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ORIGINAL CONTRIBUTION

Contribution of creatine to protein homeostasis in athletes after endurance and sprint running

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Abstract
Purpose Few studies have focused on the metabolic changes induced by creatine supplementation. This study investigated the effects of creatine supplementation on plasma and urinary metabolite changes of athletes after endurance and sprint running.
Methods Twelve male athletes (20.3 ± 1.4 y) performed two identical (65–70 % maximum heart rate reserved) 60 min running exercises (endurance trial) before and after creatine supplementation (12 g creatine monohydrate/day for 15 days), followed by a 5-day washout period. Subsequently, they performed two identical 100 m sprint running exercises (power trial) before and after 15 days of creatine supplementation in accordance with the supplementary protocol of the endurance trial. Body composition measurements were performed during the entire study. Plasma samples were examined for the concentrations of glucose, lactate, branched-chain amino acids (BCAAs), free-tryptophan (f-TRP), glutamine, alanine, hypoxanthine, and uric acid. Urinary samples were examined for the concentrations of hydroxyproline, 3-methylhistidine, urea nitrogen, and creatinine.
Results Creatine supplementation significantly increased body weights of the athletes of endurance trial. Plasma lactate concentration and ratio of f-TRP/BCAAs after recovery from endurance running were significantly decreased with creatine supplementation. Plasma purine metabolites (the sum of hypoxanthine and uric acid), glutamine, urinary 3-methylhistidine, and urea nitrogen concentrations tended to decrease before running in trials with creatine supplements. After running, urinary hydroxyproline concentration significantly increased in the power trial with creatine supplements.
Conclusions The findings suggest that creatine supplementation tended to decrease muscle glycogen and protein degradation, especially after endurance exercise. However, creatine supplementation might induce collagen proteolysis in athletes after sprint running.

Keywords Purine metabolites · Glutamine · Alanine · Hydroxyproline · 3-Methylhistidine · Urinary urea nitrogen

Introduction

Creatine has been used as an ergogenic supplement. It is produced endogenously in kidneys, liver, and pancreas, or obtained from exogenous sources such as meat, poultry, and fish [1]. Creatine is stored in the cytosol of muscle cells as phosphocreatine (PCr) that prevents the rapid depletion of ATP by providing a readily available high-energy phosphate to regenerate ATP from ADP [2]. During exercise, muscle cells obtain energy via several pathways, which involve ATP-PCr system, anaerobic glycolysis, TCA cycle, and electron transfer system [3]. When ATP hydrolysis rate exceeds ADP rephosphorylation rate, ATP is then resynthesized via the myokinase reaction, resulting

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in the formation of adenosine monophosphate (AMP) [4]. AMP is rapidly deaminated to inosine monophosphate (IMP) and ammonia via AMP deaminase. IMP can also be further degraded to hypoxanthine, xanthine, and uric acid. Consequently, these purine metabolites are released into the circulation and used as biomarkers of localized energy deficiency [5]. In fact, plasma ammonia and hypoxanthine levels are considered as markers of adenine nucleotide loss during exercise [6]. Ammonia is then transferred to glutamate which is subsequently converted to glutamine [7].

Although lack of ergogenic effect in aerobic condition, creatine supplementation was assumed to benefit sprint performance, such as repeated or short bouts of high-intensity exercise [8], and their mechanisms of actions have also been postulated [9]. The impact of creatine supplementation on the metabolite changes after endurance exercise, however, remains to be understood. When ATP is depleted with endurance exercise, branched-chain amino acids (BCAAs, i.e., leucine, isoleucine, and valine) in the muscle are oxidized to meet the energy demand [10], which induces muscle proteolysis [11]. Meanwhile, BCAAs are also removed from blood, resulting in an increased ratio of free-tryptophan (f-TRP, the precursor of serotonin) to BCAAs in plasma [12], which enhances serotonin (a neurotransmitter which may induce a sense of drowsiness, possibly associated with fatigue [12]) synthesis in the brain. This f-TRP to BCAAs ratio, therefore, is assumed to be associated with central fatigue during prolonged exercise [13]. During endurance exercise, gluconeogenesis is also enhanced. Alanine is formed by transamination of pyruvate (produced by glycolysis) and exported to the liver for gluconeogenesis [14]. Candow et al. [15] found that creatine supplementation resulted in decreased urinary 3-methylhistidine (3MH) excretion due to reduced muscle proteolysis. The excretions of 3MH [16] and urinary urea nitrogen (UUN) [17] are used as indicators of protein and/or amino acid degradation [11]. Thus, analysis of plasma concentrations of hypoxanthine, uric acid, glutamine, alanine, f-TRP, and BCAAs, along with the urinary 3MH and UUN excretions after exercise, will be helpful for understanding how creatine supplementation may be related to energy metabolism and protein homeostasis.

Most previously related studies have emphasized the effects of creatine supplementation on athletic performance; few publications have been on the metabolism and protein homeostasis. It is difficult to make the comparisons among the associated studies, due to the differences in participants' characteristics and experimental design. The present study, therefore, was designed to investigate the influences of creatine supplementation on the concentrations of plasma and urinary metabolites after exercise, and make comparisons between endurance and power exercise of well-trained athletes after creatine supplementation. The hypothesis of this investigation was that creatine supplementation would decrease carbohydrate and protein degradation in muscle during exercise.

Methods

Participants

Twelve male athletes with a mean (±SD) age of 20.3 ± 1.4 years, height of 174.0 ± 6.0 cm, and weight of 66.4 ± 8.0 kg were recruited from National Taiwan Normal University track and field team to participate in this study. The Ethics Committee of Taipei Medical University approved this blind study with repeated subject measures, which was performed according to the Declaration of Helsinki. Participants were fully informed about the experimental procedures as well as the potential risks involved. After safety confirmation, subjects signed an informed consent form prior to their participation in the study. Throughout the study, each participant was instructed to refrain from any medication that would affect dependent variables; from strenuous exercise, smoking, and alcohol and caffeine consumptions. In addition, participants were asked to maintain their routine dietary habits and exercise activity of moderate/low intensity during the entire study.

Experimental design

A cyclical supplementary strategy of 20 g creatine/day for 4–6 days followed by 5 g creatine/day for 2–3 weeks (totally ~187 g) [1] has been adopted by most athletes. However, Vandebuerie et al. [18] reported that the loading dose of 20 g creatine/day for 5 days might be expected to induce gastrointestinal discomforts (stomach upsets, diarrhea, and vomiting, etc.). In order to avoid the potential side effects and be comparable with the amount of total creatine intake (187 g) described in the literature, the dosage of creatine supplements given in the present 15-day study was 12 g/day (187 g/15 day). The total dosage of creatine given (180 g) to each participant then was similar to that prescribed in the literature [1]. All participants performed two identical 60 min running exercises (*endurance trial*) before and after 15 days of creatine supplementation (12 g creatine monohydrate/day in capsules, from day 8 to day 22 of the experimental period, as shown in Fig. 1), followed by a 5-day washout period (day 23–27). Subsequently, they performed two identical 100 m sprint running exercises (*power trial*) before and after another 15 days of creatine supplementation in accordance with the supplementary protocol of the endurance trial.

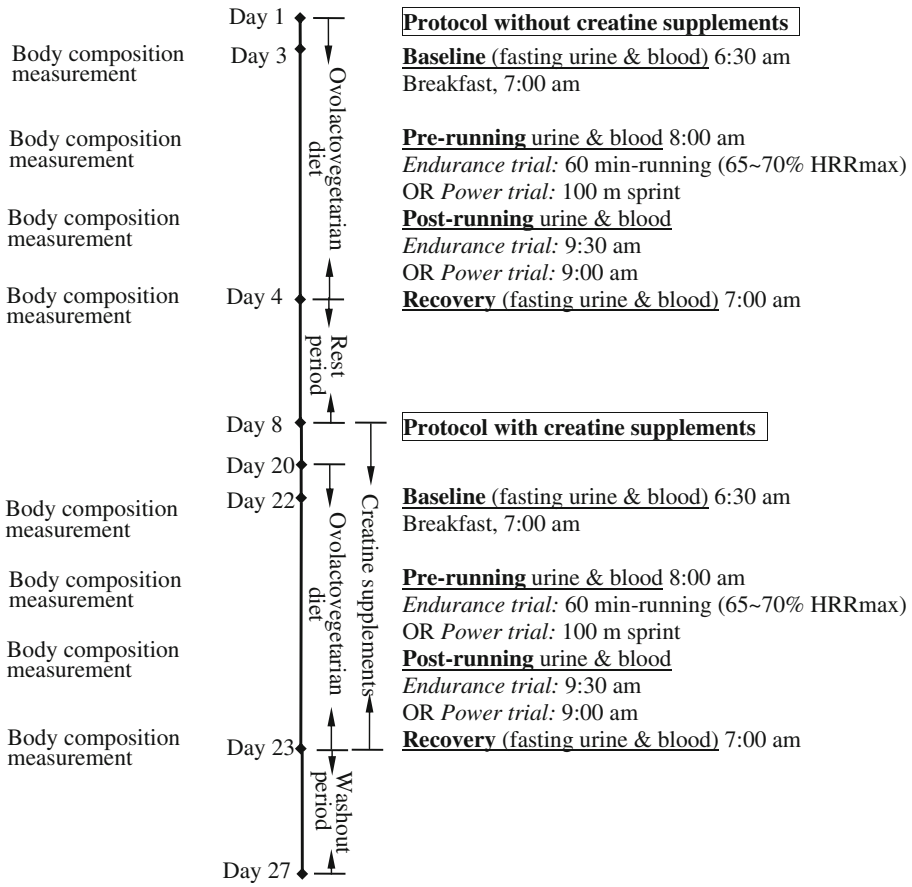

Fig. 1 Experimental diagram. Two trials (endurance and power), separated by a 5-day washout period, proceeded the same treatment except exercise test. Each athlete totally received four-cycle tests. The sequence was (1) without creatine supplement endurance trial + rest period (2) with creatine supplement endurance trial + washout period; (3) without creatine supplement power trial + rest period (4) with creatine supplement power trial (end of the cycle test). Within each trial (two protocols per trial, separated by a 5-day rest period), two identical exercises were conducted to examine the effect of creatine supplementation. HRRmax: maximum heart rate reserved. Creatine supplements: 12 g of creatine monohydrate/day. Ovolactovegetarian diet: carbohydrate 59 ± 3 %, lipid 26 ± 3 %, protein 15 ± 1 %; ~39 kcal/kg body weight

Neither the participants nor the executive researchers knew the content of the capsules. Within each trial (two protocols per trial, separated by a 5-day rest period), two identical exercises were performed by athletes on day 3 (without creatine supplementation) and day 22 (with creatine supplementation). No water or other beverages were allowed during running. Participants for endurance running maintained their exercise intensity within 65–70 % HRRmax (maximum heart rate reserved, Polar AccurexPlus^{TM}, Polar Electro Oy, Kempele, Finland) during the 60-min running exercise. In the power trial, the running time at 100 m of each participant was determined by an experienced coach using a stopwatch. To avoid the exogenous 3MH and creatine contributed from meat, poultry, and fish, all participants consumed the ovolactovegetarian diets (carbohydrate 59 ± 3 %, lipid 26 ± 3 %, protein 15 ± 1 %; ~39 kcal/kg body weight) which were prepared by our research team, for 3 days prior to the running exercise. On the day of each running exercise, urinary samples (baseline) and 10 h overnight fasting blood samples (baseline) were collected before breakfast. One hour after the breakfast (~7:00 a.m.), the pre-running urinary and blood samples were collected (~8:00 a.m.). Post-running urinary and blood samples were collected immediately after the 60 min running (endurance trial) or after the 100 m sprint running (power trial) was completed. Urinary samples (recovery) and 10 h overnight fasting blood samples (recovery) were collected the following morning. Body composition (SBIA, InBody 3.0, Biospace Co., Ltd., Seoul, Korea) was determined between the collection of each urinary sample and blood sample. During this time, compliance to the supplementation protocol was monitored by researchers and by verbal confirmation.

Sample analysis

Blood samples were collected from the forearm antecubital vein. The hematocrit was determined immediately (Clay Adams 4207 QBC Centrifuge, Rankin Biomedical Corp., USA) and used for the calibration of blood volume which would be affected by dehydration due to endurance exercise. Blood samples were centrifuged (150×*g*, 20 min at 4 °C, Allegra X-22, Beckman Coulter, USA), and the plasma samples were immediately stored at −80 °C until they were analyzed. Prior to HPLC analysis, plasma samples were deproteinized with 20 mM HCl, filtered (ultrafree-MC, UFC3LGC00, Millipore, USA) and centrifuged. Urinary samples were hydrolyzed with 6 N HCl, at 110 °C

for 3.5 h, and analyzed for free hydroxyproline (HP, a reliable index of bone resorption and collagen degradation [19, 20]) and 3MH. Plasma valine, isoleucine, leucine, f-TRP, alanine, and glutamine concentrations were determined by HPLC (AccQ TAG method, Waters Co., Milford, MA, USA). Plasma glucose and lactate concentrations were determined by the enzymatic method (Beckman Coulter Synchron LX20 Analyzer, Beckman Coulter Inc., Fullerton, CA, USA). Plasma hypoxanthine and uric acid concentrations were determined by a modified HPLC method [21–23]. Urinary creatinine concentration was determined by a modification of alkaline picrate method [24] and used to calibrate all other urinary constituents [11, 25]. Urinary HP and 3MH concentrations (HPLC, AccQ TAG method, Waters Co., Milford, MA, USA), and urea nitrogen concentration (urease methods, Beckman Coulter Synchron LX20 Analyzer, Beckman Coulter Inc., Fullerton, CA, USA) were also determined.

Statistical analysis

Statistical analysis was performed by SAS (Version 9.1 for Windows, SAS Institute Inc., Cary, NC, USA), and the results were expressed as mean ± SD at each sampling point. One-way ANOVA with repeated-measures and the paired *t* test were used to investigate the influences of creatine supplementation and exercise treatment on the dependent variables. Duncan's new multiple range test was performed for all comparisons at a significant level of $P < 0.05$. Within each measurement, data with different letters are significantly different (Table 1; Figs. 2, 3). Purine metabolites, which represented the sum of hypoxanthine and uric acid, were statistically analyzed to study the effect of creatine supplementation on protein metabolism after exercise.

Results

Body composition analysis and sprint performance

During the entire study period, maintaining routine dietary habits and exercise activity were confirmed by the participants, except the ovolactovegetarian diets which were provided by the research team. No gastrointestinal discomforts were observed among the participants after study achievement. Creatine supplementation resulted in a significant increase in body weights ($P < 0.05$) and tended to increase the fat-free-mass and total body water content of the athletes of the endurance trial, but not the athletes of the power trial (Table 1). Fat-mass significantly decreased ($P < 0.05$) after either exercise treatment, regardless of creatine supplementation. Before 100 m sprint running, the body weights of the athletes of the power trial with creatine supplementation were not different from those of the power trial without creatine supplementation. The explanation for the difference in body weight changes between the two trials may be attributable to the inadequacy of the washout period, which was due to the tight training and/or competition schedules of the participants. There was no significant enhancement on 100 m sprint performance (12.42 ± 0.49 vs. 12.33 ± 0.53 s, $P > 0.05$) after creatine supplementation. This lack of effect of creatine supplementation on performance is similar to the finding of Hickner et al. [26].

Plasma analysis

Creatine supplementation and/or exercise treatment did not affect plasma glucose concentrations. The significantly decreased glucose concentration after breakfast was due to the insulin response. After 100 m sprint running, plasma lactate concentration reached its highest value, regardless of creatine supplementation (Fig. 2). After endurance running, the clearance of plasma lactate was significantly enhanced ($P < 0.05$) at recovery with creatine supplementation. Creatine supplementation tended to decrease the ratios of f-TRP/BCAAs in the endurance trial, whereas they were increased in the power trial. Before running, the plasma purine metabolite (the sum of hypoxanthine and uric acid) concentrations of the endurance trial with creatine supplementation were significantly lower ($P < 0.05$) than those of the endurance trial without creatine supplementation. After endurance running, the purine metabolite concentrations significantly increased ($P < 0.05$) with creatine supplementation but were similar to those of the endurance trial without creatine supplementation. Creatine supplementation tended to decrease the purine metabolite concentrations in the power trial. Immediately after endurance running, the plasma glutamine concentration with creatine supplementation was significantly lower ($P < 0.05$) than that without creatine supplementation. At baseline, the plasma glutamine concentration was significantly lower ($P < 0.05$) after 15 day creatine supplementation in either trial. The plasma alanine concentration was not affected by creatine supplementation in the endurance trial, but it was affected in the power trial at recovery ($P < 0.05$), which significantly decreased and was lower than that of the power trial without creatine supplementation.

Urinary analysis

As shown in Fig. 3, creatine supplementation did not affect the urinary HP concentration of the endurance trial, but tended to increase the urinary HP concentration of the

Table 1 Body composition and plasma glucose concentration changes induced by creatine supplementation and exercise treatment

	Baseline	Pre-running	Post-running	Recovery
Endurance trial				
Body weight (kg)				
Without creatine supplementation	66.4 ± 8.0^{b}	66.7 ± 8.0^{a}	65.0 ± 7.9^{c}	66.2 ± 7.7^{b}
With creatine supplementation	$^{\#}67.1 \pm 7.9^{b}$	$^{\#}67.5 \pm 8.1^{a}$	$^{\#}66.2 \pm 7.9^{c}$	$^{\#}66.9 \pm 8.0^{b}$
Fat-mass (kg)				
Without creatine supplementation	8.3 ± 1.8^{a}	8.5 ± 1.9^{a}	7.1 ± 1.7^{b}	8.3 ± 1.8^{a}
With creatine supplementation	8.1 ± 1.5^{b}	8.7 ± 1.6^{a}	7.2 ± 1.6^{c}	8.4 ± 1.7^{ab}
Fat-free-mass (kg)				
Without creatine supplementation	58.1 ± 7.4^{a}	58.2 ± 7.4^{a}	57.9 ± 7.2^{a}	58.0 ± 7.2^{a}
With creatine supplementation	$^{\#}58.9 \pm 7.1^{a}$	58.8 ± 7.4^{ab}	$^{\#}59.0 \pm 7.4^{a}$	58.5 ± 7.3^{b}
Total body water (kg)				
Without creatine supplementation	40.3 ± 5.2^{a}	40.4 ± 5.2^{a}	40.2 ± 5.0^{a}	40.2 ± 5.1^{a}
With creatine supplementation	$^{\#}40.9 \pm 5.0^{a}$	40.8 ± 5.2^{ab}	$^{\#}40.9 \pm 5.2^{a}$	40.6 ± 5.1^{b}
Plasma glucose (mg/dL)				
Without creatine supplementation	87.0 ± 5.4^{a}	66.3 ± 10.7^{b}	80.5 ± 14.7^{a}	85.2 ± 4.8^{a}
With creatine supplementation	$^{\#}80.8 \pm 6.2^{a}$	70.6 ± 16.7^{b}	85.5 ± 12.7^{a}	85.7 ± 5.6^{a}
Power trial				
Body weight (kg)				
Without creatine supplementation	67.1 ± 8.0^{ab}	67.3 ± 8.1^{a}	66.6 ± 8.1^{c}	66.8 ± 8.2^{bc}
With creatine supplementation	67.4 ± 8.4^{b}	67.8 ± 8.4^{a}	$^{\#}67.2 \pm 8.3^{b}$	$^{\#}67.4 \pm 8.4^{b}$
Fat-mass (kg)				
Without creatine supplementation	8.0 ± 1.5^{a}	8.3 ± 1.5^{a}	7.4 ± 1.6^{b}	8.2 ± 1.7^{a}
With creatine supplementation	8.4 ± 1.8^{ab}	8.7 ± 1.8^{a}	$^{\#}8.0 \pm 2.2^{b}$	8.6 ± 1.9^{a}
Fat-free-mass (kg)				
Without creatine supplementation	59.0 ± 7.4^{ab}	59.1 ± 7.5^{ab}	59.2 ± 7.5^{a}	58.6 ± 7.5^{b}
With creatine supplementation	59.0 ± 7.5^{a}	59.2 ± 7.4^{a}	59.3 ± 7.3^{a}	58.8 ± 7.7^{a}
Total body water (kg)				
Without creatine supplementation	41.0 ± 5.2^{ab}	41.0 ± 5.2^{ab}	41.1 ± 5.2^{a}	40.7 ± 5.2^{b}
With creatine supplementation	40.9 ± 5.3^{a}	41.0 ± 5.2^{a}	41.2 ± 5.1^{a}	40.8 ± 5.4^{a}
Plasma glucose (mg/dL)				
Without creatine supplementation	87.8 ± 3.5^{a}	75.5 ± 16.2^{b}	92.6 ± 5.6^{a}	86.4 ± 3.9^{a}
With creatine supplementation	$^{\#}82.1 \pm 6.4^{a}$	72.0 ± 35.7^{b}	$^{\#}84.5 \pm 8.8^{a}$	88.4 ± 4.1^{a}

Within each measurement and trial, data (mean $\pm$ SD, $n = 12$; measured after 3 days of ovolactovegetarian diet: carbohydrate 59 $\pm$ 3 %, lipid 26 $\pm$ 3 %, protein 15 $\pm$ 1 %; $\sim$39 kcal/kg body weight) with different superscript significantly differ, $P < 0.05$, as determined by one-way ANOVA with repeated-measures. $^{\#}$Within the same measurement, significantly different from the corresponding data measured without creatine supplements, $P < 0.05$, as determined by paired t test

Recovery: Fasting data collected the following morning after exercise treatment

power trial. Immediately after running, the urinary HP concentration of the power trial with creatine supplementation was significantly higher ($P < 0.05$) than that of the power trial without creatine supplementation. In the endurance trial, creatine supplementation tended to decrease urinary 3MH and urea nitrogen concentrations. But this tendency was only observed in the UUN concentration, not the urinary 3MH concentration of the power trial. Immediately after the endurance running, UUN excretion significantly decreased ($P < 0.05$), regardless of creatine supplementation.

Discussion

Creatine is primarily found in meat products, creatine intake from our ovolactovegetarian diets, therefore, was negligible as compared with the creatine supplements in the present study. It has often been reported that short-term creatine supplementation was accompanied by an increase in body mass [27–31]. Although intramuscular creatine and PCr concentrations were not determined, a gain in body weight was observed after creatine supplementation in the endurance trial. Such an increase indicated that the

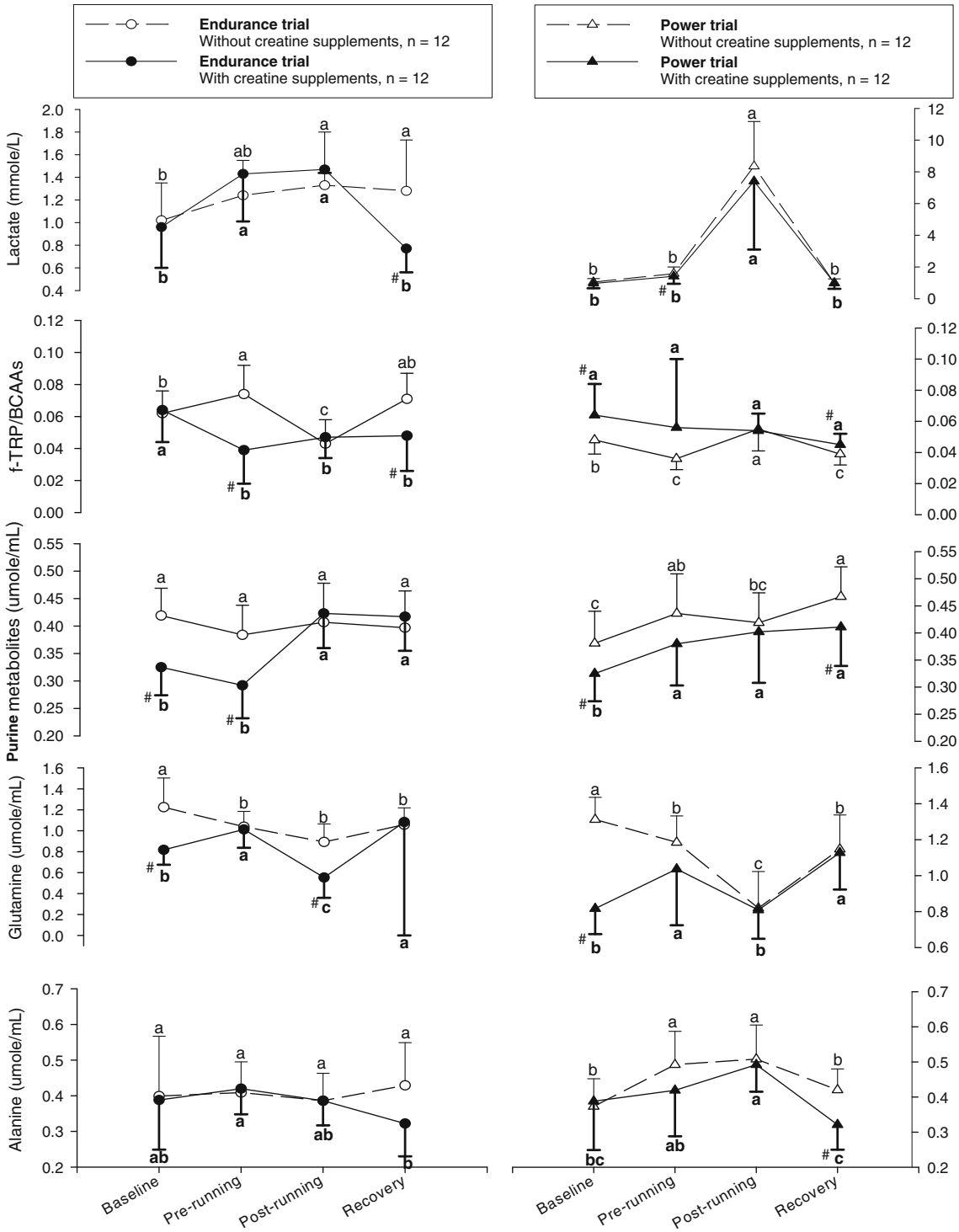

Fig. 2 Plasma composition changes induced by creatine supplementation and exercise treatment. *Circle* represents 60 min running (endurance trial). *Triangle* represents 100 m sprint (power trial). *Filled symbol*, *bold error bar* and *letters* represent creatine supplements. *f-TRP* free-tryptophan, BCAAs branched-chain amino acids, *Purine metabolites* the sum of hypoxanthine and uric acid. Within each measurement and trial, data (mean ± SD) with different letters significantly differ, $P < 0.05$, as analyzed by one-way ANOVA with repeated-measures. #Within the same measurement, significantly different from the corresponding data measured without creatine supplements, $P < 0.05$, as determined by paired t test. Recovery: fasting data collected the following morning after exercise treatment

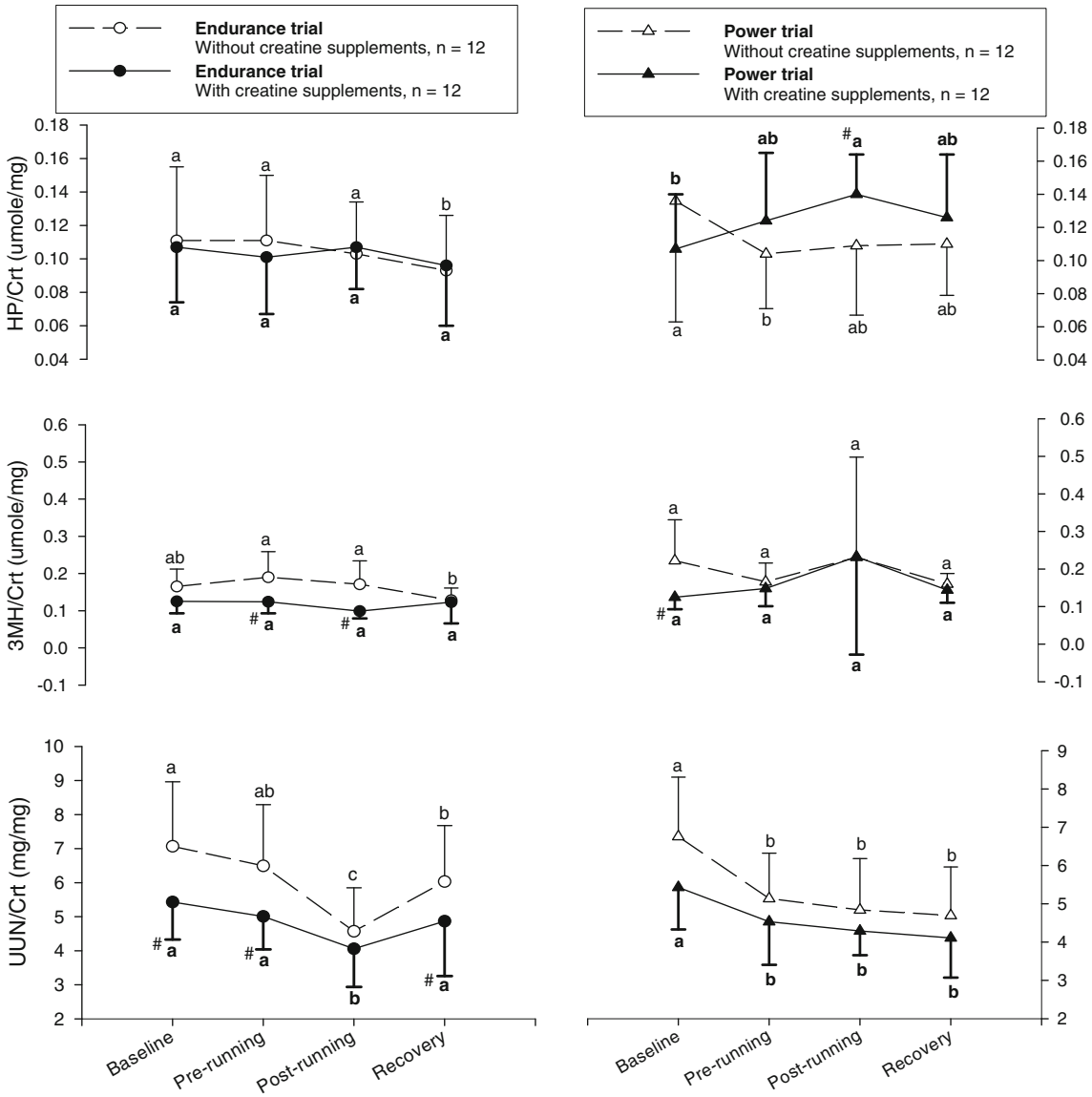

Fig. 3 Urinary composition changes induced by creatine supplementation and exercise treatment. *Circle* represents 60 min running (endurance trial). *Triangle* represents 100 m sprint (power trial). *Filled symbol*, *bold error bar* and *letter* represent creatine supplements. *HP* hydroxyproline, *3MH* 3-methylhistidine, *UUN* urinary urea nitrogen, *Crt* creatinine. Within each measurement and trial, data (mean ± SD) with different letters significantly differ, $P < 0.05$, as analyzed by one-way ANOVA with repeated-measures. # Within the same measurement, significantly different from the corresponding data measured without creatine supplements, $P < 0.05$, as determined by paired *t* test. Recovery: fasting data collected the following morning after exercise treatment

supplemental strategy (12 g creatine/day for 15 days) was effective. The increase in body mass after creatine consumption was explained by increasing in fat-free-mass [32, 33] and water retention in muscle [34, 35]. The inadequacy in washout period, leading to the water (accumulated from the endurance trial with creatine supplementation) retained in the athletes of power trial without creatine supplementation, resulted in an increase in the body weight before sprint running. The body weights of the athletes before the sprint running, therefore, were not different, regardless of creatine supplementation. This study, however, focused on the effects of creatine supplementation on plasma and urinary metabolite changes after exercise, whereas the influence of inadequacy of the washout period (5 days) was only limited to intramuscular (intracellular) water retention which was induced by creatine locked in the muscle cells. The influence of the inadequate washout period, therefore, affected the results of body weight measurement, but not the extracellular metabolite concentrations.

Literatures [36–38] indicate that, after high-intensity exercises, lactate accumulation in the blood was decreased with creatine supplementation. The mechanisms were postulated to be due to a higher initial PCr content availability and an increased rate of ATP resynthesis during

recovery periods. However, several studies have failed to confirm the decrease of plasma lactate concentration after creatine supplementation [39–41]. In our study, creatine supplementation did not alter lactate concentration immediately after running, regardless of the exercise treatment. However, in the endurance trial with creatine supplementation, the plasma lactate concentration not only decreased at recovery, but was also significantly lower than that of the endurance trial without creatine supplementation, and further returned to its baseline, which was not seen in the endurance trial without creatine supplementation. These observations indicated that creatine supplementation played a role in the clearance of plasma lactate during recovery from endurance exercise. Lactate and pyruvate are the products of glycolysis. With creatine supplementation, the decrease in plasma lactate concentration at recovery indicated a reduction in relying on carbohydrate utilization after endurance exercise. In other words, creatine supplemented provided the substrate for ATP-PCr system, which decreased muscle glycogen degradation during recovery from endurance exercise. This is in line with the finding of Roschel et al. [41] who found that creatine supplementation spared muscle glycogen during a high-intensity intermittent exercise in rats.

It is interesting that, with creatine supplementation, plasma f-TRP to BCAAs ratio at recovery was significantly lower in the endurance trial, but significantly higher in the power trial. Literatures have shown that creatine supplementation reduced mental fatigue by increasing oxygen utilization [42] or energy availability [43] in the brain. Both studies [42, 43] were examined with brain performance (mathematical calculations and/or intelligence tests), not associated with muscle performance. There is less evidence to demonstrate the benefit of creatine supplementation on mental fatigue associated with physical (muscle) exercise. This could be due to the fact that creatine has been widely used for sprint events which acquire energy mainly from the ATP-PCr system and anaerobic glycolysis [3], rather than from BCAA oxidation in the muscle.

It is well known that high-intensity exercise results in a decrease in muscle adenine nucleotide pool ([ATP] + [ADP] + [AMP]) and an increase in IMP, ammonia, and hypoxanthine concentrations [9]. Bellinger et al. [44] found that plasma ammonia and hypoxanthine concentrations were lower in the creatine group and suggested that oral creatine supplementation decreased plasma markers of adenine nucleotide degradation. It is in line with our results on purine metabolite concentrations observed before running in either trial. At recovery, the plasma purine metabolite concentration of the power trial with creatine supplementation was significantly lower than that of the power trial without creatine supplementation. It was due to the enhancement of creatine on ATP resynthesis, intracellular adenine nucleotide, therefore, could be protected from degradation during sprint events.

After endurance running, however, the significantly increased purine metabolite concentrations were only observed in the trial with creatine supplementation. Beis et al. [45] found that significant increases in body mass and total body water with a combined creatine and glycerol supplementation did not affect the running economy (defined as sub-maximal oxygen consumption at a given running velocity) of 30 min running at 60 % maximal oxygen uptake in hot and cool conditions. Other studies [46, 47], however, indicated the endurance running performance can be improved by way of reducing body weight or body water, due to the reduction of the energy cost of movement at a sub-maximal velocity. In our study, after 60 min running at 65–70 % HRRmax, the increased purine metabolite concentrations in the trial with creatine supplementation, therefore, may result from an increase of the energy cost of moving extra body weight increased by creatine supplementation. It gives the possible reason for the seldom using of creatine in endurance running. In his book, Williams [35] also addressed that a gain in body mass with creatine supplementation could possibly impair performance in running.

During protein metabolism, alanine is generated after the catabolism or transamination of BCAAs to pyruvate in muscle. At recovery, creatine supplementation tended to decrease the plasma alanine concentration in the endurance trial and significantly decreased the plasma alanine concentration in the power trial. A decrease in plasma alanine concentration after exercise could be due to a decrease in BCAA oxidation or proteolysis. It seems that, in spite of exercise intensity and duration, creatine supplementation decreased muscle proteolysis.

In addition to fatigue, ammonia is also associated with protein deamination [48]. After deamination, ammonia reacted with glutamate to form glutamine. The plasma ammonia concentration should be determined with fresh samples [49]. However, this could not be accomplished in our study because the running exercise was performed in the field. Plasma glutamine, urinary 3MH, and urea nitrogen concentrations, therefore, were measured to investigate the protein metabolism during exercise. The significantly decreased UUN concentration observed immediately after the endurance running could be due to the urea nitrogen loss via heavy sweating. Decreases in the concentrations of plasma glutamine, urinary 3MH, and urea nitrogen immediately after endurance running with creatine supplementation, indicated that creatine supplementation decreased proteolysis. Hence, creatine supplementation exerted an advantage on protein preservation during endurance exercise. Although Jowko et al. [50], by determining urinary

nitrogen, were able to verify that creatine did not affect the retention of body nitrogen, our findings are still in line with the studies of Candow et al. [15] who found that participants receiving creatine supplements experienced a decrease in urinary 3MH excretion, and Parise et al. [51] who reported that creatine supplementation reduced whole body protein breakdown. The possible mechanisms for these phenomena could be due to the adequate ATP and PCr availability, which protected body from protein degradation. This also explained the association of creatine supplementation with the decrease in the ratios of f-TRP/BCAAs in the endurance trial.

After 15 days of creatine supplementation, decreases in the concentrations of plasma purine metabolites, glutamine, urinary 3MH, and urea nitrogen, at the baseline of either trial, indicated that creatine alone may play a role in preventing proteolysis. This is in agreement with the review of Tarnopolsky and Safdar [52] who indicated that creatine supplementation may enhance the fat-free-mass and muscle strength by resistance training in elderly adults. After sprint running, however, a significantly increased body weight and high urinary HP excretion were found in athletes who received creatine supplements. The increased body weight load could be a burden to the knees or legs [53], especially during high impact running exercise. It resulted in the increase of urinary HP excretion, since HP is almost exclusively related with collagen found in various tissues [54]. It explained that creatine supplementation was less associated with the high impact endurance exercise, as was demonstrated in our study. With the 65–70 % HRR-max 60 min endurance running, the urinary HP concentration was not affected by creatine supplementation; whereas, with the 100 m sprint, the urinary HP concentration was affected by creatine supplementation.

In terms of metabolism, creatine supplementation possessed the advantages of decreasing muscle glycogen and protein degradation, especially after endurance exercise, although it might not benefit the endurance performance. Further, based on plasma and urinary data at baseline, creatine supplementation could be adopted by non-athletes or clinical populations [55], including elderly adults with sarcopenia [52], patients with muscular dystrophies [56], and patients with McArdle's disease (a kind of myopathies) who cannot use muscle glycogen [57] for energy production efficiently. However, studies with these kinds of clinical applications are required for verification.

Conclusions

The data obtained in this study provide substantial support to the hypothesis that creatine supplementation is beneficial to the decrease of carbohydrate and protein degradation in muscle, especially after endurance running. However, long term creatine supplementation might induce collagen proteolysis in athletes after sprint running.

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Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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