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Original article

Investigating the Impact of Creatine and Vitamin C Supplementation on Insulin Sensitivity and Glycated Hemoglobin Levels in Male Rabbits

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Abstract
Insulin resistance and impaired glucose metabolism are key features of metabolic disorders such as type 2 diabetes mellitus. Natural supplements such as creatine and ascorbic acid have shown potential in improving glycemic control through different biochemical mechanisms. This study aimed to evaluate the effects of creatine supplementation (CrS), ascorbic acid, and their combination on glucose metabolism, insulin sensitivity, and long-term glycemic control in male rabbits. Male rabbits were randomly assigned to four groups: control, creatine-treated, ascorbic acid-treated, and combination-treated. Fasting blood glucose, plasma insulin levels, homeostasis model assessment of insulin resistance (HOMA-IR), and glycated hemoglobin (HbA1c) were measured to assess metabolic status. Treatments were administered over a specific period, and data were analyzed using standard biochemical and statistical methods. Creatine supplementation resulted in a significant hypoglycemic effect and improved insulin sensitivity, with a notable reduction in HOMA-IR (0.85 ± 0.104) compared to the control group (1.12 ± 0.144). Ascorbic acid exerted a milder glucose-lowering effect but contributed to improved antioxidant defenses, potentially protecting pancreatic β-cells from oxidative damage. Plasma insulin levels did not significantly differ among groups (3.27 ± 0.356 to 4.00 ± 0.707 g/dL), suggesting enhanced insulin action rather than increased secretion. HbA1c levels were significantly reduced in all treatment groups, with the combination group achieving the lowest level (3.21 ± 0.43%), followed by creatine (3.8 ± 0.4%) and ascorbic acid (4.6 ± 0.45%). Creatine and ascorbic acid, individually and in combination, effectively improved markers of glucose metabolism in male rabbits. Creatine demonstrated a stronger effect in enhancing insulin sensitivity and lowering glucose, while ascorbic acid played a supportive role by reducing oxidative stress and preserving insulin function. These findings highlight the potential therapeutic value of combining antioxidant and creatine supplementation for managing insulin resistance and hyperglycemia.
Keywords. *Ascorbic Acid, Creatine, Insulin Sensitivity, Rabbits.*

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Introduction

Insulin resistance and elevated glycated hemoglobin (HbA1c) levels are key markers of metabolic dysfunction and are strongly associated with the pathophysiology of type 2 diabetes mellitus and cardiovascular diseases [1]. Recent attention has been directed toward nutritional and biochemical interventions that may ameliorate these metabolic disorders through the modulation of glucose metabolism and oxidative stress [2]. Creatine, a nitrogenous organic acid synthesized primarily in the liver, kidneys, and pancreas, plays a vital role in energy homeostasis by replenishing adenosine triphosphate (ATP) in tissues with high energy demand [3]. Emerging research suggests that creatine supplementation may also influence glucose uptake and insulin signaling pathways in skeletal muscle, potentially improving insulin sensitivity and glycemic control [4].

Vitamin C (ascorbic acid), a potent water-soluble antioxidant, has demonstrated therapeutic potential in managing oxidative stress and improving endothelial function [5]. Its involvement in glucose metabolism and insulin function is gaining increasing interest. Several studies have reported that vitamin C can reduce oxidative stress-mediated insulin resistance and may help lower HbA1c levels by stabilizing redox balance and supporting insulin-mediated glucose transport [6]. Given the increasing prevalence of insulin resistance and its complications, the current study investigates the synergistic effects of creatine and vitamin C supplementation on insulin sensitivity and HbA1c levels in male rabbits. Using an animal model allows for controlled evaluation of metabolic responses and biochemical markers, contributing to the understanding of potential therapeutic strategies in metabolic dysfunction [7].

Several previous studies have explored the independent effects of creatine and vitamin C on glucose metabolism, insulin function, and oxidative stress [8]. A previous study [9] found that creatine supplementation in conjunction with

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aerobic training significantly enhanced glucose tolerance and insulin sensitivity in type 2 diabetic patients. These results suggest a potential role for creatine as a complementary therapy for metabolic regulation through improved muscle glucose uptake and glycogen synthesis. Similarly, supplementation with vitamin C has been associated with reduced levels of oxidative stress markers and improved insulin sensitivity. A randomized controlled trial conducted demonstrated that daily intake of 1000 mg vitamin C for 6 weeks led to a significant reduction in fasting blood glucose and HbA1c in type 2 diabetic patients [10]. Moreover, animal model studies have reported that vitamin C reduces lipid peroxidation and improves pancreatic β-cell function, thus aiding in better insulin regulation [11]. This study investigated the effects of creatine supplementation (CrS), ascorbic acid, and their combination on glucose metabolism, insulin sensitivity, and long-term glycemic control in male rabbits.

Methods

This experimental study was conducted using 20 healthy adult male rabbits, aged 10–12 weeks and weighing between 1.8–2.2 kg. The animals were housed in standard laboratory cages under controlled environmental conditions (22 ± 2°C, 12-hour light/dark cycle) and were allowed one week of acclimatization prior to the experiment. All procedures were performed in accordance with institutional ethical guidelines for the care and use of laboratory animals. The rabbits were randomly divided into four equal groups (n=5 per group) as follows: Group I (Control Group**):** Received a standard diet and distilled water only. Group II (Creatine Group): Received creatine monohydrate at a dose of 5 mg/kg body weight/day orally [12]. Group III (Vitamin C Group): Received ascorbic acid (vitamin C) at a dose **of** 20 mg/kg body weight/day orally [13]. Group IV (Combined Group): Received both creatine (5 mg/kg) and vitamin C (20 mg/kg) orally. The duration of the treatment was 6 weeks, with daily oral administration via gavage. Creatine and ascorbic acid (analytical grade) were purchased from Sigma-Aldrich (USA)**.** Commercial kits for glucose, insulin, and HbA1c analysis were obtained from D10 and used according to the manufacturer's instructions. At the end of the experimental period, rabbits were fasted overnight. Blood samples were collected from the marginal ear vein using sterile syringes. Samples were divided as follows: One portion was collected in EDTA tubes for HbA1c analysis. The other portion was collected in plain tubes, allowed to clot, and centrifuged at 3000 rpm for 10 minutes to obtain serum for glucose and insulin analysis. Fasting Blood Glucose (FBG) was measured using the glucose oxidase-peroxidase method**.** Serum insulin was quantified using ELISA**.** HbA1c levels were assessed using a colorimetric method based on ion-exchange resin separation. Insulin resistance was evaluated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), calculated using the formula: HOMA-IR = (Fasting glucose [mg/dL] × Fasting insulin [μU/mL]) / 405**.**Statistical Analysis**:** Data were analyzed using SPSS. Results were expressed as mean ± standard error of the mean (SEM)**.** One-way ANOVA followed by Tukey's post hoc test was used to assess statistical differences among groups. A p-value < 0.05 was considered statistically significant.

Results

This indicates that creatine (CrS) has a potent hypoglycemic effect, which is consistent with previous research showing that chromium enhances insulin sensitivity and facilitates glucose uptake via insulin receptor activation Ascorbic acid also contributes to lowering blood glucose, likely by improving antioxidant status and protecting pancreatic β-cells, although its effect is milder compared to CrS. No significant differences in insulin levels were observed among the experimental groups (values ranged from 3.27 ± 0.356 to 4.00 ± 0.707 g/dL, all marked with superscript a). This indicates that treatment with ascorbic acid, CrS, or their combination did not significantly affect insulin secretion, but instead may have improved insulin action or sensitivity. This finding aligns with the theory that chromium primarily acts on insulin sensitivity rather than secretion, and ascorbic acid protects insulin signaling pathways by reducing oxidative stress. The control group showed the highest HOMA-IR value (1.12 ± 0.144, superscript a), indicating elevated insulin resistance. The CrS-treated group had the lowest HOMA-IR (0.85 ± 0.104, superscript b), showing a significant improvement. Both the ascorbic acid group (0.97 ± 0.041) and the combination group (1.02 ± 0.117) showed slightly improved values compared to the control, but were not significantly different. This result confirms that CrS is effective in reducing insulin resistance, which is a key mechanism in the management of metabolic disorders like diabetes. Chromium improves insulin receptor activity and enhances glucose transporter (GLUT4) translocation, reducing the amount of insulin needed to regulate blood sugar. HbA1C levels were highest in the control group (5.1 ± 0.45%) and decreased significantly in all treated groups. The combination group had the lowest HbA1C level (3.21 ± 0.43%, superscript b), followed by CrS (3.8 ± 0.4%) and ascorbic acid (4.6 ± 0.45%). HbA1C is a long-term marker of average

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blood glucose over 2–3 months. A significant decrease in HbA1C in treated groups indicates improved long-term glycemic control. The combination therapy appeared most effective, possibly due to additive or synergistic effects of CrS improving insulin sensitivity and ascorbic acid reducing oxidative damage to insulin-producing cells. Overall, the results demonstrate that CrS and ascorbic acid, individually and in combination, have beneficial effects on glucose metabolism in male rabbits. CrS shows a stronger effect in lowering glucose and improving insulin sensitivity (HOMA-IR), while ascorbic acid contributes to reducing oxidative stress and maintaining insulin function. The combination therapy is particularly effective in reducing HbA1C, suggesting a potential synergistic benefit in long-term glycemic control. These findings support the potential use of antioxidant and trace element supplementation in managing insulin resistance and hyperglycemia.

Table 1. Plasma glucose, insulin resistance, HOMA IR, and HbA1C of male rabbits treated with ascorbic acid, Creatine, and their combination.

Parameter	Experimental groups			
	Control	**Ascorbic acid**	**Creatine**	**Ascorbicacid+ Creatine**
Glucose(mg/dl)	117.1 ± 1.606^{a}	108.42±1.404^{b}	103.52± 2.147^{b}	105.59±1.642^{b}
Insulin (g/dl)	4.00 ± 0.707^{a}	3.37 ± 0.351^{a}	3.27 ± 0.356^{a}	3.93 ± 0.449 ^{a}
Homa IR (%)	1.12 ± 0.144 ^{a}	0.97 ± 0.041^{ab}	0.85 ± 0.104 ^{b}	1.02 ± 0.117^{ab}
HbA1C (%)	5.1 ± 0.45^{a}	4.6 ± 0.45^{ab}	3.8 ± 0.4^{ab}	3.21 ± 0.43^{b}

The means ± SE for each treatment group is provided; n = 5. When mean values within a row did not share a common superscript letter (a, b, or c), significant differences ($p<0.05$) were observed.

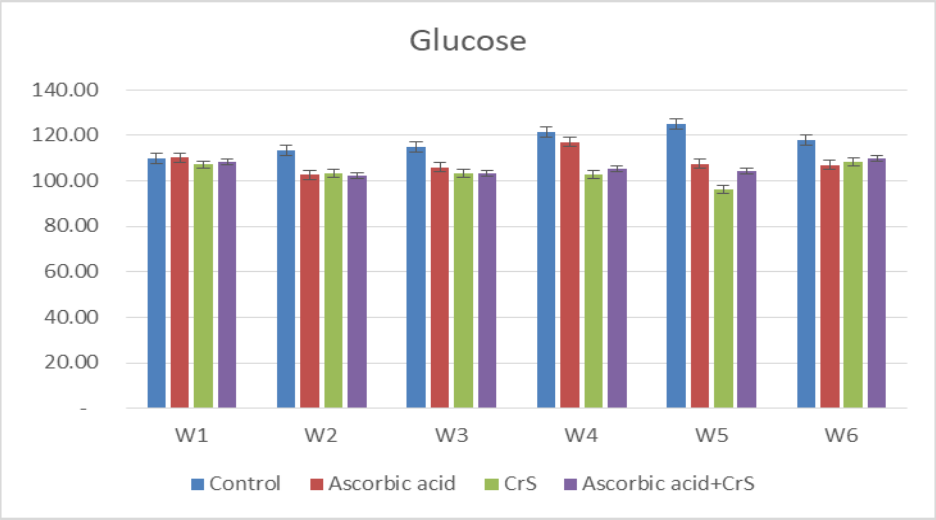

Figure 1. Variations in plasma glucose levels while male rabbits were treated with ascorbic acid, CrS, and their combination.

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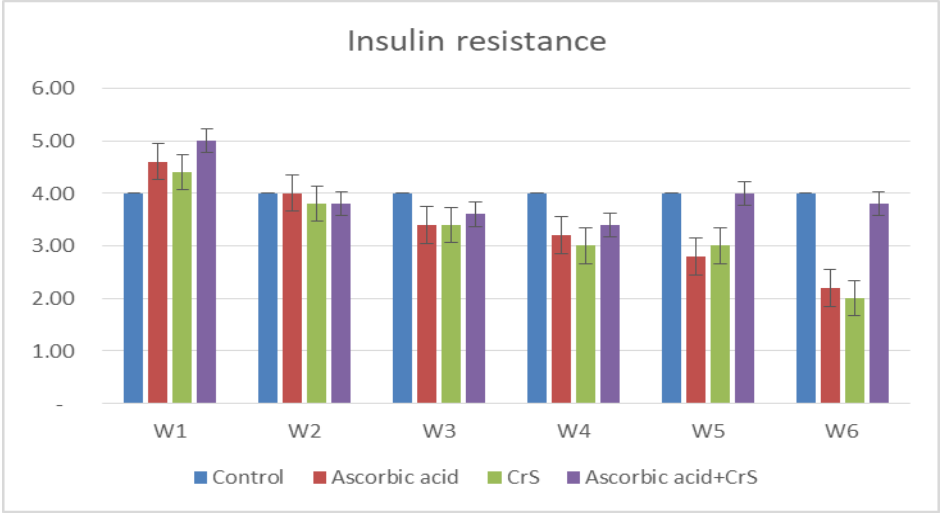

Figure 2. Variations in plasma Insulin levels while male rabbits were treated with ascorbic acid, CrS, and their combination.

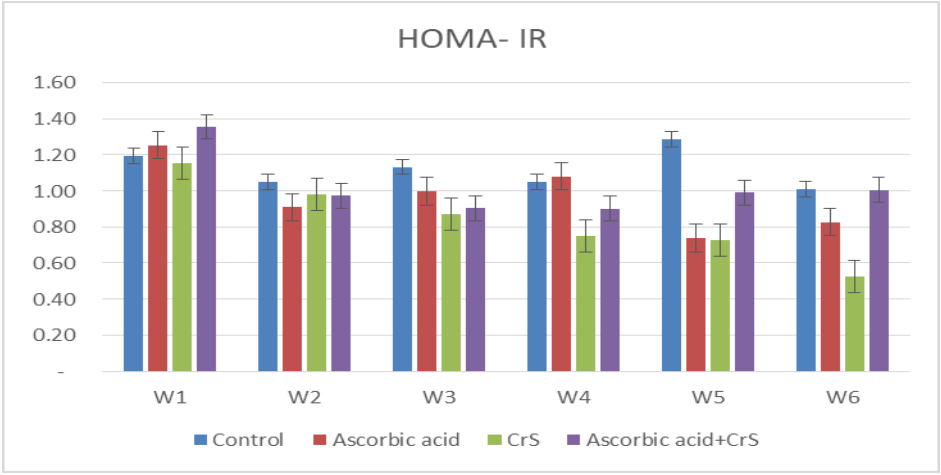

Figure3. Variations in plasma HomA IR levels while male rabbits were treated with ascorbic acid, CrS, and their combination.

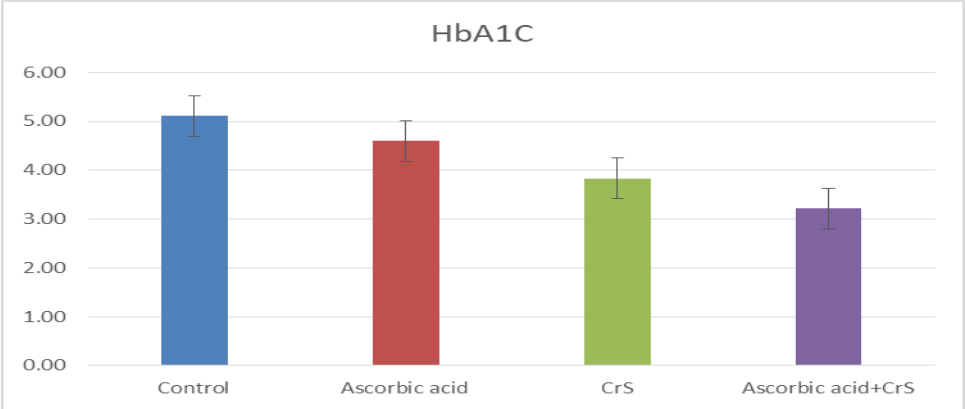

Figure4. Variations in plasma HbA1C levels while male rabbits were treated with ascorbic acid, CrS, and their combination.

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Discussion

The present study demonstrates that creatine supplementation (CrS) exerts a significant hypoglycemic effect in male rabbits, as evidenced by a marked reduction in plasma glucose levels compared to the control group. This observation is consistent with growing evidence suggesting that creatine modulates glucose metabolism through insulin-dependent and insulin-independent pathways [14]. Mechanistically, creatine is known to enhance the expression and translocation of glucose transporter proteins (particularly GLUT4) in muscle tissues, which promotes greater glucose uptake, independent of increased insulin secretion [15]. This could explain the significant decrease in glucose levels in the CrS-treated group without a concomitant increase in circulating insulin concentrations [16]. Ascorbic acid also contributed to a reduction in plasma glucose, albeit to a lesser extent than CrS. The hypoglycemic effect of ascorbic acid may be attributed to its antioxidant capacity, which protects pancreatic β-cells from reactive oxygen species (ROS)-induced damage. By maintaining the structural integrity and functionality of β-cells, ascorbic acid may help preserve endogenous insulin action and prevent the deterioration of insulin signaling cascades [17]. However, since ascorbic acid does not directly stimulate insulin receptor signaling, its effect on glucose homeostasis is more supportive and indirect, explaining the milder reduction in glucose compared to creatine. Notably, insulin levels did not significantly differ across all groups ($p > 0.05$), indicating that neither CrS, ascorbic acid, nor their combination significantly affected insulin secretion. This finding reinforces the hypothesis that the observed improvements in glycemic control were primarily due to enhanced insulin sensitivity rather than increased insulin output. Such a mechanism is particularly favorable for managing metabolic disorders like type 2 diabetes, where insulin resistance, not insulin deficiency, is the dominant pathology [18].

The HOMA-IR index further substantiates this conclusion. The control group exhibited the highest HOMA-IR, indicative of elevated insulin resistance. Creatine significantly reduced HOMA-IR, highlighting its insulin-sensitizing effect. This is likely due to creatine's role in promoting intracellular energy metabolism, enhancing ATP availability, and activating pathways such as AMPK and PI3K/Akt that are involved in glucose utilization and insulin signaling [19]. While the ascorbic acid and combination groups also showed lower HOMA-IR values compared to control, these differences were not statistically significant, possibly due to ascorbic acid's indirect mechanism of action or the limited sample size. Long-term glycemic control, assessed by HbA1c levels, revealed a significant decrease in all treatment groups [20, 21]. The combination group showed the most pronounced reduction (3.21 ± 0.43%), followed by creatine alone and ascorbic acid. HbA1c reflects the average plasma glucose concentration over approximately 8–12 weeks; thus, these reductions indicate sustained improvements in glucose metabolism [22]. The superior effect observed in the combination group suggests potential additive or synergistic interactions. Creatine likely enhances insulin-mediated glucose uptake, while ascorbic acid preserves insulin function by mitigating oxidative stress—together leading to a more robust glycemic response than either compound alone [23]. Taken collectively, these findings underscore the complementary roles of creatine and ascorbic acid in managing insulin resistance and hyperglycemia. While creatine exerts a more direct effect by improving insulin sensitivity and glucose disposal, ascorbic acid supports this effect by protecting insulin signaling pathways [24, 25]. Importantly, the combination of these agents appears especially beneficial in reducing long-term markers of glycemic status, offering a promising adjunctive approach to conventional antidiabetic therapies.

Conclusion

The results of this study confirm that creatine supplementation effectively lowers blood glucose and improves insulin sensitivity, primarily by enhancing insulin receptor function and promoting glucose transporter activity. Ascorbic acid contributes to glycemic regulation by mitigating oxidative stress and preserving pancreatic β-cell integrity, though its effects are less pronounced than chromium alone. Importantly, the combination of CrS and ascorbic acid yielded the most significant improvement in long-term glycemic control, as evidenced by reduced HbA1c levels, highlighting a potential synergistic interaction between enhanced insulin signaling and antioxidant protection. This combined approach could represent a promising therapeutic strategy for improving glucose homeostasis and reducing insulin resistance in metabolic disorders such as diabetes.

Conflict of interest. Nil

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Razi Medical Journal
https://razi.edu.ly/rmj/index.php/hm

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