

# Evaluation of Some Physiological Kidney Function Tests in Athletes Underuse of Protein Supplementation: Pilot Study

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## Abstract

**Objective:** To evaluate the impact of protein supplementation on kidney health among athletes, focusing on the relationship between protein supplements and kidney biomarkers.

**Methods:** The study analyzed kidney biomarkers—creatinine, urea, and uric acid levels—in serum and urine samples, as well as the albumin-to-creatinine ratio (ACR) in urine. Participants included adult male athletes from Sulaymaniyah City, Iraq, divided into two groups: Those who did not consume protein supplements (-ve protein supplementation) and those who did (+ve protein supplementation).

**Results:** Statistical analysis showed no significant difference ( $P < 0.05$ ) in kidney biomarker levels between the -ve and +ve protein supplementation groups, indicating no adverse effects of protein supplements on kidney health.

**Conclusion:** The study concludes that protein supplementation does not appear to have detrimental effects on kidney biomarkers in athletes. Excluding other lifestyle factors, such as smoking and alcohol consumption, is important to prevent additional alterations in kidney function.

**Keywords:** ACR, athlete, kidney function tests, protein supplementation

## Introduction

Athletes use protein supplements for weight loss, muscle growth, and dynamic performance.<sup>1</sup> For energy production, athletes may require more protein; that is why protein supplements are used daily to fill the requirement, but today those who work out at the gym regularly take protein supplements without any professional instructions from physicians, which can later lead to health issues,<sup>2</sup> health issues like a chronic renal failure,<sup>3</sup> cancer, nephrotoxicity, and neurotoxicity. Protein supplements are used to get the right amount of protein needed for the body. Still, taking it incorrectly and excessively without medical supervision can cause serious issues.<sup>4</sup> In this research, the effect of protein supplements on the kidney will be evaluated to detect their harmful impact on the physiological and functional state of the kidneys. After this, the kidney biomarker tests will be compared with athletes who do not take protein supplements. The health risks which come in parallel with protein supplement consumption originate from its contents that typically will encompass a source of protein either plant-based as soybeans and/or substituted in eggs or milk with other filler ingredients, namely artificial sugars, flavoring, minerals along with vitamins, except for the newly found detectable amount of heavy metals in them which exceeds the health risks of a hazard index less than one ( $HI < 1$ ) in which is mainly carried by cadmium and arsenic.<sup>5</sup> Along with this, another study explicitly investigates the protein powders and the adverse effects and health issues caused by their contents in athletes.<sup>6</sup> Some similar articles and research have been conducted to detect the effect of protein supplements on the human body,<sup>7,8</sup> targeting athletic and non-athletic individuals who tend to consume larger quantities leading to substantial health risks. The research focused on the efficacy of protein supplements that athletes have used and also focuses on how excessive consumption of these protein enhancers are used as a substitute for steroids to reach the desired

results, with the consequences that can cause some severe health issues such as kidney damage, gout, as well as physiological effects as dehydration.<sup>5</sup>

## Purpose of the Study

The lack of knowledge and misuse by the consumers of these protein supplementations have made it necessary to expand the research and data regarding the potential dangers and consequences of protein supplement consumption misuse. Regardless, the already existing data is focused on different regions of the world, making it intriguing for us to investigate in our community.

## Methods

Data were obtained from male adult gym athlete participants who underuse protein supplements without any use of secondary enhancing supplements or illness-specific medications. The samples were collected in different intervals of location and time; due to the time-sensitivity of some of the samples, they were analyzed as soon as they were collected. The data in the result presented as a mean  $\pm$  95% confidence interval. The comparison between the two groups was generated using the statistical software GraphPad Prism (version 8.0.2, GraphPad Prism, IBM, USA) using paired two-tailed *t*-test, considering *P*-value  $< 0.05$  as a significant difference.

## Participants

The process of acquiring samples was initiated by specifying criteria for the participants as target patients who will be eligible for this project. The general target athlete participant criteria consist of adult males between the ages of 21–50 years old with no pre-existing complications in their medical history, as well as no lifestyle risk factors such as cigarette/narghile smoking or alcohol consumption that would influence body physiology and test results. Other points that

participants were set to fall under are the duration of which they have been an athlete and have been active in the gymnasium facilities, thus limited to the duration of one year and above. In addition to these criteria, the protein-consuming participants also abide by the duration of protein consumption, which was also limited to a year and above. Further factors were not limited to a criterion, but the overall participants' standard included a height range between (168–190 cm) and weight between (65–114 kg).

A total of 32 athletes participated in two groups equally separated based on their consumption of protein supplementation. All participants were adult males and residents of Sulaymaniyah City in the Kurdistan region of Iraq from five different gyms in the city, with some volunteers students from Komar University of Science and Technology who met the criteria to participate in this study.

At the same time, the sample size was reduced due to other factors, such as an inappropriate collection of the samples, which led to hemolysis, thus remaining a total of 26 participants whose results are included in this work.

- Group 1: 13 Participants, a negative group of non-protein supplement consumers
- Group 2: 13 Participants, a positive group of protein supplement-consuming participants.

Each participant was asked to fill out a consent form in advance and before obtaining any samples, stating their information and signature in agreement with their understanding and cooperation with the project. The consent form contained questions such as the participant's name, age, height, medical condition, use of medications or protein supplements, and how long participants have been working out (more than one year or not), as well as their phone numbers, date and time of sample collection, the amount of sample taken from the participants, and in the bottom of the consent form an area was left precisely for the participants and the author to sign (Table 1). Most of the participants who uptake protein supplements claim that using these supplementation before starting the training session mixed with milk or citrus juice.

This study adhered to the ethical standards settled by the KUST committee of research approval, which used STROBE guidelines to ensure the quality of the study, and granted (KUSTS2203MLS01) as an approval number for the study.

### Data Collection and Analysis

The blood samples were obtained abiding by safety measures and standard procedure of venipuncture, using blue 5 ml, 23G syringes, and yellow 5 ml gel-clot activator tubes; after 30 minutes of clotting time, the obtained samples were centrifuged at 3400 rpm (round per minute) for 10 minutes to extract the plasma, subsequently separating plasma into 1.5 ml Eppendorf tubes. Alongside the blood sample, participants were given standard 10 ml urine tubes for urine samples, which were used in creatinine, urea, uric acid, and albumin: creatinine ratio (ACR). On the other hand, the tests performed with the blood samples were all the same, excluding the urinary ACR test, which consists of seven kidney biomarker tests in both urine and blood. The creatinine, urea, and uric acid levels were quantified using chemical reagent kits (BioLabo, France) according to the instruction in manual sheet for both plasma and urine samples. In addition, Albumin concentration was

quantified in urine for ACR test using chemical reagent kit (BioSystem, Spain). All these biochemical tests were performed using spectrophotometer (Jenway 6305, UK).

## Results

### Serum Creatinine

The creatinine test results in serum showed the -ve protein supplementary group with  $1.37 \pm 0.1$ , and the +ve protein supplementary group with  $1.41 \pm 0.2$ . The *P*-value between these two groups was 0.7227, indicating no significant difference (Figure 1A).

### Serum Urea

The result of urea tests in serum showed the -ve protein supplementary group with  $42.4 \pm 3.3$ , while the +ve protein supplementary group with  $45.5 \pm 8.7$ , and there was no significant difference because the *P*-value between the two supplementary groups was 0.5626 (Figure 1B).

### Serum Uric Acid

Test results for uric acid serum showed that the -ve protein supplementary group was  $8.0 \pm 0.6$ , the +ve protein supplementary group was  $7.2 \pm 0.9$ , and the *P*-value between the two groups was 0.2179, indicating there was no significant difference between the two groups (Figure 1C).

### Urinary Creatinine

The result of creatinine tests in urine showed the -ve protein supplementary group with  $165.5 \pm 39.0$ , and the +ve protein supplementary group result was  $247.8 \pm 95.0$ . The *P*-value between the two groups was 0.1082, indicating no significant difference (Figure 2A).

### Urinary Urea

The results of urea tests in urine showed the -ve protein supplementary group with  $20.4 \pm 3.3$ . The +ve protein supplementary group result was  $25.8 \pm 4.4$ ; there was no significant difference between the two groups because the *P*-value was 0.0995 (Figure 2B).

### Urinary Uric Acid

The result of the uric acid test in urine for the -ve protein supplementary group was  $67.4 \pm 13.8$  mean value. The +ve protein supplementary group result showed a value of

Table 1. The type and amount of protein supplementation consumed by the participants in this study

Protein supplement type and amount per training session	Number of +ve protein supplementation participants
Whey protein (30 gram)	6
ISO (30 gram)	2
Mass Pro (90 gram)	1
Creatine (5 gram)	1
Glutamine (40 gram)	1
Amino (3 gram)	1
Unrecorded	1

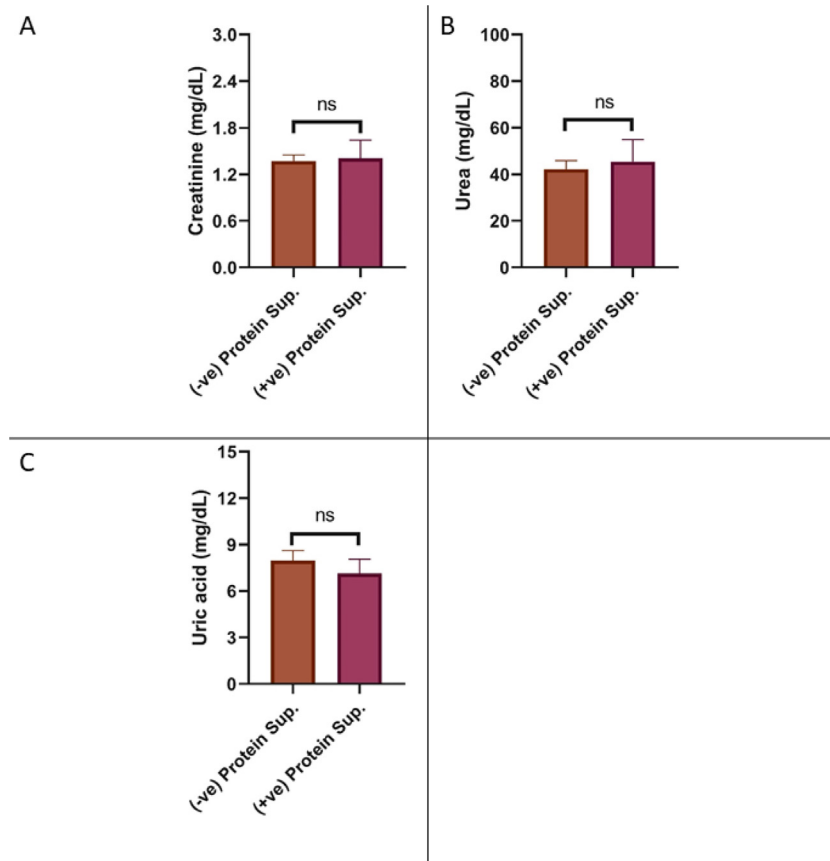


Fig. 1 The bar chart showing test results for serum creatinine, urea, and uric acid levels in both -ve and +ve protein supplementary groups.

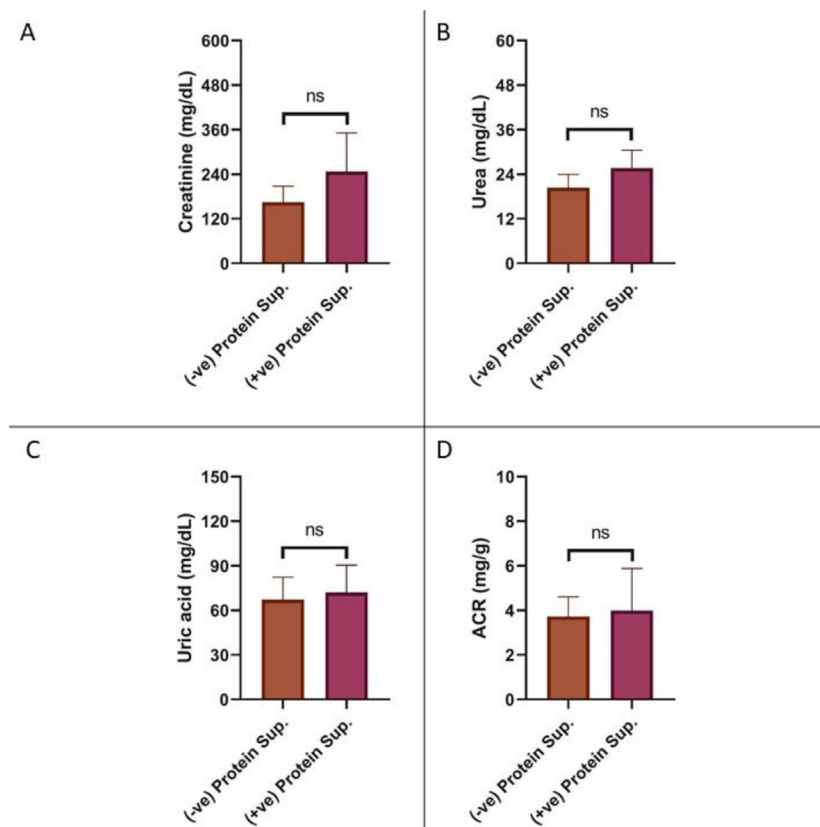


Fig. 2 The bar chart shows the levels of urinary creatinine, urea, uric acid, and Albumin to creatinine ratio in both -ve and +ve protein supplementary groups.

72.2  $\pm$  16.9. The *P*-value between the two sup groups was 0.7012, showing no significant difference (Figure 2C).

### Urinary Albumin to Creatinine Ratio (ACR)

The results of urinary ACR showed the -ve protein supplementary group with 3.8  $\pm$  0.8 and +ve protein supplementary group with a value of 4.1  $\pm$  1.7. The *P*-value was 0.8172, meaning there was no significant difference between the groups (Figure 2D).

## Discussion

As the outcome of this research showed no significant difference between the -ve protein supplementation group and +ve protein supplementation group, it would indicate the lack of effect of protein supplementation on the kidney biomarkers such as creatinine, urea, and uric acid in both urine and serum samples, as well as albumin: creatinine ratio in the urine of athletes who follow standard guidelines or instruction from personal trainers. The results presented agree with other conducted analysis, which exhibits the insusceptibility of renal function to these isolated agents.<sup>9</sup> Furthermore, in favor of this statement, pre-existing data implies that the combination of protein consumerism in healthy resistance-trained athletes/individuals, and despite increasing the level of protein intake, studies have demonstrated no significant difference in kidney functions.<sup>10-12</sup> Even supposing the discovery of heavy metal cadmium (Cd) and other artificial components in the manufacturing of these supplements could theoretically cause adverse health risks and effects on the kidney, it also showed insignificant toxicity when consumed excessively and proved insufficient to alter the mechanism of the renal system.<sup>15</sup>

In opposition to this notion, contrasting studies have investigated the adverse effects of protein supplementation in high-protein diets on renal functions, with the differential points of excessive, chronic, and lack of professional medical guidance on consumption of these supplementations over a long time and when in combination with sedentary lifestyles.<sup>13</sup> Alongside the considerable difference in the type of sample, these analyses were performed on two groups, one of them as humans and the other as animal models.<sup>14</sup> The presented results were not differentiated or separated to distinguish which is for which.<sup>13</sup> In addition, mear data is accessible based on derivatives in these supplements, namely ones that are plant-based versus animal protein dietary supplements that inevitably contain an equivalent amount of total protein, which validate contradistinctive efficacy on renal function, concluding the significant rise of glomerular filtration rate (GFR) and platelet-rich fibrin (PRF) when consuming

animal-based protein supplements. At the same time, there were insignificant deviations in these parameters for plant-based protein consumers.<sup>15</sup>

## Conclusion

Protein supplements are one of the most favored dietary supplements and are commonly used by athletes for muscle growth and several other benefits. Protein supplement consumption can lead to serious health issues when used too much without any medical guidance. Evaluation of kidney biomarkers was done on both -ve and +ve protein supplementary groups and then compared; the results concluded that there were no significant differences in both supplementary groups and that consuming protein supplements did not have any effect on the kidney's functional ability or its physiology when both -ve and +ve protein sup groups were compared.

## Recommendations & Limitations

Extended investigations are advised to understand the influence of protein supplementation on the kidney.

Facilitate an enormous variety of tests such as cystatin c, glomerular filtration rate (GFR), inflammatory biomarkers (interleukin-1, tumor necrosis factor-alpha), as well as a kidney gene panel to exclude the genetic aspects of pre-existing damage to the kidneys or higher susceptibility when exposed to the infiltrating supplementations.

Enlarge the sample size with an extensive limitation for participant qualification and establishing detailed information regarding the type and amount of protein consumed, along with specifying the period of continues consumerism. Another limitations of this study is the difficulty of find the (-ve protein supplementation) participants, since few atheltes in gyms who prattice fitness and body building excersies for more than one year without using protein supplementatn. In addition, The types and duration of training and excersies for participants is not recorded in this study.

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## Conflict of Interest

None. ■

## References

- Bowen, A., Denny, V. C., Zahedi, I., Bidaisee, S. D. V. M., & Keku, E. M. D. (2018). The whey and casein protein powder consumption: The implications for public health. *International Public Health Journal*, 10(2):131–136.
- Kårlund, A., Gómez-Gallego, C., Turpeinen, A. M., Palo-Oja, O. M., El-Nezami, H., & Kolehmainen, M. (2019). Protein Supplements and Their Relation with Nutrition, Microbiota Composition and Health: Is More Protein Always Better for Sportspeople?. *Nutrients*, 11(4):829. <https://doi.org/10.3390/nu11040829>.
- Pomerantz, A., Blachman-Braun, R., Vital-Flores, S., Berebichez-Fridman, R., Aguilar-Mendoza, J. P., & Lara-Villalón, D. (2016). Consumo de suplemento proteico y su posible asociación con daño renal en atletas mexicanos de alto rendimiento [Protein supplement consumption and its possible association with kidney damage in Mexican elite athletes]. *Revista medica del Instituto Mexicano del Seguro Social*, 54(1):42–47. <https://pubmed.ncbi.nlm.nih.gov/26820197/>.
- El Khoury, D., & Antoine-Jonville, S. (2012). Intake of Nutritional Supplements among People Exercising in Gyms in Beirut City. *Journal of Nutrition and Metabolism*, 2012, 703490. <https://doi.org/10.1155/2012/703490>.
- Bandara, S. B., Towle, K. M., & Monnot, A. D. (2020). A human health risk assessment of heavy metal ingestion among consumers of protein powder supplements. *Toxicology Reports*, 7, 1255–1262. <https://doi.org/10.1016/j.toxrep.2020.08.001>.

6. Goston, J. L., & Correia, M. I. (2010). Intake of nutritional supplements among people exercising in gyms and influencing factors. *Nutrition* (Burbank, Los Angeles County, Calif.), 26(6):604–611. <https://doi.org/10.1016/j.nut.2009.06.021>.
7. Graf, S., Egert, S., & Heer, M. (2011). Effects of whey protein supplements on metabolism: Evidence from human intervention studies. *Current Opinion in Clinical Nutrition and Metabolic Care*, 14(6):569–580. <https://doi.org/10.1097/MCO.0b013e32834b89da>.
8. Lam, F. C., Bukhsh, A., Rehman, H., Waqas, M. K., Shahid, N., Khaliel, A. M., Elhanish, A., Karoud, M., Telb, A., & Khan, T. M. (2019). Efficacy and Safety of Whey Protein Supplements on Vital Sign and Physical Performance Among Athletes: A Network Meta-Analysis. *Frontiers in Pharmacology*, 10, 317. <https://doi.org/10.3389/fphar.2019.00317>.
9. Li, Z., Treyzon, L., Chen, S., Yan, E., Thames, G., & Carpenter, C. L. (2010). Protein-enriched meal replacements do not adversely affect liver, kidney or bone density: An outpatient randomized controlled trial. *Nutrition Journal*, 9, 72. <https://doi.org/10.1186/1475-2891-9-72>.
10. Lugaesi, R., Leme, M., de Salles Painelli, V., Murai, I. H., Roschel, H., Sapienza, M. T., Lancha Junior, A. H., & Gualano, B. (2013). Does long-term creatine supplementation impair kidney function in resistance-trained individuals consuming a high-protein diet?. *Journal of the International Society of Sports Nutrition*, 10(1):26. <https://doi.org/10.1186/1550-2783-10-26>.
11. Reis C. E. G. (2021). Discussion of “Whey protein supplementation and its potentially adverse effects on health: A systematic review”- Unsubstantiated claims of adverse effects of whey protein supplementation on human kidney and liver function. *Applied Physiology, Nutrition, and Metabolism*, 46(1):90–91. <https://doi.org/10.1139/apnm-2020-0674>.
12. Hattori, C. M., Tiselius, H. G., & Heilberg, I. P. (2017). Whey protein and albumin effects upon urinary risk factors for stone formation. *Urolithiasis*, 45(5):421–428. <https://doi.org/10.1007/s00240-017-0975-0>.
13. Vasconcelos, Q. D. J. S., Bachur, T. P. R., & Aragão, G. F. (2021). Whey protein supplementation and its potentially adverse effects on health: A systematic review. *Applied physiology, Nutrition, and Metabolism*, 46(1):27–33. <https://doi.org/10.1139/apnm-2020-0370>.
14. Nunes, R., Silva, P., Alves, J., Stefani, G., Petry, M., Rhoden, C., Dal Lago, P., & Schneider, C. D. (2013). Effects of resistance training associated with whey protein supplementation on liver and kidney biomarkers in rats. *Applied Physiology, Nutrition, and Metabolism*, 38(11):1166–1169. <https://doi.org/10.1139/apnm-2013-0004>.
15. Bernstein, A. M., Treyzon, L., & Li, Z. (2007). Are high-protein, vegetable-based diets safe for kidney function? A review of the literature. *Journal of the American Dietetic Association*, 107(4):644–650. <https://doi.org/10.1016/j.jada.2007.01.002>.

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