SECTION – SPORT SCIENCES

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# EFFECTS OF EXERCISE TRAINING AND CREATINE MALATE SUPPLEMENTATION ON VENTILATORY THRESHOLD AND ANAEROBIC WORKING CAPACITY IN LONG-DISTANCE RUNNERS

Authors' contribution:
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B. Data collection/entry
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**Key words:** runners, creatine malate, ventilatory threshold, $VO_2$max, total anaerobic work, body composition.

## Summary

**Purpose**. The aim of this study was to investigate the influence of a 6-week physical training programme and creatine malate supplementation on forming aerobic and anaerobic exercise capacity in long-distance runners in an experimental group (n = 7) and control group (n = 7).

**Basic procedures.** The ventilatory threshold, the distance covered to the threshold, oxygen uptake per minute, heart rate, the percentage of maximal oxygen uptake, as well as the total distance, and maximal oxygen uptake were analysed. In a jumping test (single jump and a series of 15 jumps), total work and height of the gravity centre elevation were registered.

**Main findings.** Only the experimental group showed a significant lengthening of the run distance to the ventilatory threshold and to the total distance, while in the jumping test (series of 15 jumps) an increase of anaerobic work was registered.

**Conclusions.** Changes observed in runners suggest an ergogenic effect of creatine malate supplementation. In the control group, no significant changes in the analyzed variables were noted.

## Introduction

Enriching one's daily diet with supplements as dictated by increased demand for energy has become a standard practice in professional athletes. This is due to, inter alia, the body's limited ability to absorb nutrients. In training, it is recommended to enrich the diet of professional athletes with ergogenic supplements [1–3].

The worldwide popular effect of creatine compounds on the human body (creatine monohydrate, creatine pyruvate, creatine citrate, creatine ethyl ester, creatine malate) gives absolutely no side effects [4–9]. Many years ago, it was observed that creatine makes an excellent phosphate medium. By creating phosphocreatine, it becomes a key substance in the process of ATP re-synthesis in the muscle cells, providing an adequate level of this high-energy compound essential in the mechanism of muscle contraction [1]. Creatine is considered a physiological aid in cellular metabolic processes that determine muscle strength and the amount of the generated power, what is particularly vital for sprinter athletes. The ATP re-synthesis in anaerobic conditions using phosphocreatine is a considerably more effective metabolic pathway than substrate phosphorylation, as it contributes to lower muscle acidification, which in turn, affects the increased workout intensity [10].

An interesting aspect of creatine supplement administration is its concentration variation in muscle cells. It is suggested that creatine supports exercise by participating in several metabolic mechanisms. Firstly, together with increased creatine concentration in the skeletal muscle, it rises the ability of ADP re-phosphorylation or ATP-re-synthesis, resulting in better performance during high-intensity activities, particularly if they are interspersed with restitution periods [11, 12]. Examples of such activities are sprint runs, weightlifting or jumping. Secondly, creatine enhances penetration of phosphate between the mitochondria and chains of myosin resulting in positive impact on muscle ability of maximum contraction. Thirdly, creatine acts as a buffer by neutralising the pH caused by progressive metabolic acidosis, utilising hydrogen ions during the ADP re-phosphate reaction to ATP catalyzed by creatine kinase. Fourthly, by lowering the PCr level due to increased ATP demand during exercise, it can re-stimulate phosphofructokinase by accelerating glycolysis, which results in increased production of ATP [11, 13]. Creatine (Cr) is produced endogenously by the liver or delivered to the body with food, whereas the daily pool of endogenous Cr is about 2 g for a person of 70 kg BM. Its resources are mainly located in the muscles; 40% is free creatine, and the remaining 60% is phosphocreatine (PCr). The PCr concentration is significantly higher in the II type of muscle fibres and depends on gender (higher concentration was observed in men [14]) and age. A slight decrease occurs with the aging of the body, however, it is not certain whether this is related to age as such, or to decreased physical activity [15]. In the body of a person of 70 kg BM, there is c. 130 g of creatine. The body loses approximately 2 g of it per day, half of which the amount is recovered by means of synthesis in the liver. The remaining 1 g should be provided through food [13]. The increase of creatine concentration, and in result the phosphocreatine concentration in the muscles, contributing to a significant intensification of the ATP rate re-synthesis [16], is the main ergogenic effect of this compound supplementation. It directly affects fatigue delay and accelerates the process of restitution after training at high or maximum intensity [17]. It also affects the increase in muscle strength and mass [18], anaerobic power, and lean body mass – LBM [5, 19, 20], and consequently, anaerobic capabilities used during running [21]. The most popular dietary supplement among athletes is a hydrated form of creatine – creatine monohydrate. The disadvantage of this supplementation is its fairly easy transformation into inactive creatinine. In order to obtain a good effect of supplementation, it should be taken in high doses, which is not always accepted by athletes or their training teams. Studies of the impact of creatine supplementation on anaerobic strength, power and endurance development in professional athletes, inter alia swimmers, athletes, sprinters, representatives of team sports, including American football players, have been conducted by many researchers [16–18, 21–28]. There is less information available on the physiological effects of low-dose athlete supplementation with creatine malate (CML) in typical endurance sports [3]. Therefore, this study has made the attempt of determining the effect of CML supplementation on the development of selected physiological indicators in long-distance runners, taking into account a typical - for this period of the macrocycle – physical training session. Such researches are legitimated by the fact that so far, in the branch literature, there are few studies on the effects of the CML supplementation on the development of physiological indicators at ventilatory threshold (VT), and anaerobic and aerobic capacity in long-distance runners. Researches focusing on professional athletes specializing in typical endurance disciplines also seem to be interesting because so far, in the branch literature, more attention has been paid to representatives of speed and strength sports, which significantly differ from long-distance runners regarding body composition, which may affect reactivity of the preparation.

## Materials and Methods

### Participants

Out of the 20 professional athletes selected for the study, finally qualified were 14 individuals aged 18–30, having practised running for a minimum of 4 years, specializing in long-distance runs >5 km, who had not received any creatine formulations for at least 1 year. All volunteers were informed on the purpose, methodology, and possible risks during the studies, and then, having been familiarised with the procedure, signed written informed consent for participation, with the right to resign at any time. The tests were conducted during an introductory mesocycle and basic preparatory period, during which the physical strain during training sessions were at a predominant percentage maintained below the ventilatory threshold (VT). The sportsmen in the experimental group (E) n = 7 were administered creatine malate (CML), and the ones in the control group (P) n = 7 received a placebo (blind test). Runners were allocated into groups E and P in pairs, so as during the experiment they could undergo a similar training programme. The runners in the groups E and P did not differ regarding their morphological body structure. In the initial research, the structural and biometric body indicators showed the following values, respectively: BH 176 ± 3.46 cm and 175 ± 4.11 cm, BM 65.88 ± 3.77 kg and 65.31 ± 3.55 kg, PF 6.44 ± 0.23% and 7.35 ± 1.82%, and LBM 61.20 ± 3.80kg and 60.34 ± 4.85kg. No significant differences were registered in their level of $VO_2$max (E = 70.10 ± 7.35 ml · $kg^{-1}$ · $min^{-1}$ and P = 69.46 ± 7.20 ml · $kg^{-1}$ · $min^{-1}$), either. The research project obtained consent from the Bioethics Commission for Scientific Research at the Regional Medical Chamber in Krakow to carry out the experiment (Opinion No. 76 KBL/OIL/2008).

### Measurements

Body height (BH) was measured with a *Martin* (USA) anthropometric device, body mass (BM) with the *Sartorius scales type F 1505 – DZA* (Germany). The percentage of fat (PF), lean body mass (LBM) and body mass index (BMI) were measured with *Densitometer DEXA 2013 Lunar Prodigy Primo Full*, with the body composition option, manufactured by *GE Healthcare Technologies* (USA). The graded exercise test was executed on a mechanical treadmill, type *Saturn-250/100R h/p/Cosmos* (Germany). It took place in an air-conditioned laboratory of the Department of Physiology and Biochemistry in an ambient temperature of 21±0.5°C, and relative humidity of 45± 3%. The warm-up was carried out using the *Corival Ergometer (Lode BV)* (Netherlands). Respiratory and cardiovascular exchange ratios were measured with *OxyconPro apparatus (Care Fusion Healthcare GmbH), Jaeger* (Germany), while in the jumping test, the amount of total work (TW) and the height (h) of rising the gravity centre was measured with the *Optojump V.3-high-Microgate* (Italy) Device.

### Jumping Test

After a 5 minute warm-up at 50% $VO_2$max intensity on an ergometre, pedalling frequency of 70 rpm, and three maximum accelerations in the last 5 seconds in the 2nd, 4th, and 5th minutes, the subjects began the jumping test. It consisted in executing on a platform one, maximum vertical jump without an arm swing (RR), and after the next 2 minutes of rest, a series of 15 jumps at a frequency dictated by a metronome; 1 jump per second. During the test, the height (h) of raising the gravity centre and total work (TW) were measured.

### Exercise test

On the second day, a graded exercise test on a treadmill was carried out. It was preceded with a 3-minute warm-up (WU) at a running speed of 2.3 $m \cdot s^{-1}$, followed by an every-three-minute increased running speed by 0.5 $m \cdot s^{-1}$. The exercise continued until refusal, i.e. to the point, when the tested person was not able to run at the imposed speed any more. During the run, the following were measured: tidal volume (TV), respiratory frequency (FR), minute ventilation ($V_E$), minute oxygen uptake ($VO_2$), volume of carbon dioxide ($VCO_2$), respiratory rate (RER), percentage of carbon dioxide in the air exhaled from the lungs ($FeCO_2$), breathing equivalent $V_E \cdot O_2^{-1}$ and $V_E \cdot CO_2^{-1}$, which were used to determine the second ventilatory threshold (VT) [28–30], and heart rate (HR).

### Procedures

The research was carried during a nine-week period, between 8:00 a.m. and 12:00 a.m.. In the first week, the athletes underwent pilot studies to eliminate the psychological factor associated with stress arising from a new situation. Physiological body reactions to graded exercise were examined. In the second week, for two days, basic examinations were done (I-test). On the first day, biometric and structural measurements of the body were performed, and a jumping test was carried out: a single jump test without arm swing (RR), and a series of 15 jumps RR swing. On the second day, a graded exercise test until exhaustion was performed. Then, for six weeks, every day, two hours after breakfast, the men were orally administered capsules containing 0.07 g · $kg^{-1}$ LBM of creatine malate manufactured by *OLIMP* (Poland), corresponding to about 5 g of this preparation for a person of lean body mass (LBM) of 70 kg [14]. The supplement was taken with 250 ml of plain, boiled water. During that time, the athletes trained according to the plan and used a standard, given diet. In an identical manner, the athletes in the control group (P) were given placebo capsules. The E and P athletes were not informed about different contents of the administered capsules (blind test). They just received information that for 6 weeks, they would be receiving an identical, permitted supplement. During that period, the athletes trained in pairs, according to the training plan, and applied a typical diet, which they registered in their record-books. During the course of the study they declared not to take other supplements. Once the saturation of the body with CML or placebo was completed, they started the second main tests. All tests were carried out in an air-conditioned laboratory.

### Statistical Analysis

For statistical analysis of numerical material, the *Statistica* 9.0 package for *Windows* by *StatSoft* was used. The results were presented as mean ($\bar{x}$) and standard deviation (SD). Changes in the indicators due to training were evaluated by using two-way analysis of variance (ANOVA) with repeated factor measurement (measurement I vs. measurement II) and one-factor intergroup (group E vs. group P). Evaluation of the differences at one level of a factor was made by using the strategy of planned comparisons. When the cases did not meet the assumptions of normal distribution, due to the small sample size (7 people), results were compared with the nonparametric *U Mann-Whitney* test for two unrelated trials and the *Wilcoxon* test for two related ones. As the results of these analyses were consistent, we decided to present the results of analysis of variance, as it is greatly more versatile and resistant to deviate from the assumptions of normality of distribution. The adopted level of statistical significance was $p < 0.05$.

## Results

After 6 weeks, there were no significant changes in the level of biometric (BH and BM) and structural (PF and LBM) body indicators among the runners from the E or P groups. In the E group supplemented with CML, despite no essential differences, a slight tendency to increase in PF and LBM was observed. Also registered was a significant increase in the running distance to the ventilatory threshold ($D_{VT}$) by about 19% ($p < 0.018$), which was not observed in the placebo group (P) undergoing a similar training programme. In both groups, E and P, in the second tests (after), the distance to the ventilatory threshold ($D_{VT}$) was extended respectively, by 555m ($p < 0.018$) and 100 m ($p < 0.654$) (Table 1).

In both groups, E and P, no significant changes in cardiopulmonary indicators were registered at $VO_2max$ level. (Tab. 2). Meanwhile, in the experimental group (E), in the graded exercise test a significant ($p < 0.017$) increase of the covered distance (D) was registered. In the

**Table 1.** Physiological indicators registered at the ventilatory threshold (VT)

| Indicators | Group | Training period | | Changes* |
|---|---|---|---|---|
| | | Before | After | (p < 0.05) |
| | | Mean ± SD | Mean ± SD | p-value |
| $HR_{VT}$ | E | 168 ± 7.67 | 166 ± 8.89 | 0.478 |
| (b · min⁻¹) | P | 173 ± 8,99 | 172 ± 7.12 | 0.386 |
| $VO_{2VT}$ | E | 3.35 ± 0.63 | 3.32 ± 0.62 | 0.328 |
| L · min⁻¹) | P | 3.76 ± 0.68 | 3.61 ± 0.98 | 0.421 |
| $\%VO_2max$ | E | 73.9 ± 6.99 | 78.9 ± 8.88 | 0.213 |
| (%) | P | 83.5 ± 5.58 | 80.5 ± 7.10 | 0.298 |
| $D_{VT}$ | E | 3159 ± 389.14 | 3714 ± 281.14 | **0.018** |
| (m) | P | 3082 ± 389.14 | 3182 ± 364.21 | 0.654 |

**Table 2.** Physiological indicators registered in the graded test at the $VO_2max$ level

| Indicators | Group | Training period | | Changes* |
|---|---|---|---|---|
| | | Before | After | Before |
| | | Mean ± SD | Mean ± SD | Mean ± SD |
| HRmax | E | 183 ± 5.40 | 178 ± 4.11 | 0.289 |
| (b · min⁻¹) | P | 178 ± 6.14 | 181 ± 5.57 | 0.265 |
| $VO_2max$ | E | 4.67 ± 0.62 | 4.62 ± 0.58 | 0.319 |
| (L ·min⁻¹) | P | 4.49 ± 0.67 | 4.46 ± 0.85 | 0.222 |
| $VO_2max$ | E | 70.10 ± 7.35 | 70.02 ± 5.44 | 0.193 |
| (mL · kg⁻¹ · min⁻¹ | P | 69.46 ± 7.20 | 68.69 ± 7.30 | 0.293 |
| D | E | 5080 ± 334.14 | 5340 ± 362.58 | **0.017*** |
| (m) | P | 4958 ± 263.06 | 5005 ± 265.98 | 0.270 |

**Table 3.** Total work and height of gravity centre elevation in the jumping test [maximum single jump ($_p$) and a series of 15 jumps ($_s$)]

| Indicators | Group | Training period | | Changes* |
|---|---|---|---|---|
| | | Before | After | Before |
| | | Mean ± SD | Mean ± SD | Mean ± SD |
| $TW_P$ | E | 196.1 ± 24.56 | 196.5 ± 26.87 | 0.389 |
| (J) | P | 185.1 ± 26.65 | 185.5 ± 26.58 | 0.361 |
| $TW_s$ | E | 2194 ± 42.89 | 2553 ± 42.14 | **0.042*** |
| (J) | P | 2208 ± 60.13 | 2103 ± 60.36 | 0.242 |
| $h_p$ | E | 32.21 ± 3.41 | 32.80 ± 4.55 | 0.197 |
| (cm) | P | 28.97 ± 3.23 | 30.06 ± 2.34 | 0.198 |
| hs | E | 22.65 ± 1.89 | 26.53 ± 2.11 | **0.037*** |
| (cm) | P | 22.33 ± 2.21 | 22.85 ± 1.36 | 0.156 |

placebo group (P), these changes were not statistically significant. The results of the covered distance (D) in the second tests (after) between E and P groups were statistically significant ($p < 0.037$).

In runners from groups E and P, in the first and second tests, in the test consisting of a single jump ($_p$) the results of total work ($TW_p$) and the elevation of the gravity centre height ($h_p$) did not differ significantly (Table 3). Whereas in the 15 jumps test (s), in the runners supplemented with CML significant increases in total work ($TW_s$) ($p < 0.042$) and elevation of the gravity centre height ($h_s$) ($p < 0.037$) were found, which was not present in the placebo group (P). In the second tests (after) the results of TWs and $h_s$ were significantly higher in the E than P group, $p < 0.045$ and $p < 0.036$, respectively.

## Discussion

In the last twenty years, a number of studies on the morphological and functional changes resulting from creatine compounds supplementation: creatine monohydrate, creatine pyruvate, creatine citrate, creatine ethyl ester, or creatine malate have appeared [2, 3, 16–18, 21–26, 29–31]. These publications focus mainly on very specific issues such as the impact of its administration on the ability to generate power as well as speed and power endurance, or training adaptation in athletes [7, 3]. Until now, it has been empirically demonstrated that administration of creatine provides an ergogenic effect in sportsmen practicing speed or strength disciplines. There are also suggestions that the ergogenic effect of creatine supplementation may be greater in men than in women. Our team has conducted research aiming at assessing the impact of CML supplementation in sprinters and long-distance runners – athletes involving the efforts of different systems for power generation. In these groups of athletes, a different ergogenic effect of the administered CML was found. In sprinters, it showed a considerable increase in peak power and total work in the *Wingate test* [32], and progression of lean body mass. However, in long-distance runners, it only showed a slightly significant increase in aerobic capacity, as in the graded exercise it showed a significant increase in the covered running distance. Much less information is available regarding its impact on exercise capacity in typical endurance sports representatives. The present study has made an attempt to verify the body's response to CML administration in long-distance runners. In order to determine the influence of CML supplementation and physical training, the runners presenting an identical sports class were selected into groups E and P in pairs, so as in\\over the 6-week period of the study they could undergo almost the same exact training program, in identical conditions of the external environment. In long-distance runners, in graded exercise, the ergogenic effect of body supplementation with CML showed a significant shift of the anaerobic threshold towards a higher speed, yet without any changes in $VO_2max$. This indicates that supplementation with CML may increase the total work in a long-term graded test. This is attributed to accelerated aerobic phosphorylation during exercise at lower intensities, which affects oxygen balance [33]. It also shows the indirect effects of CML supplementation on aerobic fitness. Engelhardt et al. [34] noted that administration of creatine in a low doseage of 2 x 3 g daily for 6 days to athletes in endurance sports does not affect the circulatory system, oxygen uptake or lactate concentration in the blood. However, although the triathletes' endurance during interval exercises increased by as much as 18%, the indicators defining aerobic capacity did not change at all. It was therefore concluded that administration of creatine could have positive impact on short-term physical activity, which might be an intrinsic element shaping endurance. This is in line with our results of the long-distance runners tests. Meanwhile, Nelson et al. [35] studied the response of the cardiorespiratory system to a graded test on a cycle ergometre. Creatine caused a significant increase in working time. Maximal oxygen uptake and maximum heart rate decreased in the test group. The ventilatory threshold occurred by a much higher percentage of oxygen consumption (increase from 66% to 72% $VO_2$max). It was concluded that creatine affects various components of the metabolic systems during initial phases of graded exercise. This contributes to the athlete's ability to endure a sub-maximal training load at a lower oxygen cost and by a reduced load of the circulatory system. Moreover, the Chwalbińska-Moneta's study [23] has shown that creatine supplementation enhances aerobic capacity, regardless of the intense endurance training effects. Jones et al. [36] examined the impact of creatine on $VO_2$max. Their research evidenced that creatine supplementation does not result in changes in maximal oxygen uptake, but significantly influences the reduction of the $VO_2$ temporary components at the initial phase of high-intensity exercise. In the above study, the vastus lateralis muscle biopsy was carried out. It turned out that the percentage of type II fibres was closely correlated with the reduction level of sub-maximal $VO_2$, which indicates that supplementing with creatine may be associated with changes in the amount of motoneurons recruited during exercise, or with the volume of activated muscles [36]. In our research, administering CML to the runners resulted in significant prolongation of the running distance to the ventilatory threshold (VT), and the total running distance (D). Similar changes were observed in our previous studies in which, however, we found no anaerobic ergogenic effect of CML supplementation. In the *Wingate test*, in the individuals supplemented with CML, no progression in peak power or total work results was observed. In the present study, after 6 weeks of training and CML supplementation, we observed progression in group E of anaerobic performance in the series of 15 jumps on a dynamographic platform, and no effect in the single jump test. Such structure of the results suggests that the improvement in aerobic endurance in runner athletes also originated from an increased speed and endurance strength. In other studies, in a jumping test – series of 10 jumps in 60 seconds – after supplementation, an increase in the average elevation of the gravity centre was observed, despite a simultaneous, negligible increase in body weight, suggesting large influence of creatine on the ability to overcome short-term high-intensity workloads [37]. In some opinions, creatine can stimulate aerobic capacity by increasing the ability to carry out intensive interval efforts [38]. It seems that for determining the peak power and anaerobic work of athlete runners, due to the nature of the movements, running or jumping tests should be employed, rather than, for example, cycling tests, e.g. *Wingate* [32]. Routine tests on runner athletes conducted in our laboratory show that the results of PP and TW in the cycling test are quite remotely correlated with the results of these indicators in running and jumping tests. Many researchers have noted that creatine supplementation combined with specific physical training has a direct relationship not only with the increase of maximum strength and ability to manage higher and higher physical loads during a resistance training, but also with lean body mass [15, 19, 39, 40]. Some reports indicate that after creatine compound supplementation and physical training, the LBM changes are not the same in sprinters and long-distance runners, which is likely due to the different nature of the training types and body composition of the athletes. In our study, we found no significant changes in the composition, however, in the runners from group E, we observed a tendency for LBM to increase.

## Conclusions

The physical training and supplementation with creatine malate applied for six weeks have led to increased aerobic and anaerobic endurance in long-distance runners, but without any significant changes in $VO_2$max and body composition (BM, PF, LBM), and has also resulted in later occurrence of ventilatory threshold and longer distance covered to VT. The positive ergogenic effect of supplementation observed in men indicates validity of supplementing the body with CML not only in speed and strength sports athletes, according to the reference literature data, but also in the long-distance runners.

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