Baltic Journal of Health and Physical Activity

Volume 8 | Issue 3 Article 1

2016

Intermittent exercise models may be basic in research of creatine complex effects in aerobic and anaerobic performance of athletes and Cr supplementation influence

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Recommended Citation

Szczesna-Kaczmarek A. Intermittent exercise models may be basic in research of creatine complex effects in aerobic and anaerobic performance of athletes and Cr supplementation influence. Balt J Health Phys Act. 2016; 8(3): 7-19. doi: 10.29359/BJHPA.08.3.01

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Abstract

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Keywords

supramaximal intermittent exercise, creatinine excretion, phosphagenic component of total work, glycolytic component of total work, recovery time between exercise bouts

Baltic Journal of Health and Physical Activity 2016;8(3):7-19
Journal of Gdansk University of Physical Education and Sport in Gdansk
e-ISSN 2080-9999

ORIGINAL

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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article details

Article statistics **Word count:** 3,751; **Tables:** 1; **Figures:** 5; **References:** 39

Received: April 2016; **Accepted:** August 2016; **Published:** September 2016

Full-text PDF: http://www.balticsportscience.com

Indexation: AGRO, Celdes, CNKI Scholar (China National Knowledge Infrastructure), CNPIEC, De Gruyter - IBR (International Bibliography of Reviews of Scholarly Literature in the Humanities and Social Sciences), De Gruyter - IBZ (International Bibliography of Periodical Literature in the Humanities and Social Sciences), DOAJ, EBSCO - Central & Eastern European Academic Source, EBSCO - SPORTDiscus, EBSCO Discovery Service, Google Scholar, Index Copernicus, J-Gate, Naviga (Softweco, Primo Central (ExLibris), ProQuest - Family Health, ProQuest - Health & Medical Complete, ProQuest - Illustrata: Health Sciences, ProQuest - Nursing & Allied Health Source, Summon (Serials Solutions/ProQuest, TDOne (TDNet), Ulrich's Periodicals Directory/ulrichsweb, WorldCat (OCLC)

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest: Author has declared that no competing interest exists.

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INTRODUCTION

Each sport has specific properties in terms of motor skills that demand specific conditions of participants, both physiologic and metabolic for that particular sport. The most important aspect of the metabolic demands is the proper contribution of the three energy systems (aerobic, anaerobic-alactic and anaerobic-lactic ones) in the adequate proportion to the total energy required by an organism for this activity. Determination of the specific demands of acyclic sports (for example: football, judo, karate, hockey and others) is not easy. In the case of metabolic requirements of these sport disciplines, we ought to take into consideration the regulation processes which join aerobic and anaerobic metabolic reactions. According to known scientific publications, the key roles of creatine-phosphocreatine system (Cr-PCr system) and creatine kinase in cellular energy translocation from mitochondria to cytoplasm and vice versa are established. Furthermore, it is known that the Cr-PCr system is also called the Cr-PCr shuttle [1, 2, 3, 4]. ATP homeostasis in skeletal muscles is maintained by PCr via the reverse reaction catalysed by creatine kinase.

It is known that the muscle store of PCr is decreased not only during high-intensity exercise but also during submaximal exercise. The decrease in muscle PCr during submaximal exercise is in proportion to the work rate [5]. PCr determined after short-term exercise is rapidly restored in a reaction catalysed by creatine kinase in which Cr and ATP are used as substrates.

Creatine is an endogenous compound produced at an amount of about 1g/24h and also is available to the body through diet about 1g/24h. As many as 95% of the body creatine is found in the skeletal muscle and the remaining 5% is placed in the brain, the liver, the kidney, and testes [6]. The majority of creatine synthesis is located in the liver and the kidney [6, 7]. Creatine must be transported from places of synthesis via blood to tissues of utilization and storage. Cr is transported from blood into tissues against a concentration gradient through a sodium- and chloride-dependent transporter [8, 9]. Catecholamines, insulin, insulin-like growth factor 1 and exercise can influence the net uptake of Cr into skeletal muscle cells. The content of Cr is dependent on the skeletal muscle fibre type. Type 2 (FT) fibres have higher levels of Cr and PCr than fibre of type 1 (ST) [10].

Creatine is the most popular dietary supplement in sports nutrition, because it determines the level of muscle phosphocreatine – high-energy phosphagen. Oral Cr supplementation can elevate human skeletal muscle creatine by 10–30% [11].

Knowledge about profitable effects of creatine on the body is gathered systematically. At present some results of research proved pleiotropic effects of creatine for the cells function and the cells protection. Endogenous creatine and Cr supplementation raised muscle cellular energy state (PCr/ATP) [3, 4, 12, 13, 14] and facilitated intracellular energy transport (PCr/ATP shuttle) between cytoplasm and mitochondria, stimulated mitochondrial respiration [13, 14] and improved the efficiency of energy utilization [15, 16]. Moreover, some researchers showed its anti-oxidant effect of improving the neurological function in elderly and young people [17] and positive effects in muscular dystrophies [18].

However, there are also studies which reported that creatine supplementation was ineffective either during repeated bouts of anaerobic exercise or during aerobic activity, and they did not state that creatine supplementation improved performance of professional athletes [19, 20, 21].

Although our knowledge about the effects of Cr supplementation on exercise performance is significant, as yet the mechanism by which creatine supplementation might improve performance is not entirely clear, even though it seems clear that this effect is related to the increased muscle CP content. The explanation of mechanisms that are principal, in particular by which creatine can achieve the energetic effects during supramaximal intermittent exercise, is still incomplete, because it does not justify the role of Cr in stimulation of mitochondrial respiration and in facilitation of intracellular energy transportation.

In the present study we aimed to assess the effects of short-term creatine supplementation on performance capacity and power output during supramaximal intermittent exercise in athletes. It was important for us to apply the exercise test in which the three energy systems could supply muscles with energy. We aimed to apply the exercise test (HIIE) consisting of three repetitions of 30s Wingate Test interspersed with 7-minute rest, on the assumption that it is the exercise test creating conditions to assess the diverse contribution of the three energy systems demanded for acyclic sports. We also assumed that the applied exercise conditions were adequate to observe not only the Cr contribution in enhancing the phosphagen system that is involved in high-intensity exercise performance but also in the creatine phosphate shuttle between mitochondria and cytosol that may enhance both anaerobic and aerobic capacity. In our exercise test the rest between consecutive exercise bouts was suitably long to enable the Cr/CrP-creatine kinase shuttle to develop.

In these conditions the physiological variables characterizing aerobic and anaerobic processes were measured in athletes and their significance was used to suggest the importance of creatine supplementation in the interaction between aerobic/anaerobic muscle metabolism at rest and during exercise. The following effects of short-term oral creatine supplementation were examined:

- on the relative contribution of the phosphagenic and glycolytic work during high-intensity intermittent exercise in athletes,
- on anaerobic performance during subsequent high-intensity exercise bouts,
- on the blood acid-base equilibrium during high-intensity intermittent exercise,
- on creatinine excretion to 24h-urine after high-intensity intermittent exercise,
- on the relationship between the value of creatinine excreted to 24h-urine after high-intensity intermittent exercise and aerobic capacity in athletes.

MATERIALS AND METHODS

SUBJECTS

Eleven active physical education students additionally competing in different training (javelin – 2 persons, discus – 2 persons, shot put – 4 persons, weight lifting – 1 person, tennis – 2 persons) volunteered in the study. Subjects' age, height, and weight were 23.01 ±2.16 yrs, 180.5 ±9.6 cm, 97.0 ±12.8 kg (means ±SE), respectively. This study had been approved by the local research ethic committee and was conducted in accordance with the Declaration of Helsinki. The results obtained in the group supplemented with placebo were the control for those obtained in the group supplemented with creatine.

Table 1. Anthropometric and physiological characteristics of subjects measured before placebo or creatine supplementation (n = 11), values are mean ±SD

Anthropometric characteristics		Physiological characteristics	
		Aerobic performance	
Age (years)	22.6 ±2.1	$\dot{V}O_2$ max (L · min^{-1})	4.53 ±0.36
Weight (kg)	97.5 ±12.9	$\dot{V}O_2$ max (ml · min^{-1} · kg^{-1})	46.74 ±7.46
Height (cm)	185.9 ±4.80	AT (mLO_2 · kg^{-1} · min^{-1})	35.55 ±6.42
BMI (kg·m^2)	22.8 ±2.46	AT (%VO_2max)	75.50 ±8.35

THE CREATINE SUPPLEMENTATION PROGRAM

All participants were prescribed 5 days of dietary supplementation with creatine monohydrate (Cr) and after 48h, which were designed for laboratory exercise test (HIIE) and recovery, they repeated next 5 days of dietary supplementation with maltodextrin – placebo (PL). The participants were blind to the order of supplementations used. During all trials the participants were not informed either about the sort of supplementation or the sequence of application.

SUPPLEMENTATION PROCEDURE

The Cr subjects (CrS) ingested 5.0 g creatine monohydrate (Olimp Sport Nutrition) 4 times a day for 5 days. The PL group consumed 5.0 g maltodextrin, which was matched with the creatine powder for taste and colour, also 4 times a day for 5 days. The participants mixed the powder in approximately 200 ml of warm water of the creatine monohydrate or maltodextrin and consumed this solution immediately after preparation together with breakfast, lunch, dinner and before sleep.

EXPERIMENTAL PROTOCOL

Participants made three visits to the laboratory over a period of 2 weeks. During the first visit – during two consecutive days a participant performed two tests: an incremental cycloergometric test (ICT) for the assessment of peak oxygen uptake and the gas exchange threshold [22]. After 24 hours of recovery participants performed three bouts of supramaximal intermittent exercise (3 x 30-s Wingate Test) (HIIE). Each bout of exercise lasted 30 s and was interspersed with 7-min recovery. 30-s Wingate Test was performed on a Monark mechanically braked cycloergometer. After warming up, the subjects began pedalling as rapidly as possible during 30 s against a heavy resistance of 74 N/

kg body mass. The load was applied before the subject started his work. This trial was repeated after each applied supplementation. During each exercise the following values were measured: total work (TW) values in kJ and kJ per kg b.m., peak and mean power output in watts and watts per kg b.m. (RPP and RPM, respectively), fatigue index (FI), time required to approach peak power (TrPP), time during which subjects sustained the peak power output, time during which subjects kept peak power output (TkPP).

Before and 4 min after each bout of exercise and after HIIE we drew arterialized blood samples to determine parameters of acid-base equilibrium, and before HIIE and after HIIE 24-h urine was collected to determine creatinine content.

STATISTICAL ANALYSIS

Statistical analyses were performed using Statistica StatSoft, version 9. Statistical significance was accepted at $p < 0.05$ level. Results are presented as mean ± standard deviation. Differences in parameters estimated between the placebo and creatine trials were analysed using Student's paired *t*-test. Statistical evaluation of the comparisons between the CR and PL trials were made using one-way ANOVA with repeated measures. When a significant F value was achieved, Fisher's LSD post hoc test was used to provide information on which means were significantly different from one another.

RESULTS

WORK CAPACITY, POWER AND FATIGUE INDEX AND EFFECT OF CREATINE SUPPLEMENTATION

Total work production during supramaximal intermittent exercise was higher after creatine supplementation than after placebo by about 7% and 4.5% in bout 1 and 2, respectively, but in bout 3 the total work done was about 2% lower. Relative values of total work were only 2% higher in subjects after Cr--supplemented in relation to PL (differences not significant). Both total values and relative values of work during bouts 2 and 3 were significantly lower than the values achieved during bout 1 both in the group after placebo and after creatine supplementation (Fig. 1A). On average, the total work done for subjects after placebo and creatine was reduced during bout 3 by about 10% and 8%, respectively. In consecutive repetitions of the Wingate test we controlled the participation of phosphagenic and glycolytic components of total work done (Fig. 1B, C). The measured shares of two components (phosphagenic work and glycolytic work) of total work during consecutive three exercise bouts as follows: 18.1% and 81.9%, 14.8% and 85.2%, 13.9% and 86.1%, respectively in PL subjects, and 32.3% and 67.6%, 20.6% and 79.4%, 28% and 72%, respectively in CrS subjects. Cr ingestion resulted in an increase in the phosphagenic work participation in total work during exercise bouts 1, 2 and 3 by about 179%, 139%, 260%, respectively as well as simultaneous cumulative decline in the glycolytic work participation by about 35.9% (Fig. 1).

Absolute and relative values of peak power output during exercise bouts 1, 2 and 3 were greater in subjects after creatine supplementation than after placebo ingestion, but differences were not significant. Values of peak power during exercise bouts 2 and 3 for both placebo and creatine subjects were significan-

tly lower than the values achieved during bout 1 (Fig. 2 A, B). Accordingly, the examined group after creatine supplementation reached greater power peak in a shorter time (differences between Cr supplemented subjects and PL were significant) and kept up the same level for a longer time (difference not significant) (Fig. 2). On average, the time in which the creatine group reached power peak during bouts 1, 2, 3 were reduced by about 19%, 11%, 6%, respectively. In contrast to the reductions in power measured in successive exercise bouts, the fatigue index was unchanged (25–27%); furthermore, changes did not show significant differences after creatine or placebo ingestion (Fig. 3C).

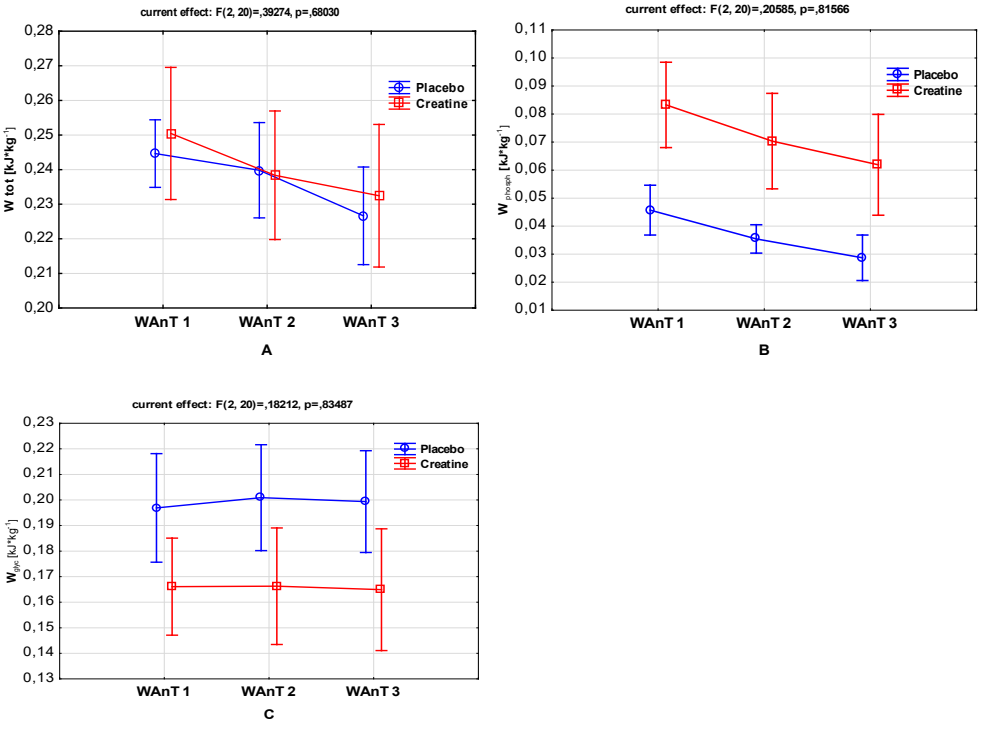


Fig. 1. Changes in relative total work (A), and values of the relative phosphagenic component (B) and the glycolytic component of total work,(C) during HIIE (3 bouts of 30-s Wingate Test, each bout of exercise was separated by 7 min of passive recovery). Values are given in the placebo group (circles) and the Cr supplemented for 5 days group (squares). Values represent means ± SE. Significant differences placebo- , and creatine-supplementation (Fig. 1B and 1C) ($p < 0.05$)

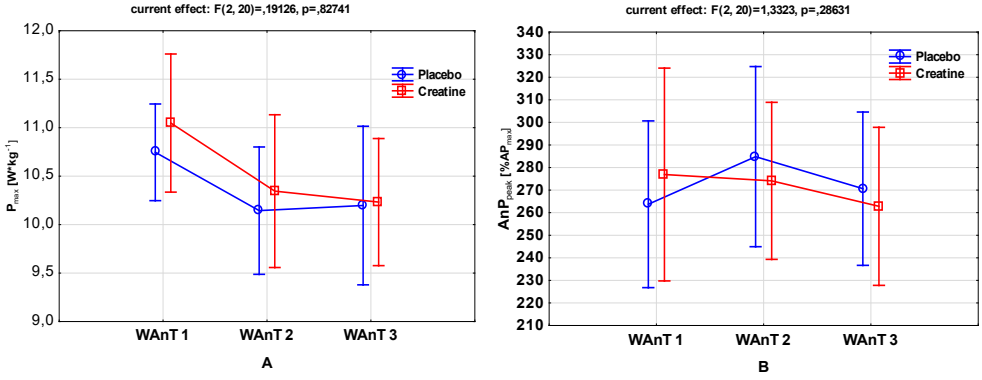


Fig. 2. Changes in Peak Power values (A – absolute values, B – relative values J x kg body mass^{-1} during HIIE (3 bouts of 30-s Wingate Test, each bout of exercise was separated by 7 min of passive recovery). Values are given in the placebo group (circles) and the Cr supplemented for 5 days group (squares). Values represent means ± SE. Significant differences placebo-, and creatine-supplementation (Fig. 2A and 2B only in the first bout) ($p < 0.05$)

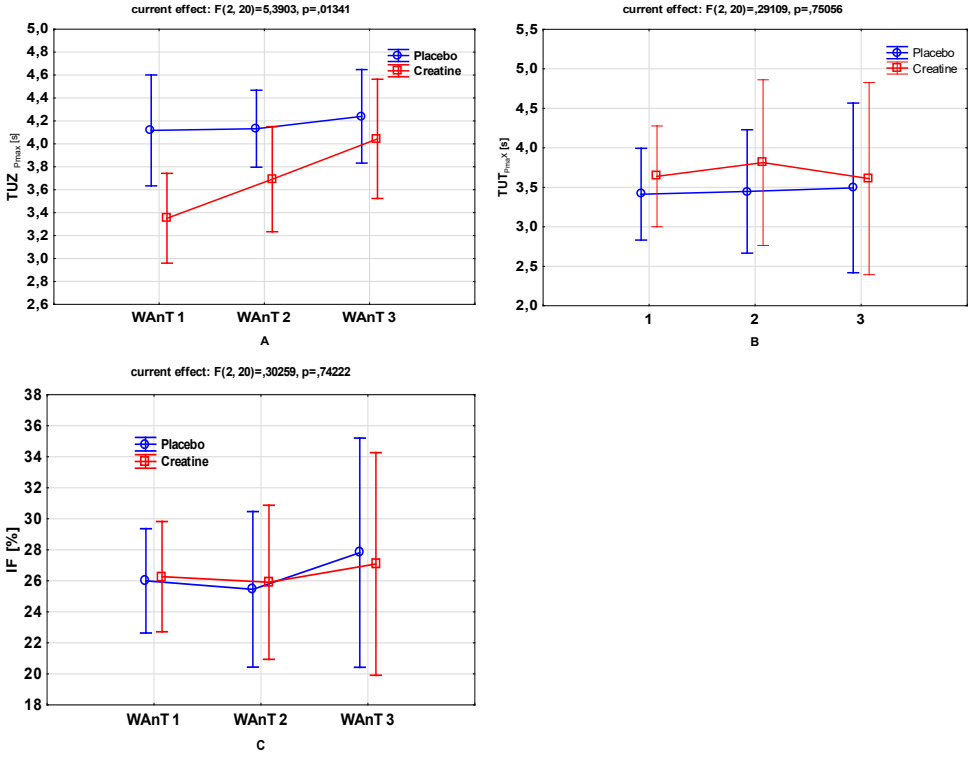


Fig. 3. Time of Peak Power approach (TUZ) (A), Time of Peak Power maintenance (TUT) (B) Fatigue index (IF) (C) during HIIE. Protocol and data analysis are as described in Fig 1. Values represent means ± SE. Significant differences placebo-, and creatine-supplementation (Fig. 3A only in the first bout) ($p < 0.05$)

BLOOD ACID-BASE STATUS

Cr supplementation caused changes in blood buffers capacity.

Blood pH decreased progressively during successive supramaximal exercise bouts and reached 7.13 ±0.3 and 7.15 ±0.5 at the end of the third exercise bout in the PL- and Cr-group, respectively ($p < 0.001$), but differences between PL-subjects and Cr-subjects were not significant (Fig. 4A).

Plasma [HCO_3^-] decreased progressively from 23.69 ±0.98 mmol/l and 23.99 ±0.69 mmol/l in PL and CrS subjects, respectively, to reach following the three exercise bouts 11.08 ±0.86 mmol/l and 11.94 ±1.56 mmol/l in subjects PL and Cr, respectively ($p < 0.001$) (Fig. 4B).

Blood BE decreased progressively during intermittent supramaximal exercise from (-)1.26 ±0.98 mmol/l at rest to (–)19.01 ±1.9 ($p < 0.001$) during the third exercise bout in subjects after placebo; however, in the same circumstances the blood BE in subjects after Cr-supplementation decreased from (-)0.79 ±1.05 to (-)18.13 ±2.67), but differences between PL- and CrS-subjects were not significant (Fig. 4C). Somewhat larger acidification in PL subjects after consecutive exercise bouts could be a result of weakened participation of work phosphagenic component in total work done.

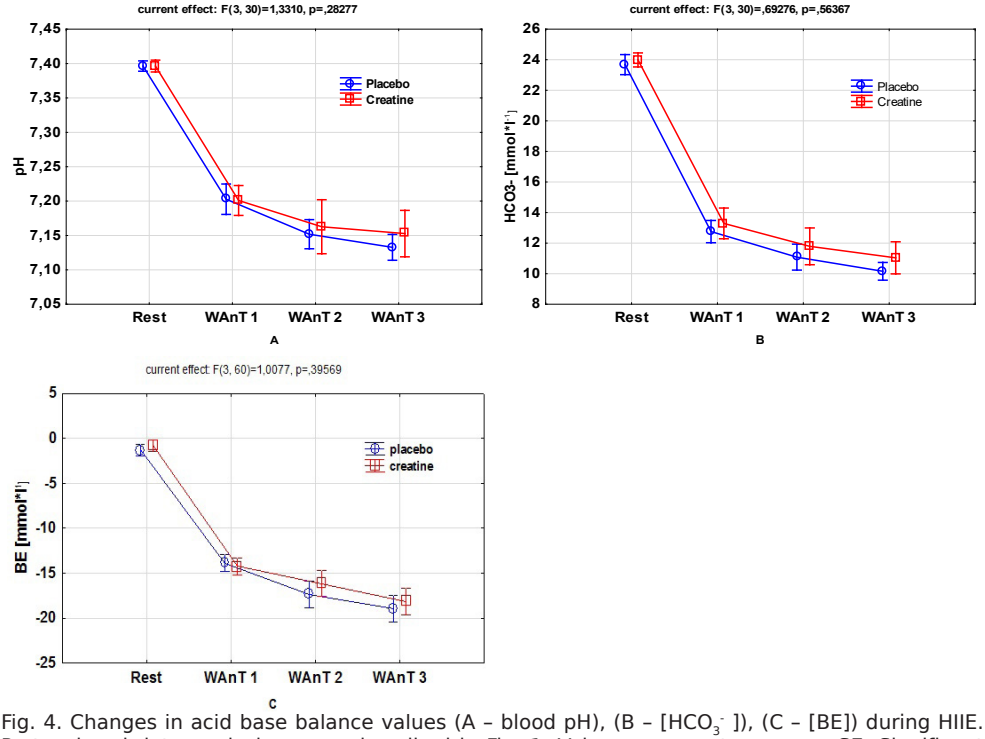
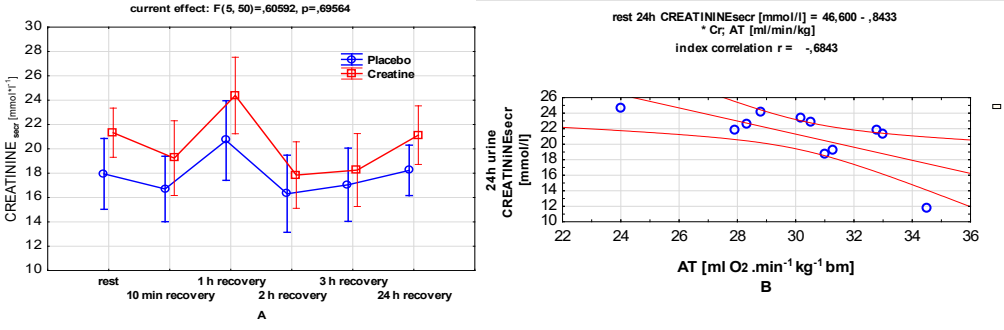


Fig. 4. Changes in acid base balance values (A – blood pH), (B – [HCO_3^-]), (C – [BE]) during HIIE. Protocol and data analysis are as described in Fig. 1. Values represent means ± SE. Significant differences placebo-, and creatine-supplementation in [HCO_3^-] (Fig. 4B) ($p < 0.001$).

CREATININE EXCRETION TO URINE



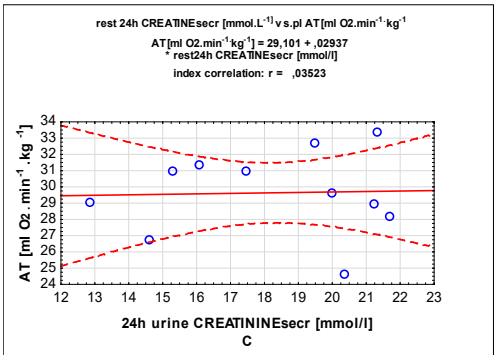


Fig. 5. Creatinine secretion to 24-urine (A), correlation between [creatinine in 24-urine] and AT values in creatine and placebo supplemented groups (B) and (C), respectively.
Values represent means ±SE. Significant differences placebo-, and creatine-supplementation in creatine secretion to 24-h urine) ($p < 0.005$). The increased creatinine concentration in 24h--urine in Cr-supplemented subjects after HIIE was negatively correlated with anaerobic threshold (r =(-)0.6849, r2 = 0.4683) (Fig. 5B). Such relationships between AT and creatinine value in 24-h urine did not appear in placebo supplementation (Fig. 5C).

As depicted in Fig. 5A, the amounts of creatinine excreted to 24h-urine at rest and after intermittent exercise were greater after creatine than after placebo supplementation. Differences were significant at rest, 1h and 24h after cessation of exercise ($p < 0.05$). The greatest creatinine excretion was deter-

mined 1h after exercise cessation, both in CrS and PL. The increased creatinine concentration in 24h-urine in Cr-supplemented subjects after intermittent supramaximal exercise was negatively correlated with relative peak VO_2 (r = (-)0.34686) and with anaerobic threshold (r = (-)0.6849, r^2 = 0.4683) (Fig. 5B, C). Such relationships between AT and creatinine value in 24-h urine were not shown in subjects after placebo supplementation; however, a weaker (r = (-)0.25038) negative correlation between VO_2max and creatinine value in 24-h urine was affirmed. The result described above showed the relationship between Cr absorption and subject's physical efficiency. Higher Cr absorption revealed in subjects was characterized by higher AT and VO_2max.

DISCUSSION

The present study investigated the effects of oral creatine supplementation on both anaerobic and aerobic performance during repeated bouts of intense exercise separated by passive recovery and verified the hypothesis that there is a relationship between the physical efficiency of athletes supplemented with creatine and the amount of creatinine excreted to urine after high-intensity intermittent exercise. The experimental procedure was based on supramaximal intermittent exercise (3 bouts of 30-s Wingate Test separated by 7-min rest) because this model of exercise more accurately reflected the activities in some acyclic sports which are characterized by bursts of high intensity aerobic activity followed by periods of both anaerobic activity and short aerobic rest. Under conditions of supramaximal intermittent exercise we investigated changes in parameters defining anaerobic power, total work done and its phosphagenic and glycolytic components, and also fatigue factors in subjects supplemented with both placebo and creatine. The obtained results reflected not only the effects of elevated muscle Cr concentration on energy contribution from the ATP-PCr system and the anaerobic glycogenolytic energy system yielding increase in $[H^+]$ and maintained physiological pH but also on a share of aerobic processes. Due to long duration (30 seconds) of high intensity repeated exercise bouts and seven minutes rest between them, the used HIIE determined the experimental conditions to develop both anaerobic processes and ATP/PCr shuttle regulating the oxidative function of mitochondria [2, 3, 5]. The obtained results showed an increase in cumulative total anaerobic work done on average by 3.5% in subjects supplemented with creatine compared to the placebo group (differences not significant). The improvement in performance after Cr supplementation was probably caused by an increased ability to resynthesize PCr during the recovery periods. The increasing availability of PCr may better maintain the required rate of ATP demand during exercise. But the revealed decline of total work done during consecutive exercise bouts could indicate the growing share of aerobic processes and only about 3% increase of glycolysis in the energy yielding during repeated bouts of exercise [23, 24]. Another result of this study demonstrated the contribution of both components of total work done, i.e. phosphagenic and glycogenolytic work in which the phosphagenic and glycogenolytic systems maintained the rate of energy required during three bouts of supramaximal exercise (Fig. 1). This result allowed us to evaluate more precisely the effect of Cr supplementation on performance. Cr supplementation caused an increase in phosphagenic work values, on average by 85%, and, simultaneously, a decrease in the contribution of glycogenolytic work, on average by 18%, during three successive exercise bouts. The mechanism behind this performance effect probably lies

in the elevated muscle CrP content, which increases the capacity for ATP rephosphorylation in a reaction catalysed by creatine kinase [2, 8, 15, 25, 26, 27]. Analysing the value of glycolytic work as part of total work done in which contribution of the glycolytic system is crucial to ATP yielding, we observed a significantly higher participation of this work in PL supplemented subjects compared with Cr supplemented ones ($p < 0.001$).

Taking in consideration changes in the quantitative contribution of glycolytic work and phosphagenic work in total work done during successive exercise bouts, we observed interesting results. The contribution level of glycolytic work remained almost constant during successive exercise bouts. Simultaneously, the contribution level of phosphagenic work in total work done decreased systematically down to 25% compared with first and third exercise bouts in subjects supplemented with both placebo and creatine (Fig. 1). Almost constant participation of glycolytic work in total work done can be explained by the fact that the glycogenolysis activity in muscle cells was inhibited by significantly increased [H^+] after the first exercise bout, which was deepened only insignificantly during successive exercise bouts.

The decrease in phosphagenic work was gradual, probably due to diminished recovery resynthesis of PCr after successive high-intensity exercise bouts. It is known that the tempo of ATP supply imposes the rate of PCr resynthesis. The PCr resynthesis rate may reflect a contribution of both glycolytic and oxidative metabolism in ATP production [23]. Muscle cells acidification affected the PCr resynthesis because it caused inhibition of glycogenolysis activity not only during successive exercise bouts, but also during successive recovery periods, when glycolysis provided ATP to the fast phase of CrP resynthesis and also weakened the slow phase of PCr resynthesis [23, 24, 28]. Inhibition of glycogenolysis by acidosis was mediated via H^+ effects on activity of phosphorylase, hexokinase and phosphofructokinase – crucial enzymes to glycolysis [24, 29, 30].

The above mentioned trend in changes was observed not only in total external work done but also in power output generated during the successive bouts of supramaximal exercise. Five days of creatine supplementation resulted in a significant increase in average peak power output. The mechanism of these changes probably lay in elevated muscles PCr concentration, because the maximal power output was reached in the initial seconds of each exercise bouts and completely depended on ATP derived from the PCr/ATP system [31]. Even though values of peak power in Cr supplemented subjects was higher compared to the placebo supplemented ones, a decrease in peak power during the second and the third exercise bout appeared in all subjects. The observed in this work gradual decline in power output during successive supramaximal exercise bouts was probably connected with muscle cells acidification and the availability of PCr or with some other fatigue factors which impaired sarcoplasmic reticulum function and reduced [Ca^{++}] and caused weakness of excitation–contraction coupling [32, 33]. The availability of PCr in contracting muscle was determined by processes controlling the cell Cr level and kinetic resynthesis of PCr in muscle cells [34]. It is known that 50% of PCr content may be restored in about 25s, while the total restoration pre-exercise levels take 5 to 8 min [34, 35]. Several studies have shown that the kinetics of PCr resynthesis is enhanced with endurance training in athletes, and that the pro-

cess is dependent on O_2 availability [25, 35, 36]. Earlier results of the same authors showed that the greater O_2 uptake during consecutive exercise bouts resulted from the increased Cr uptake rate in the fast oxidative fibres type recruited to muscle contraction [26, 37]. In oxidative fibres Cr is involved in ATP production through the PCr system engaged in shuttling of ATP from the mitochondria into the cytosol [3, 5, 35, 38]. In the light of the above mentioned knowledge, our result concerning a negative relationship between the value of creatinine excreted to urine during 24-h rest after intermittent supramaximal exercise and the value of anaerobic threshold ($r = (-)0.6849$; $p < 0.05$) in Cr–supplemented subjects was understandable.

The ergogenic effect of creatine applied in both anaerobic and aerobic activity was probably connected with the muscle fibres structure. A number of distinct mechanisms of this phenomenon were activated during recovery after cessation of exercise bouts. The earlier results of scientists have shown that the total creatine content of skeletal muscle differs more than twofold from other fibre types [26, 33]. Furthermore, individuals with higher intramuscular Cr and PCr levels and those with fewer type II muscle fibres were less responsive to supplementation [37]. There is important evidence that the Cr uptake rate during interval rest was significantly elevated by Cr depletion in high-oxidative fast-twitch muscle fibres [33]. Our results could suggest that humans with high content of oxidative slow and oxidative fast twitch muscle fibres and higher anaerobic threshold connected with the type of muscle fibres could be more responsive to Cr uptake. Our explanation is supported by previous achievements of some authors. Earlier research by Chwalbińska-Moneta [38] showed that creatine supplementation improves endurance in elite rowers by an increase in the individual lactate threshold. Results of Volek et al. [27] have shown an increase in muscle fibre diameter in type 1 and type 2 muscle fibres by 35% in Cr supplemented men after 12 weeks of resistance training. A positive effect of creatine supplementation on aerobic exercise has been shown in Branch's paper [39]. Branch showed that oxidative phosphorylation was an energy supplier during exercise lasting longer than 2.5 min and suggested that creatine supplementation may lead to a change in energetic substrate utilization and an increase in endurance performance.

CONCLUSION

The model of supramaximal intermittent exercise (HIIE) applied in this research, consisting of three repetitions of 30s Wingate Test with 7-minutes rest periods between tests, enabled us to measure parameters characterizing aerobic and anaerobic processes in which creatine/phosphocreatine shuttle maintained muscle cells energetic homeostasis. Under these conditions the influence of creatine supplementation on the interaction between aerobic and anaerobic muscle metabolism was studied. In this experimental system we revealed a relationship between the absorbed level of supplemented creatine and athletes' efficiency.

Author is aware that further research should be conducted on that issue.

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Cite this article as:
Szczęsna-Kaczmarek A. Intermittent exercise models may be basic in research of creatine complex effects in aerobic and anaerobic performance of athletes and Cr supplementation influence. Balt J Health Phys Act. 2016;8(3):7-19.