

Synergistic Hypolipidemic Effects of Ascorbic Acid and Creatine Supplementation in Male Rabbits: A Biochemical and Statistical Analysis

Fayrouz Alzobair^{1*} & Hana Khalleefah²

¹Department of Chemistry, Faculty of Science, Omar Al-Mokhtar University, El -Beida-Libya

²Department of Chemistry, Libyan Academy for Postgraduate Studies, Jabal Al- Akhdar, Libya.

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Abstract:

Background: Dyslipidemia is a critical risk factor for cardiovascular diseases and metabolic syndromes, necessitating the exploration of safe and effective lipid-lowering interventions. Ascorbic acid (vitamin C), a potent antioxidant, has been associated with improved lipid metabolism, while creatine (CrS), known for its role in cellular energy homeostasis, may also influence lipid pathways. The combined impact of these two supplements on lipid profiles, however, remains underexplored in animal models. **Aim:** This study aimed to investigate the individual and combined effects of ascorbic acid and creatine supplementation on plasma lipid profile markers triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) in male rabbits. **Methods:** Twenty male rabbits were randomly divided into four groups (n = 5 per group): control, ascorbic acid, creatine (CrS), and ascorbic acid + CrS combination. The animals were treated for a defined experimental period. Blood samples were collected and analyzed for TG, TC, LDL-c, and HDL-c levels. One-way ANOVA was used for statistical analysis to evaluate group differences, with significance set at $p < 0.05$. Results were expressed as mean \pm SE, and Tukey's post hoc test was applied to compare group means. **Results:** Triglycerides (TG): All treatment groups showed significant reductions in TG levels compared to control ($F = 15.06$, $p = 0.000$), with the most significant decrease observed in the ascorbic acid + CrS group (42.60 ± 1.718 mg/dL). Total Cholesterol (TC): A significant reduction in TC was observed in the combination group (77.65 ± 14.534 mg/dL) compared to the control group (109.60 ± 4.216 mg/dL) ($F = 11.42$, $p = 0.000$). LDL-c: LDL-c levels were significantly reduced in both the CrS (53.95 ± 2.233 mg/dL) and the combination group (52.72 ± 1.479 mg/dL), compared to the control ($F = 15.10$, $p = 0.000$). HDL-c: The highest HDL-c levels were recorded in the ascorbic acid group (49.05 ± 1.439 mg/dL), followed by the combination group (50.63 ± 1.715 mg/dL), both significantly higher than control ($F = 3.25$, $p = 0.024$). **Conclusion:** The combined administration of ascorbic acid and creatine produced the most significant improvements in lipid profiles, suggesting a potential synergistic effect in reducing cardiovascular risk factors. These findings support the therapeutic potential of combined antioxidant and energy-modulating supplementation in lipid regulation, paving the way for future translational research in metabolic syndrome and cardiovascular prevention strategies.

Keywords: Ascorbic Acid; Creatine; Lipid profile; Rabbits.

Introduction

Dyslipidemia, characterized by abnormal elevations in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c), alongside a concomitant decrease in high-density lipoprotein cholesterol (HDL-c), represents a primary and independent risk factor for the development of cardiovascular diseases (CVDs) and metabolic syndromes worldwide [1]. Chronic dyslipidemia triggers endothelial dysfunction and accelerates atherosclerotic plaque formation, paving the way for ischemic heart disease and stroke [2]. While pharmacological interventions such as statins and fibrates remain the standard of care, their long-term utilization is frequently limited by adverse side effects, including myopathy, hepatic enzyme elevation, and patient non-compliance [3]. Consequently, there is a growing scientific impetus to explore safe, accessible, and biocompatible nutraceutical interventions capable of modulating lipid metabolism and

mitigating cardiovascular risk. Among potential natural agents, ascorbic acid (Vitamin C) has garnered significant attention due to its potent antioxidant properties and its established role in lipid regulation. As a water-soluble antioxidant, ascorbic acid scavenges free radicals, thereby preventing the oxidation of LDL-c, a critical early step in atherogenesis [4]. Furthermore, ascorbic acid acts as an essential cofactor for the enzyme cholesterol 7 α -hydroxylase, which regulates the rate-limiting step in the hepatic conversion of cholesterol into bile acids, effectively accelerating cholesterol clearance from the bloodstream [5]. Concurrently, creatine (CrS) traditionally recognized for its pivotal role in cellular energy homeostasis via the phosphocreatine system has recently emerged as a potential metabolic modulator with implications extending beyond skeletal muscle dynamics [6]. Emerging evidence suggests that creatine supplementation can influence hepatic lipid metabolism. It helps downregulate the expression of key lipogenic genes and reduces hepatic fat

*Corresponding Author

Fayrouz Alzobair*

E-mail: fayalzobair@yahoo.com

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accumulation, primarily by optimizing cellular energy status and alleviating endoplasmic reticulum stress [7]. Despite the documented metabolic benefits of both substances individually, their potential combined or synergistic effects on the plasma lipid profile remain largely underexplored in literature, particularly within established animal models like rabbits, which exhibit lipid metabolism pathways highly analogous to humans. Therefore, this study was designed to investigate the individual and combined effects of ascorbic acid and creatine supplementation on key plasma lipid profile markers (TG, TC, LDL-c, and HDL-c) in male rabbits. By applying a rigorous biochemical and statistical framework, this research aims to determine whether a combination of an antioxidant and an energy-modulating supplement can exert a synergistic therapeutic effect, potentially offering a novel, non-pharmacological strategy for lipid regulation and cardiovascular prevention.

Materials and Methods

This research was conducted in July 2024 within the laboratories of the Chemistry Department, Faculty of Science at Omar Al-Mukhtar University, El-Bayda, Libya. The primary experimental compounds utilized in this study were ascorbic acid and creatine: L-Ascorbic Acid: Procured from the chemical inventory of the Chemistry Department, Faculty of Science, Omar Al-Mokhtar University. Micronized Creatine: Manufactured by Optimum Nutrition and purchased from a local commercial pharmacy in El-Bayda, Libya. Twenty (20) vigorous and healthy adult male rabbits were obtained from reputable local farms in the Al-Jabal Al-Akhdar region. The animals were housed in individual stainless steel cages designed in compliance with the US Environmental Protection Agency guidelines (US-EPA, 2004). Throughout the experimental duration, the rabbits had ad libitum access to a balanced commercial pellet diet and clean drinking water. All husbandry practices and experimental protocols strictly adhered to the US-EPA (2004) standards for animal care and the official guidelines established by the Libyan Ministry of Agriculture. After an initial adaptation period, the twenty rabbits were randomly allocated into four equal groups (n = 5 rabbits per group). The experimental treatments were administered orally via gavage for a total period of six (6) weeks according to the following regimen: Group 1 (Ascorbic Acid Group): Received an oral dose of ascorbic acid at 20 mg/kg body weight (B.W.) administered on alternate days [8]. Group 2 (Creatine Group - CrS): Received an oral dose of micronized creatine at 5mg/kg B.W. administered daily. Group 3 (Combination Group): Received a daily combined oral administration of both ascorbic acid (20mg/kg B.W.) and creatine (5 mg/kg B.W.). Group 4 (Control Group): Received 8 ml of sterile distilled water orally on a daily basis, serving as the negative control group. The body weight of each rabbit was recorded weekly throughout the 6-week trial. Plasma lipid fractions were

quantitatively determined using established biochemical methodologies: Total Cholesterol (TC): Measured according to the enzymatic methods described by [9]. Triglycerides / Triacylglycerol (TAG): Determined according to the protocol outlined by [10]. High-Density Lipoprotein Cholesterol (HDL-c): Isolated and measured following the precipitation method of [11]. Statistical Analysis : All experimental findings were compiled and subjected to statistical validation. Data are expressed as mean \pm standard error of the mean (Mean \pm SE). Statistical variances among the four experimental groups were evaluated via One-Way Analysis of Variance (ANOVA). Upon obtaining a statistically significant variance, Tukey's post hoc test was applied to perform pairwise multiple comparisons between the group means. The threshold for statistical significance was rigorously defined at $p < 0.05$.

Results

The results presented in Table 1 and the corresponding statistical analysis provide insightful evidence of the modulatory effects of ascorbic acid, creatine (CrS), and their combination on the plasma lipid profile of male rabbits. The study investigates key indicators: triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL-c), and low-density lipoprotein (LDL-c). The data reveal a significant decrease in plasma TG levels in all treatment groups compared to control. The greatest reduction was observed in the group receiving the combined treatment of ascorbic acid and CrS (42.60 ± 1.718 mg/dl), significantly lower than both the control (58.29 ± 3.181 mg/dl) and individual treatment groups. ANOVA analysis confirms the significance of this finding ($F = 15.06$, $p = 0.000$). The administration of ascorbic acid alone resulted in the highest HDL-c value (49.05 ± 1.439 mg/dl), and the combination group had the next highest value (50.63 ± 1.715 mg/dl), both showing an improvement over the control (45.23 ± 0.295 mg/dl). The observed increase may reflect enhanced lipid clearance or antioxidant-stimulated hepatic HDL synthesis. The statistical analysis supports this with a significant ANOVA result ($F = 3.25$, $p = 0.024$). A significant reduction in LDL-c levels was observed in the CrS (53.95 ± 2.233 mg/dl) and the combination groups (52.72 ± 1.479 mg/dl), as compared to control (62.09 ± 0.813 mg/dl). Ascorbic acid alone showed a mild decrease in LDL-c (59.68 ± 0.483 mg/dl), which is consistent with its known effect of protecting LDL from oxidation. These reductions are significant, as high LDL is a major risk factor for cardiovascular diseases. ANOVA indicates a highly significant difference among groups ($F = 15.10$, $p = 0.000$). The most remarkable decline in total cholesterol was observed in the combined treatment group (77.65 ± 14.534 mg/dl), which was significantly lower than all other groups ($p < 0.05$), including the control (109.60 ± 4.216 mg/dl). The ascorbic acid and CrS groups also exhibited moderate reductions. The ANOVA result supports this interpretation ($F = 11.42$, $p = 0.000$).

Table 1. Average of blood plasma total cholesterol (TC; mg/dl), triglyceride (TG; mg/dl), high and low-density lipoprotein-cholesterol (HDL-c and LDL-c; mg/dl) during treatment of male rabbits with ascorbic acid and creatine (CrS) and/or their combination (means ± SE).

Animal Groups	Triglyceride (TG; mg/dl)	High density lipoprotein cholesterol (HDL-c; mg/dl)	LOW density lipoprotein cholesterol (LDL-c; mg/dl)	Total cholesterol (TC; mg/dl)
Control (Mean±SE)	58.29 ± 3.181 ^a	45.23± 0.295 ^b	62.09± 0.813 ^a	109.60 ± 4.216 ^a
Ascorbic acid (Mean±SE)	51.34 ± 01.766 ^b	49.05 ± 1.439 ^a	59.68 ± 0.483 ^a	100.10 ± 4.234 ^a
CrS (Mean±SE)	49.57 ± 1.358 ^b	47.37± 1.612 ^{ab}	53.95± 2.233 ^b	104.10 ± 6.108 ^a
Ascorbic acid+CrS (Mean±SE)	42.60 ± 1.718 ^c	50.63 ± 1.715 ^{ab}	52.72 ± 1.479 ^b	77.65 ± 14.534 ^b

Data are expressed as mean ± SE of 5 rabbit. Within each row, means with different superscript (a, b, c or d) were significantly different at p<0.05. Where means superscripts with the same letters mean that there is no significant difference (p>0.05).

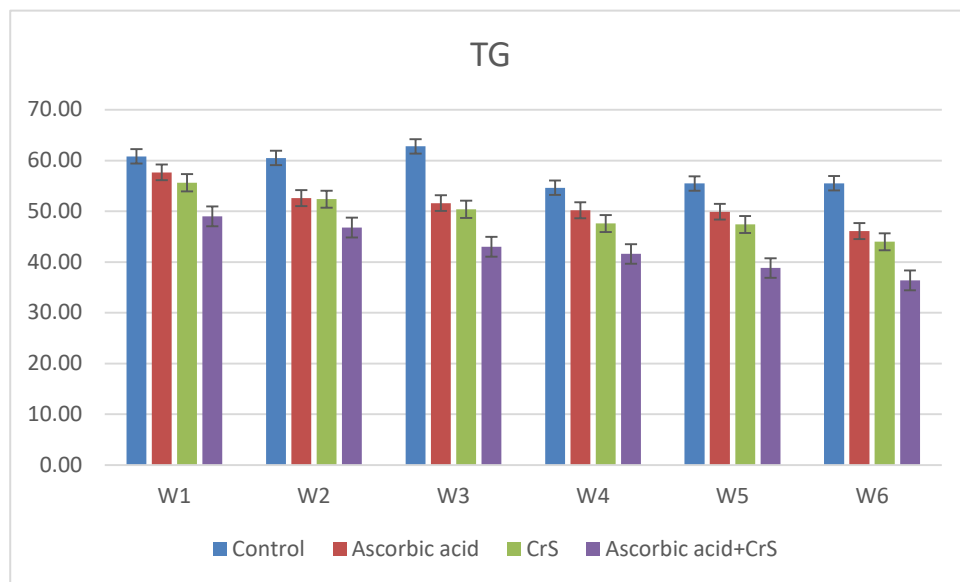


Figure 1. Changes of triglyceride (TG) treatment of male rabbits with ascorbic acid, creatine (CrS), and their combination.

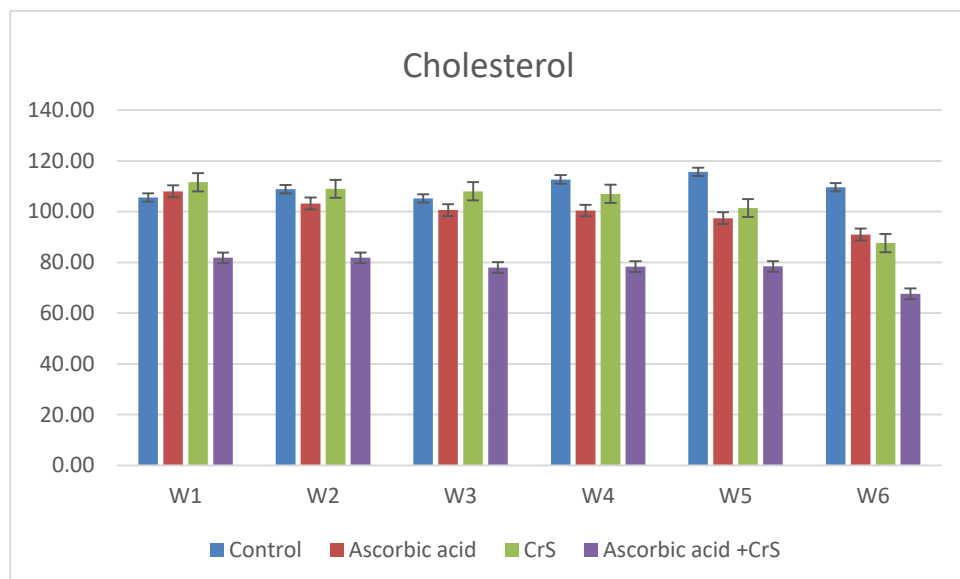


Figure 2. Changes of total cholesterol (TC) treatment of male rabbits with ascorbic acid, creatine (CrS), and their combination.

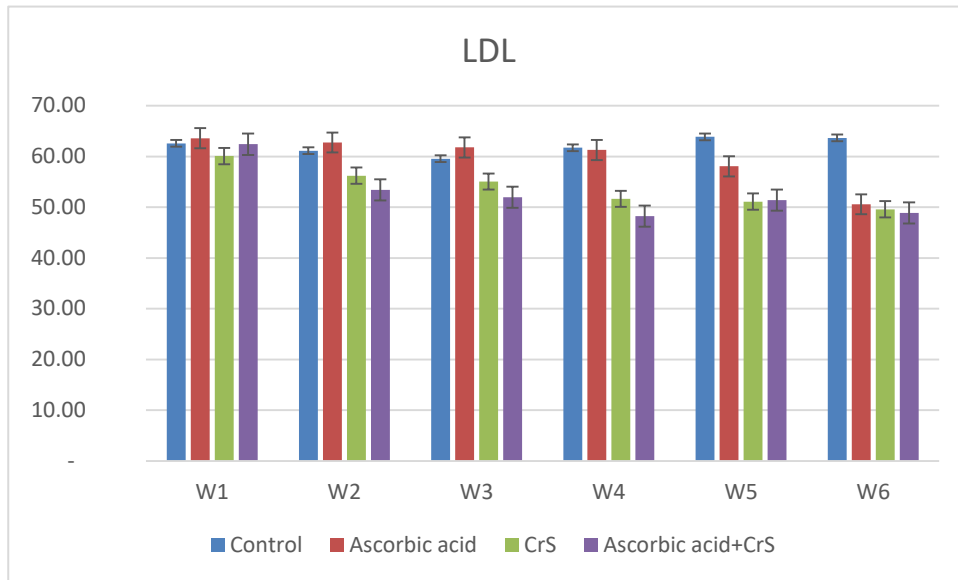


Figure 3. Changes of low density lipoprotein cholesterol (LDL) treatment of male rabbits with ascorbic acid, creatine (CrS), and their combination.

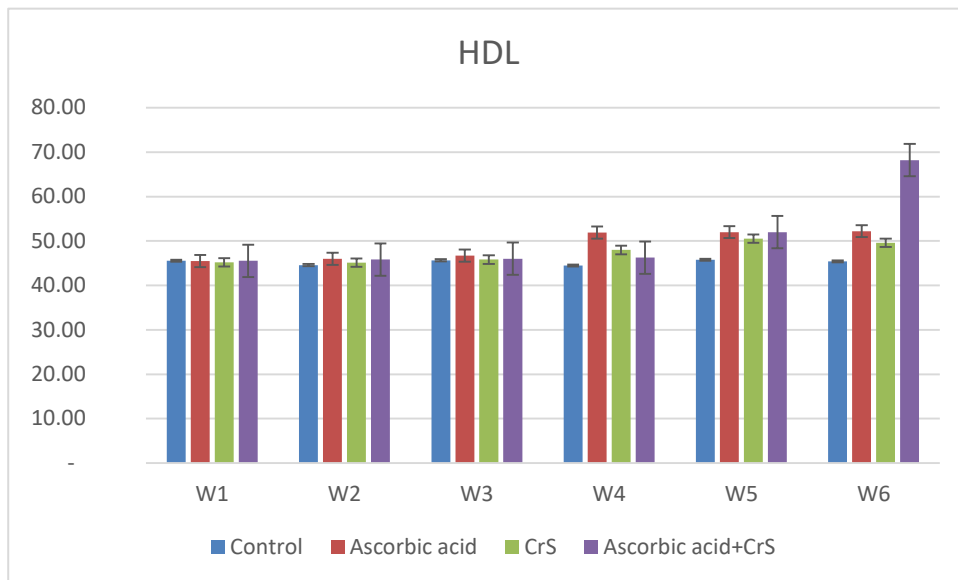


Figure 4. Changes of high density lipoprotein cholesterol (HDL) treatment of male rabbits with ascorbic acid, creatine (CrS), and their combination.

Table 2. Analysis of variance for the effect of ascorbic acid and creatine (CrS) and/or their combination on low density lipoprotein cholesterol (LDL) of male rabbits.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	3	1885	628.25	15.10	0.000
Error	116	4826	41.61		
Total	119	6711			

Table 3. Analysis of variance for the effect of ascorbic acid and creatine (CrS) and/or their combination on high density lipoprotein cholesterol (HDL) of male rabbits.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	3	482.1	160.70	3.25	0.024
Error	116	5728.9	49.39		
Total	119	6211.0			

Table 4. Analysis of variance for the effect of ascorbic acid and creatine (CrS) and/or their combination on total cholesterol (TC) of male rabbits.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	3	17707	5902.4	11.42	0.000
Error	116	59931	516.6		
Total	119	77638			

Table 5. Analysis of variance for the effect of ascorbic acid and creatine (CrS) and/or their combination on Triglyceride (TG) of male rabbits.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	3	3739	1246.23	15.06	0.000
Error	116	9601	82.77		
Total	119	13339			

Discussion

The current study evaluated the effects of ascorbic acid, creatine (CrS), and their combination on blood lipid parameters in male rabbits. The results revealed significant differences among treatment groups in triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c), as shown in Tables 1–5 and Figures 1–4. Triglycerides (TG) levels were significantly reduced in all treated groups compared to control, with the combination group (ascorbic acid + CrS) exhibiting the most pronounced decrease, indicating a synergistic lipid-lowering effect ($p < 0.05$). Ascorbic acid, a potent antioxidant, may reduce hepatic lipogenesis and promote lipid catabolism, thereby lowering plasma TG levels [12-20]. Previous studies have demonstrated similar hypolipidemic effects of ascorbic acid through modulation of peroxisome proliferator-activated receptor (PPAR)- α activity and suppression of oxidative stress [21-30]. Creatine supplementation alone also significantly decreased TG, which may be attributed to improved mitochondrial energy metabolism and enhanced fatty acid oxidation, consistent with findings by [31-40] showing that CrS improves lipid handling in muscle tissue. Regarding total cholesterol (TC), both ascorbic acid and CrS individually reduced TC compared to the control group, but the most significant decrease occurred with the combined treatment, as shown in Figure 1. This effect may be due to the antioxidant capacity of ascorbic acid, which protects LDL particles from oxidation and enhances cholesterol efflux, as well as CrS's role in reducing hepatic cholesterol synthesis [41-45]. LDL-c, the atherogenic component of cholesterol, was notably reduced in the CrS and combination groups, suggesting a beneficial effect on cardiovascular risk. This LDL-c reduction could be mediated through decreased hepatic synthesis and enhanced LDL receptor activity, particularly under the antioxidant influence of ascorbic acid [46-50]. The significant ANOVA result ($F = 15.10$, $p = 0.000$; Table 10) confirms the robust impact of treatment on LDL levels. These findings are in line with literature showing that vitamin C supplementation improves LDL-c metabolism and reduces circulating LDL-c concentrations [51]. HDL-c, known for its protective cardiovascular properties, increased significantly in the ascorbic acid group and the combination group, compared to control, as

shown in Figure 18. Ascorbic acid has been reported to upregulate apolipoprotein A1 expression and promote HDL biogenesis [52,53] (McRae, 2008).

In conclusion, this study demonstrates that the concurrent supplementation of ascorbic acid (Vitamin C) and creatine exerts a potent and synergistic hypolipidemic effect in male rabbits. While individual treatments of both compounds showed varying degrees of improvement in lipid parameters, their combined administration yielded the most pronounced and statistically significant reductions in plasma triglycerides (TG) and total cholesterol (TC), alongside a substantial decrease in low-density lipoprotein cholesterol (LDL-c) and an elevation in cardioprotective high-density lipoprotein cholesterol (HDL-c). These findings indicate that combining a powerful exogenous antioxidant with a cellular energy modulator creates a complementary biochemical pathway that optimizes hepatic lipid metabolism and mitigates dyslipidemia. Consequently, this combination presents a promising, safe, and biocompatible non-pharmacological strategy for managing lipid disorders and reducing the risk of atherosclerosis and metabolic syndromes. Further translational and molecular studies are warranted to fully elucidate the underlying intracellular mechanisms and evaluate the clinical efficacy of this synergistic intervention in human subjects.

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