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The Effect of Creatine Supplementation with Resistance Training on the Levels of Renin and Aldosterone in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SH and GRS designed, conducted and supervised the study. Author MK participated in the writing and editing of the paper. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The aim of the present study was to compare the effect of creatine 2% and 5% supplementation with resistance training on the levels of renin and aldosterone
Methods: The 60 Wistar rats were randomly assigned to six groups (resistance training, creatine 2% and creatine 5%, resistance training plus creatine 2% supplementation, resistance training plus creatine 5% supplementation, control). The training included 8 weeks and 5 sessions a week climbing a 1-meter ladder with 26 rungs with 2-cm distances. The One – way analysis of variance (ANOVA) test was used for comparing the variables between groups and LSD test was used for pair-wise comparisons.
Results: There was no significant difference between mean differences of renin level in different groups compared to the control group. Butthere was a significant difference between mean difference of aldosterone level in creatine 5% groups and control group as well as between resistance training plus creatine 2% and creatine 5% groups ($p < 0.05$). Also, there was significant

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difference between mean difference of aldosterone level in resistance training and creatine 5% groups ($p < 0.05$).

Conclusion: As regard to result of the present study consumption of creatine 2% and 5% supplementation may be useful and safe for human so further studies is suggested.

Keywords: Creatine; resistance training; rennin; aldosterone.

1. INTRODUCTION

The effects of long-term use (over one-week) of creatine supplementation on the performance of various sports athletes particularly speed and strength sports have been investigated previously. Studies indicated that increased muscle phosphocreatine due to creatine supplementation most probably leads to rephosphatization of adenosine diphosphate (ADP) during exercise. The accumulation of ammonia and hypoxanthine decreases during maximal-intensity exercise after creatine supplementation [1-7]. To conduct preliminary studies, creatine is isolated from animals meat that was expensive. Nowadays, creatine supplements are synthesized chemically and creatine monohydrate is the most available creatine supplement and was used in most studies [6-12]. Athletes usually consume creatine to achieve two purposes; first, to conduct the repeated strength exercise (e.g. weight training and biking) that have effects on the increase in the volume and strength of the muscles; secondly, to retention of water and fluids in the body [13-22]. Normally, liver and kidneys receive 27% and 22% of the circulating blood, that due to heavy training decline to 5% and 3%, respectively. The long-term decline in blood supply to the liver and kidneys can lead to adverse consequences such as fatigue due to sub maximal continuous exercise (7-12). The renin-angiotensin-aldosterone system helps to maintain blood pressure, normal acidity, the balance of electrolytes and fluids [22-31].

However, there are still debates on the effects and potential side effects of the short and long-term use of such supplements on health indices and the body's different organs. Regarding the above mentioned; we conducted this study to compare the effects of 8-week supplementation with creatine 2% and 5% with resistance training on the renin-aldosterone level in small laboratory rats.

2. MATERIALS AND METHODS

In this experimental study, 60 Wistar rats that were genetically similarwere purchased from the Iran Pasteur institute, Karaj, Iran, with a weight range of 225-275 g, and were kept in the animal house of Islamic Azad University Khorasgan for fourteen days for acclimatization. Wistar rats had free access to water and ad libitum. The temperature of animals' house was 22± 2°C with 12 hr light-dark cycle. Animals were randomly divided into six groups including resistance training (Group 1), creatine supplementation 2% (Group 2), creatine supplementation 5% (group 3) resistance training plus creatine 2% supplementation (group 4) resistance training plus creatine 5% supplementation (Group 5) and control group. The training was climbing a 1-meter ladder included 26 rungs with 2-cm distances 5 sessions in a week for 8 weeks. The number of sets was 4- 5 times repetition with 1-min intervals and 2-min intervals between the sets. In this training, a weight was clipped to the rats' tails 2-3 cm from the proximal end and then the rats were encouraged to go up a vertical ladder with 85°C steep (Table 1). Muscle Pharm Creatine (Denver, CO, USA) was feed for 8 weeks (Code: 91-12-6).

2.1 Biochemical Assays

At the end of eight weeks,and 48 hours after the last training session the animals were anesthetized with chloroform, and then blood samples were taken by cardiac puncture for biochemical analysis. Renin and angiotensin concentration were measured by ELISA methodusingkit (Pars Azmoon) according to the manufacturer's protocol.

2.2 Ethical Issues

The research followed the tenets of theDeclaration of Helsinki. This project was approved by Ethics Committee of Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran. Also, protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran (Ethical cod#23821404931022).

2.3 Statistical Analysis

The mean±SD was used for the quantitative variable. The normal distribution was evaluated using the one-sample Kolmogorov test. The one – way analysis of variance (ANOVA) test was used for comparing the variables between groups and Bonferroni test was used for pairwise comparisons. Statistical significance was defined as $P<0.05$ and analysis were done using SPSS (version 20; SPSS Inc., Chicago, USA).

3. RESULTS

Table 1 shows the mean (standard error) values of rennin and aldosterone in different groups. The highest level of renin was seen in the resistance training group (8.6) and the highest level of aldosterone was seen in the creatine 5% supplementation group (224.43) (Table 1). There was no significant difference between mean difference of renin level in different groups compared to the control group (F=2.39, p =0.06). But there was a significant difference between mean difference of aldosterone level in different groups compared to the control group (F= 8.93, p<0. 001) and this significance is related to difference between creatine 5% groups and control group as well as between resistance training plus creatine 2% and creatine5% groups (p < 0.001), also difference between resistance training and creatine 5% groups (p<0.001) (Table 2).

Table 1. The renin and aldestrone concentration (mean and standard error) in different groups

Variable	Renin (mg/dl)	Aldosterone (mg/dl)
I	8.60 ± 3.00	148.50 ± 11.40
II	4.61 ± 3.00	196.37 ± 11.40
III	4.10 ± 3.20	224.43 ± 12.20
VI	4.38 ± 3.00	159.13 ± 11.40
V	4.59 ± 3.00	179.50 ± 11.40
C	15.91 ± 3.00	151.38 ± 11.40

I; resistance training c, II; creatine supplementation 2%, III; creatine supplementation 5%, VI; resistance training plus creatine 2% supplementation, V; resistance training plus creatine 5% supplementation, and C; control group

4. DISCUSSION

The findings of this study demonstrated that resistance training, either with or without creatine 2% and 5% supplementation, had no significant effect on changes in renin levels ($p>0.05$). But there was a significant difference between mean difference of aldosterone level in different groups compared to the control group and this is related to difference between creatine 5% groups and control group as well as between resistance training plus creatine 2% and creatine 5% groups, also difference between resistance training and creatine 5% groups. The result is consistent with Mitchell et al. [32] study but inconsistent with Volek et al. [33] study. Volek et al. studied physiologic responses to short-term exercise in hot weather after creatine loading. Cortisol, aldosterone, renin, and angiotensin I and II increased significantly in both groups.

In the light of renin's role in the physiological system and regulating water in the body, we can argue that resistance training causes dehydration in the body so mean changes in renin levels did not decrease in the resistance training. No change in renin levels in rats under creatine 2% and 5% supplementation, with or without resistance training, indicated that creatine supplementation causes an increase in plasma volume and a decrease in osmolarity in the body. In fact, this process occurred with increasing creatine level in the rats's bodies. Creatine is an active osmotic substance, and therefore increases in total creatine, free creatine, and intracellular phosphocreatine directs water flow into the cell. More clearly, in creatine supplemented groups, water retention increased due to decreased renin. Besides that, our results demonstrated that there was no significant difference in the mean levels of renin between the creatine 2% supplementation group and the creatine 5% supplementation group [34-36].

In addition, increased intracellular water can be a marker for anabolic cell development. Increased intracellular water induced by creatine supplementation may increase protein synthesis and decrease protein degradation; therefore, body mass and adipose-free mass increase, which can be important to athletes and people who seek to lose weight or increase muscle mass volume [37].

Changes in aldosterone underwent a decreasing trend compared to groups treated with creatine 2% and 5%. More clearly, aldosterone increased in both creatine-supplemented groups compared to the control group but decreased in the resistance training group. In addition, there was no significant difference in aldosterone change between creatine 2% supplementation plus

Table 2. Comparison rennin and aldosterone concentration between control and each group at the end of study

	I	P	II	P	III	P	VI	P	V		Control
Renin, mg/dl	8.60±9.34	0.23	4.61±6.36	0.05	4.1±8.37	0.07	4.38±4.72	0.14	4.69±4.64	0.16	15.9±13.75
Aldosterone, mg/dl	148.5±32.79	0.86	196.37±22.9	0.12	224.43±41.2	<0.001*‖‡	159.13±28.4	0.12	179.5±35.4	1.00	151.37±31.0

I; Resistance training c, II; c Reatine supplementation 2%, III; Creatine supplementation 5%, VI; Resistance training plus creatine 2% supplementation, V; Resistance training plus creatine 5% supplementation, and C; Control group
** Significant differences between III and VI groups*
‖ Significant differences between III and I groups
‡ Significant differences between III and control groups

training group and creatine 5% supplementation plus training group as well as between the creatine 2% and 5% supplementation groups and the control group, which is consistent with Volek et al. [36] study regarding creatine supplementation but inconsistent with this study in the light of resistance training. As the most important mineralocorticoid from the adrenal cortex, aldosterone affects the kidneys and helps to maintain the balance of electrolytes, especially sodium and potassium, in the extracellular fluids. Aldosterone maintains the body's sodium through increasing renal reabsorption of sodium and excretion of potassium and causes reabsorptionof chlorine and water through absorbing sodium [12-22], which were clearly confirmed in the current study. These hormones, therefore, appear not to cause different responses in creatine 2% and 5% supplementation plus training groups. In addition, creatine supplementation can cause an increase in aldosterone levels and therefore water retention in the body. It is clear that the contents of supplements are essential to rehydration phases and fluids restoration [10-18,33-37]. Using recovery drinks can prevent tissue and functional damage. If lost water and electrolytes are not appropriately compensated after training, dehydration and lack of balance between water and electrolytes are intensified and therefore sports performance and health are adversely influenced [38-40]. To resolve this problem, creatine 2% and 5% supplementation, according to the current study findings, can be used. However, this recommendation deserves further investigation.

5. CONCLUSION

As regard to result of the present study consumption of creatine 2% and 5% supplementation may be useful and safe for human so further studies is suggested.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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