, we did not find scientific studies that have investigated the use of beetroot extract in athlete performance. Given the above, this study aims to investigate the effect of acute supplementation of beetroot extract (1g) on taekwondo athletes submitted to progressive maximal testing. To the best of our knowledge, this study has an original question regarding the possible ergogenic effect of beetroot extract supplementation on: a) a maximum taekwondo test; and b) spirometric variables measured during the test. We hypothesized that acute supplementation promoted an ergogenic effect on test performance and spirometric measurements.

Methods

Experimental approach and participants

This cross-sectional study implemented a randomized, double-blind crossover design to investigate the sup-

plementation of beetroot extract in taekwondo athletes. To do so, athletes were measured under two conditions: a) Beetroot extract, or b) Placebo. The experiment was divided into two days. On the first day, half of the participants were randomized to the Beetroot intake or the other Placebo, and then their conditions were reversed on the second day of the experiment (4-days after first test). All participants were initially informed of the procedures to be followed in the study and signed a free and informed consent form following the 1964 Helsinki statement. The ethics committee of the University in which the experiment was conducted approved this study (protocol 4072649).

After analyzing specific literature [Breese et al. 2013; Callahan et al. 2017; Zafeiridis 2014], we estimated a minimal sample size based on the VO_{2peak}. We calculated a minimum sample of 11 participants to reach a 0.3L/min when comparing the conditions in order to achieve 80% statistical power (5.2 Granmo, IMIM, Barcelona, Spain). The subjects were required to meet the following inclusion criteria in order to be eligible for this study: a) being male; b) being a taekwondo athlete; c) \geq 18 yrs.; d) training \geq 3 x per week; e) no consumption of any supplements or drugs; f) no history of using medications which could alter the hypothalamic-pituitary-gonadal (HPG) axis, such as anabolic steroids; g) no history of chronic disease, bronchospasm or atopy; h) no respiratory infections during the previous month; and i) no recognized asthma or allergy during the 3 years preceding the study. All participants were instructed by a nutritionist to maintain their habitual diet. The subjects were excluded if they: a) did not complete the entire protocol; b) had a problem during data collection; or c) did not reach the maximum on the testing. Thus, 20 athletes were invited to participate in this study, and 13 of them accepted, however, one was excluded due to errors during achieved the spirometric data. Therefore, the final sample consisted of 12 male athletes (26.8±8.8 yrs.; 77.8±11.7 kg; 1.8±0.1 m; 25.3±3.2 kg/m²; 10.0±5.3 Fat%).

Test Procedures

Participants were separately randomized and instructed to perform a 2-hour fasting before data collection. Next, 1g of Beetroot extract (Nature's Way', Green Bay, USA) or 1g of Placebo (dextrose) was consumed in capsules (double-blind), followed by a standard snack consisting of a medium-sized apple, chocolate milk, two slices of bread and two slices of ham (~430 Kcal). All conditions were packed in identical capsules. An independent researcher was involved and he/she was the only one who knew the processing order and the exact nature of the capsules.

After the anthropometry was performed, we measured body weight (Welmi 104A Scale, SP, Brazil), height (Auturexata Stadiometer, SP, Brazil) and skinfold thickness (Cescorf caliper, SP, Brazil). We applied

the seven-fold equations of Jackson and Pollock [1978] and Siri equation [Johnson 1996] to estimate the fat percentage.

Spirometric variables were measured during the Progressive Specific Taekwondo Test (PSTT), a specific test for Taekwondo athletes validated with the incremental treadmill test (r=0.86) by Sant'Ana et al. [2019]. It is a progressive test to exhaustion and the participants were familiarized with the PSTT and respective movements. The test was performed in an area of 2x2 meters and consisted of alternately kicking (Bandal-tchagui) a punching bag (height between the navel and nipples). The participant must remain in step (combat stance hopping) during the test. Six alternate kicks are performed (starting with the right leg) in the first stage, and then four kicks are added in each completed stage. An application developed for the test was used (ITSriker, ETS4.ME, Brazil) for intensity control. The test was interrupted when: a) the frequency of kicks was not maintained; b) they did not reach the mark determined on the punching bag; or c) they voluntarily stopped. Participants were advised to avoid exercise and refrain from caffeine and alcohol consumption 48 h before the experiment. Moreover, participants were instructed to maintain their regular dietary habits. The experimental protocol was carried out at an ambient temperature of 25°C.

The spirometer was calibrated before each test according to the manufacturer's indications (breath-to-breath gas analyzer, Metalyzer 3BR2, Cortex', Leipzig, Germany). The following criteria described by Laursen *et al.* [2002] were followed for a test to be considered as maximum: a) Blood lactate ≥8.0 mmol·L-¹ (Accutrend Plus, Roche', Rotkreuz, Switzerland); b) respiratory exchange ratio (RER) >1.1; and c) heart rate (HR) > 90% of predicted (Polar', RS800, Kempele, Finland). Figure 1 shows a chart of test procedures.

Spirometric measures were calculated after the test. The exported software spreadsheet was analyzed for each participant, and the outliers were removed every 15 s of measurement. VO_{2Peak} (absolute and relative)

was the highest value observed at each of these intervals according to Sant'Ana *et al.* [2019]. VE/VO_2 and VE/VCO_2 were calculated according to Baba *et al.* [1996]. VO_2 at anaerobic threshold (AT) absolute and relative was calculated by the V-slope method according to Schneider *et al.* [1993].

Statistical analysis

We initially performed exploratory data to identify and correct extreme values. Normality and homoscedasticity were tested by the Kolmogorov-Smirnov test and by the Bartlett criterion, respectively. The two-way repeated measure ANOVA was applied to establish the difference for blood lactate and HR (for these variables we measured the isolated effect of the supplement, moment of measurement and interaction of supplement and moment of measurement). The Mauchly sphericity test was implemented (the Greenhouse-Geisser correction was used when necessary) for validating the repeated measurements. The post-hoc Bonferroni test was adopted when a significant difference was observed in ANOVA. The paired T-test was used for the other variables. Furthermore, the Eta squared (η^2) was calculated as effect size for ANOVA, presenting 0.01 (small), 0.09 (medium) and 0.25 (large). Cohen's d was also used to estimate the magnitude of the effect for the T-test, presenting 0.1 (small), 0.3 (medium) and 0.5 (large). Lastly, $p \le 0.05$ significance level was adopted in all analyzes, and all analyses were performed with the Statistical Package for the Social Sciences (version 20.0, Chicago, IL, USA).

Results

The variables AT and RER presented 2 outliers each, for these the extreme values were replaced by the mean. Table 1 shows the results for spirometry and complete stages in the effort test. There was a difference between

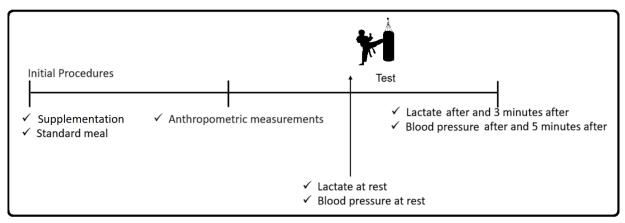


Figure 1. Procedures followed during Beetroot and Placebo tests.

Table 1. Results for spirometry variables and total stages completed by Beetroot and Placebo conditions.

Variable	Condition	Mean ± standard deviation	Statistic	Effect size
VO _{2Peak} (L/min)	Beetroot	4.0±0.6*	t=2.093; p=0.048	0.39
	Placebo	3.6±0.5		
VO _{2Peak} (mL/kg·min)	Beetroot	52.3±7.0	t=1.651; p=0.116	0.32
	Placebo	46.8±9.4		
Anaerobic threshold (L/min)	Beetroot	3.7±0.6*	t=2.136; p=0.044	0.39
	Placebo	3.2±0.6		
Anaerobic threshold (mL/kg·min)	Beetroot	47.9±5.1	t=1.99; p=0.059	0.38
	Placebo	43.2±6.3		
VE/VO ₂ /AT(mL/kg·min)	Beetroot	35.4±4.9	t=0.324; p=0.749	0.06
	Placebo	34.7±6.5		
VE/VCO ₂ /AT (mL/kg·min)	Beetroot	29.7±3,1	t=0.634; p=0.81	0.04
	Placebo	30.0±3.5		
RER Final Stage (a.u)	Beetroot	1.3±0.2*	t=0.388; p=0.609	0.05
	Placebo	1.3±0.3		

AT – anaerobic threshold. RER – respiratory exchange ratio. * p≤ 0.048 vs. PLA.

groups for the absolute $VO_{2Peak}(p=0.048)$ and VO_2 at the anaerobic threshold (p=0.044) with a large effect size.

Figure 2 shows the results for complete stages for each condition; there was a difference between groups (p=0.009).

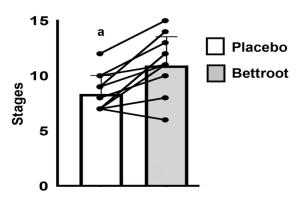


Figure 2. The final stage for Placebo (white bar) and Beetroot extract (gray bar). ^a p=0.009 vs. Beetroot.

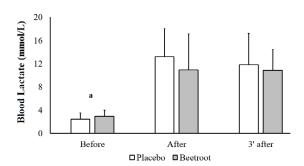


Figure 3. Blood lactate before, after, and 3minutes after the test, a p \leq 0.001 for this moment of measurement vs the others.

There was an isolated effect for the moment of measurement for the HR, with a large effect size

($F_{1.48}$ =5.24; p≤0.001; $η^2$ =0.6), where the means measured before were statistically lower compared to those after (106.4±5.4 and 104.0±5.9 vs. 204.6±5.6 and 205.6±6.5 BPM for Placebo and Beetroot before and after the test). All criteria considered for a test to be maximum were achieved. Figure 3 shows the results for blood lactate before the test, after and 3 minutes after the test. There was an isolated effect of the moment of measurement with a large effect size ($F_{1,72}$ =52.515; p≤0.001; $η^2$ =0.705), where the means observed before were statistically lower than those observed after and 3 minutes after (p≤0.001 for both comparisons).

Discussion

Due to the importance of aerobic metabolism for taekwondo athletes, supplements which increase aerobic capacity may result in better athletic performance [Lopes-Silva et al. 2015]. Thus, the present study investigated the effect of beetroot on the performance of athletes submitted to a specific maximal aerobic test. The main results indicated that the Beetroot condition presented a higher absolute VO_{2Peak} and VO_{2} at AT. Furthermore, only one athlete (Figure 2) completed lower number of stages in the Beetroot condition compared to the Placebo. To the best of our knowledge, this is the first study with this research focus; although in a review study, Stebbins [2016] suggests there is an ergogenic effect of nitrate supplementation in taekwondo athletes, however, no article that directly investigated the effect in this combat sport was revised. Nonetheless, an ergogenic effect has been previously demonstrated in predominantly aerobic sports [Christensen et al. 2013; McMahon et *al.* 2017] and in intermittent exercise [Wylie *et al.* 2016; Wylie *et al.* 2013].

This study verified the sample size in order to minimize the bias associated with this data collection, controlling the data with a randomized, double-blind, placebo-controlled trial to minimize possible external factors. The observed results agree with a systematic review (nine papers) by Wylie et al. [2016], who observed that Beetroot supplementation may result in better performance in intermittent exercises by delaying the onset of fatigue and increased muscle power, but the mechanisms are poorly understood. The primary ergogenic mechanism of beetroot is associated with a large amount of NO₃ per gram [Ormsbee et al. 2013]. NO₃ improves aerobic performance via the organic conversion of this compound to nitric oxide (NO). NO acts as a vasodilator by increasing blood flow [Maiorana et al. 2003], which indirectly increases the oxygen supply to the muscle during exercise [Breese et al. 2013]. In addition, NO₃ is effective in reducing the cost of muscle O, and ATP in submaximal exercises via increased mitochondrial production of esterified phosphate radicals, especially in type II fibers [Breese et al. 2013; Larsen et al. 2011]. Breese et al. [2013] observed that six days of supplementation with beetroot juice (140 ml/day) increased the kinetics of pulmonary and muscle VO₂. Our results indicate that this effect also occurred in supplemented athletes, as we observed a higher consumption of O₂ at the anaerobic threshold.

Despite numerous studies proving the effectiveness of Beetroot intake in VO, [Campos et al. 2012; Lopes-Silva et al. 2015; Ormsbee et al. 2013; Sant'Ana et al. 2018], the results of studies directly related to sports performance are inconsistent [McMahon et al. 2017]. This result is possibly associated with the heterogeneity of factors that interfere with sports performance [D'Isanto et al. 2019]. The heterodox characteristics of sports performance can also be seen in taekwondo athletes [Moreira et al. 2014], thus, even if there is an ergogenic effect of the supplement, the combat performance can be affected by technical or psychological barriers. In fact, two studies with supplementation in taekwondo showed opposite results in performance, caffeine [Lopes-Silva et al. 2015] and sodium bicarbonate [Lopes-Silva et al. 2018] improve the glycolytic metabolism, but only sodium bicarbonate increases the performance during combat.

Few studies investigated the effect of beetroot supplementation in intermittent exercises. Possibly, ergogenic effects are associated with the vasodilator capacity of nitric oxide and higher creatine phosphocreatine resynthesis [Dominguez et al. 2018], since, the deficiency of O₂ supply results in a lower muscle creatine resynthesis [McMahon, Jenkins 2002]. In team sports athletes who performed intermittent sprints on a cycloergometer, Wylie et al. [2013] observed an ergo-

genic effect (70mL, 8.2 mmol NO_3) only in very short sprints (24 x 6s) vs. short sprints (7 x 30s and 6 x 60s). Williams *et al.* [2020] observed an increase in power, velocity and performance until failure in the bench press (28.0 \pm 5.6 vs. 30.6 \pm 5.8 reps.). In a systematic review (9 papers), Dominguez *et al.* [2018] observed that single or repeated doses tend to increase the work output in intermittent exercises, but the ergogenic mechanisms are speculative. In combat sports specifically, which also have the main characteristic of intermittence [Miarka *et al.* 2018], no study to the best of our knowledge has tested this type of supplementation.

An important result of the present study was the better performance test in the beetroot condition (Figure 2). This result is in agreement with other studies that showed the ergogenic effect of beet in efforts until failure [Breese et al. 2013; Callahan et al. 2017; McMahon et al. 2017; Ormsbee et al. 2013]. Recently, Cocksedge et al. [2020] observed in 10 healthy male subjects undergoing moderate submaximal exercise, that beetroot juice (210 mL, 18.6 mmol NO₃⁻) increases the transport of O₂ to the muscle under hypoxia condition, without improving physical performance. Similarly, Breese et al. [2013] observed an increase of 22% in the kinetics rate of deoxyhemoglobin in exercise until failure, when compared six days of supplementation (140 ml~ 8mmol of NO₃-) versus placebo, but the same did not occur in submaximal exercise. Therefore, we hypothesized that the difference between the Placebo and Beetroot observed in our study is related to the higher capacity to use oxygen in the final stages of the test, thus increasing the resynthesis capacity of muscle phosphocreatine [Bailey et al. 2010].

Our results for the lactate showed only a significant effect of the moment of measurement. The effect of supplementation on blood lactate is unclear. Our results are in agreement with others previously published [Bond et al. 2012; Wylie et al. 2013]. In a study with sport team athletes, 490 mL (8.2 mmol NO₃⁻) beetroot juice resulted in a higher performance in the Yo-Yo test [Wylie et al. 2013]. However, other studies have found an ergogenic effect of supplementation, especially in the final stages of exercise [Dominguez et al. 2017; Wylie et al. 2016]. Possibly, vasodilation from supplementation may increase the speed of lactate buffering [Wylie et al. 2016], thus, it is expected that the increased blood flow to the type II fibers will result in an increased strength close to fatigue [Williams et al. 2020]. We believe that further studies are needed to clarify the effect of supplementation on blood lactate, as there are studies, with a single dosage [Dominguez et al. 2017], or multiple dosages [Wylie et al. 2016], that show ergogenic effects. However, the mechanisms are speculative.

The protocol adopted in the present study is one of the first to use beetroot extract, however, Callahan *et al.* [2017] previously observed an increased NO₃ buffer in cyclists supplemented with beetroot crystals (300 mg)

and sodium bicarbonate (0.3 g/kg), but there was no difference in performance when submitted to 4 km Time Trial. There was a difference between the total activity time, where the study mentioned the athletes performed approximately 5 min of effort (~338 s), whereas the athletes in the beetroot condition in our protocol performed about 11 stages (~800 s). Thus, the ergogenic effect of supplementation possibly demands longer physical stimulation time. In this line, the metanalysis by McMahon et al. [2017] on nitrate supplementation and performance indicated that time trial tests generally have no beneficial effect by supplementation, but tests up to exhaustion tend to have a beneficial effect from beetroot (ES=0.33, 95%CI = 0.15–0.5, p \leq 0.01). Specifically, for taekwondo supplementation benefits can be obtained under training and competition conditions. Official matches are disputed in 3 rounds of 2 min. with 1 min. interval [Bridge et al. 2014], in addition, in the world championship it is necessary to win 5 combats to be champion. Thus, the athlete's ability to recover between rounds and combats is very important. In this sense, the use of supplementation, as it shows an ergogenic effect regarding the improvement of aerobic capacity, can assist the athlete in moments of recovery and also increase performance in moments close to fatigue. In addition, ergogenic effects are also observed in high-intensity efforts [Dominguez et al. 2018; Wylie et al. 2016] and muscle power [Williams et al. 2020], thus, the ergogenic effect can benefit during the competition, as the athlete will perform more strikes at high power.

We believe that future studies can test this supplementation protocol in training or competition models. We also observed in the literature the absence of studies with female athletes. We also recommend measuring NO₃ concentration, which was a limitation of the present study. The results of studies have been consistent in showing an ergogenic effect in healthy subjects [McMahon et al. 2017] and athletes [Dominguez et al. 2018]. However, in highly trained athletes, studies have failed to show the benefits of NO₃ [Bourdillon et al. 2015; MacLeod et al. 2015; Nyback et al. 2017]. Our study was carried out in regional level athletes, we also recommend for future studies, to test if the ergogenic effects also occur in high-level taekwondo athletes. Finally, this was the first study to use beetroot capsules, we chose to use the dosage indicated by the manufacturer of the supplement. However, we do not know the amount of NO₃ in each capsule. The absence of this measure should be interpreted as a limitation.

Conclusion

Through the established aims and applied methods in this study, we concluded that the supplementation of 1g of beetroot increased the $\rm O_2$ uptake in taekwondo athletes submitted to a maximum aerobic test.

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Ekstrakt z buraka ćwikłowego poprawia swoistą wydolność i pobór tlenu u zawodników taekwondo: Badanie typu crossover z podwójnie ślepą próbą

Słowa kluczowe: sztuki walki, ćwiczenia aerobowe, wydolność sportowa, suplement diety

Streszczenie

Wprowadzenie. Suplementy bogate w azotany, takie jak ekstrakt z buraka ćwikłowego, mogą poprawić wydolność aerobową poprzez zwiększenie funkcjonowania nerwowo-mięśniowego. Jednakże niewiele wiadomo na temat wpływu ekstraktu z buraka na sporty walki, takie jak taekwondo.

Problem i cel. W niniejszej pracy badano ostry efekt suplementacji ekstraktu z buraka ćwikłowego (1g) na zawodników taekwondo poddanych maksymalnemu testowi aerobowemu. Metody. Dwunastu sportowców (26,8±8,8 lat; 77,8±11,7 kg; 1,8±0,1 m; 25,3±3,2 kg/m2; 10,0±5,3 Fat%) poddano szczegółowemu protokołowi aerobowemu taekwondo. Wszyscy uczestnicy wykonywali naprzemienny protokół maksymalnych kopnięć (bandal-chagi) w dwóch randomizowanych warunkach, pod wpływem: a) ekstraktu z buraka; oraz b) placebo. Wyniki. Przy zastosowanie ekstraktu z buraka zanotowano wyższą bezwzględną wartość VO_{2Peak} (4,0±0,6 vs. 3,6±0,5 L/min; p=0,048), bezwzględną wartość $\mathrm{VO}_{\mathrm{2max}}$ na progu beztlenowym (3,7±0,6 vs. 3,2±0,6 L/min; p=0,044) oraz całkowite/zakończone etapy (10,9±2,6 vs. 8,3±1,7 L/min; p=0,009). Stężenie mleczanów wzrastało istotnie pomiędzy pomiarami wykonanymi przed, po i 3 min po (p≤0,001) teście, bez różnic pomiędzy warunkami (p=0,46).

Wnioski. Uzyskane wyniki sugerują ergogeniczny wpływ suplementacji ekstraktu z buraka ćwikłowego (1 g) na wydolność tlenową i wyniki testów specjalnych u zawodników taekwondo.