



ISSN: 0067-2904

Effect of Whey Protein Supplement on Physiological Parameters in Building Body Athletes

Amal K. Abbas^{*1}, Qasim M. Fathi²

¹Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

²Department of Biology, College of Education, University of Anbar, Anbar, Iraq.

Abstract

This study was conducted to investigate the adverse effect of whey protein supplement taken by athletes in some physiological parameters. (75) blood sample were collected from athletes whose take supplements of whey protein (experimental group), (25) blood sample of athletes who don't take supplement (control group). Results showed a significant decrease ($P \leq 0.05$) in the mean of hemoglobin level (Hb) and percentage of Packed Cell Volume (PCV) in the athletes group who take food supplement compared to control. The results also showed a significant increase ($P \leq 0.05$) in the mean of White Blood Cells count (WBCs) in experimental group, Also the results show significant changes in differential white blood cells in the experimental group when compared with control group. There was a significant increase ($P \leq 0.05$) in the number of neutrophils and monocyte and significant decrease ($P \leq 0.05$) in the mean of lymphocyte in the experimental group compared with control group. There was also a significant increase ($P \leq 0.05$) in the capacity of phagocytic activity of neutrophils in experimental group in compared with control group. There was a significant increase ($P \leq 0.05$) in the levels of Aspartate amino Transferase (AST), Alanine amino Transferase (ALT), Alkaline Phosphatase (ALP) and Bilirubin in experimental group compared with control group.

Keywords: Whey Protein, Physiological Parameters, Building Body Athletes.

تأثير المكمل الغذائي Whey Protein على بعض المعايير الفسلجية لدى لاعبي كمال الاجسام

آمال خضير عباس^{*1}، قاسم محمد فتحي²

¹ قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.

² قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة الانبار، الانبار، العراق.

الخلاصة

اجريت هذه الدراسة لتقييم الاثار السمية للمكمل الغذائي بروتين مصلى اللبن Whey protein المأخوذ من قبل الرياضيين في بعض المعايير الفسلجية. تم جمع (75) عينة دم الرياضيين الذين يتناولون المكمل الغذائي (مجموعة التجربة) و (25) عينة دم من الرياضيين الذين لا يتناولون المكمل الغذائي (مجموعة سيطرة). اظهرت النتائج انخفاض معنوي ($P \leq 0.05$) في معدل خضاب الدم (Hb) والنسبة المئوية لحجم الدم الانضغاطي (PCV) في مجموعة الرياضيين الذين يتناولون المكمل الغذائي مقارنة بمجموعة التجربة، كما بينت النتائج زيادة معنوية ($P \leq 0.05$) في معدل التعداد الكلي لخلايا الدم البيض (WBCs) في مجموعة التجربة، اظهرت النتائج تغيرات معنوية في التعداد النفاذلي لكريات الدم البيض في مجموعة التجربة مقارنة بمجموعة السيطرة، كما كانت هناك زيادة معنوية (P)

($P \leq 0.05$) في معدل التعداد النفاضلي للخلايا العدلة ووحيدة النواة، وحصول انخفاض معنوي ($P \leq 0.05$) في معدل التعداد النفاضلي للخلايا اللمفاوية في مجموعة التجربة مقارنة بمجموعة السيطرة، كما لوحظ زيادة معنوية ($P \leq 0.05$) في النسبة المئوية للخلايا العدلة الملتزمة في مجموعة التجربة مقارنة بمجموعة السيطرة. كما اظهرت النتائج حصول ارتفاع معنوي في كل من Alanine ، Alkaline Phosphatase (ALP) ، amino Transferase (ALT) ، Aspartate amino Transferase (AST) والبيلبيروبين Bilirubin في مجموعة التجربة مقارنة بمجموعة السيطرة.

Introduction

Whey Protein is a high-quality protein that contains high amounts of all the essential amino acids needed compared to other dietary sources of protein. It promotes protein synthesis and prevents its degradation as the ingestion of whey protein causes a rapid increase in plasma levels of essential amino acids, causing an increase in protein synthesis and a slight change in protein degradation [1]. Whey protein supplements are considered one of these nutritional supplements, containing high concentrations of essential amino acids from other protein sources [2].

Whey and beef proteins are high-quality protein sources with the formation of amino acids similar to those found in skeletal muscles that include sulfuric acid like Taurine, Methionine, and Cysteine which have been associated with an effective immune state[3], [4]. As a result, ingestion of high quality proteins through the provision of amino acids can support the immune response of individuals participating in comprehensive and intensive training programs However, recent results indicate that dehydrated whey has biochemical properties beyond what is expected from digestion and absorption, thereby altering metabolism and causing significant physiological changes [5]. Whey protein has a high biological value and has been given attention because of the benefits reported in the field of sports nutrition, which includes effects on the composition of the body and physical performance, as well as its ability to prevent fat tissue and oxidation protein after exercise [6]. Whey protein contains high concentrations of Branched Chain Amino Acids (BCAAs) which is important for its role in preserving the fabric and preventing demolition measures during exercise [7], [8]. Studies on the effects of these supplements on blood parameters are limited and are mostly concentrated on amino acid supplements. Whey Proteins consists of proteins that are effective, complete, and fast with high biological value. These proteins are rich sources of amino acids, vitamins, and minerals required for athletes. They may be applied to various biologically active ingredients that include growth factors [9].

Materials and methods

The study samples of blood were collected by taking a tour to the gymnasiums for bodybuilders who ate dietary supplement whey protein for one year and above and aged between 20 - 30 years. The study samples included two groups, the first: the experimental group consists of 75 players taking the nutrition supplement whey protein, the second: control group consists of 25 players who do not take nutrition supplement whey protein. The samples were subjected to W.B.Cs total count, W.B.Cs differential count test [10] and The phagocytosis was performed in vitro and had been done according to Furth et al., (1985) as follows [11]: Equal volumes (250 μ l) of heparinized blood and bacterial suspension of *S. aureus* (106/ml) (1:1) were mixed into sterile test tube. The mixture was incubated in water bath at 37°C for 30 min with continuously shaking. Smear had been prepared by taking a drop of the mixture on the slide; duplicate slides were made for each tube. Slides had been air dried, fixed by absolute methanol, stained by giemsa stain for 10 minutes and then washed by D.W. The slides had been examined by oil immersion lens to calculate the number of neutrophils engulfed microorganism. The percentage of phagocytic cells had been calculated. It's the ratio between the number of phagocytic cells and the number of 100 cells agocytic and non phagocytic. Determination of the effectiveness of the (AST, ALT) enzyme with the measurement kit processed by RANDOX [12]. Determination of the effectiveness of the (ALP) enzyme with the measurement kit processed by BioMerieux [13]. Determination of total bilirubin with the measurement kit processed by RANDOX [14].

Statistical analysis

The results are analyzed statistically using SPSS program, the variance is analyzed using the ANOVA and T. test schedules to extract the differences between the group of athletes taking the dietary supplement and the control group with emphasis on these differences and standard error extraction [15].

Results and discussion:**Hemoglobin (Hb) rate and Packed cell Volume (PCV)**

The results of the present study showed significant differences ($P < 0.05$) in hemoglobin Hb level of the experimental group compared to control group, the results showed an significant decrease ($P \leq 0.05$) in the level of Hb, which is (10.94 ± 0.15) when it is compared with the rate of hemoglobin of the control group, which is (13.68 ± 0.23) Table-1, the results shown in Table-1 recorded an significant decrease ($P \leq 0.05$) in the rate of Packed Cell Volume (PCV) in the experiment group, which was (36.11 ± 0.25) when compared to the average percentage of Packed Cell Volume (PCV) in the control group in which the rate was (44.04 ± 0.18) . This is consistent with the findings of [16]; it was found that eating dietary supplement whey protein for six months led to a reduction in the percentage of red blood cells and reduces the rate of hemoglobin and the percentage of Packed Cell Volume (PCV). The reduction in the rate of hemoglobin and the percentage of Packed Cell Volume (PCV) can be due to oxidative injuries in red blood cells [17]; It can be related with some deviations in the synthesis of red blood cells path [18] this is contrary to many research on the importance of dietary supplements, including Whey Protein however, it may have a negative effect on hemoglobin and in the Packed Cell Volume (PCV) of red blood cells. To prove this, we need prospective studies involving the use of doses of Whey Protein with different time periods to study its effect on these criteria.

Table 1-Changes in the means of Hb and PCV in experimental group compare with control group.

Group	Hb, gm/dl M \pm SE	PCV% M \pm SE
Control	13.68 \pm 0.23	44.4 \pm 0.18
Experimental	10.94 \pm 0.15 *	36.11 \pm 0.25*

*Significant $P < 0.05$ M \pm SE mean \pm Standard error**The rate of White Blood Cells (WBCs) and the Differential count of WBC**

The results showed an significant increase in the number of white blood cells when measured with control group, the total number of white blood cells in the experimental group showed an significant increase ($P < 0.05$) in the total number of white blood cells that reached (11621 ± 372.0) cell /mm³ of blood when compared to the total cell count of white blood cells in the control group, which reached (8398 ± 392) cell /mm³ of blood. Variations were also found in the differential number of white blood cells, the results showed a significant increase ($P < 0.05$) in the percentage of the neutrophil cells of the experimental group that reached (64.33 ± 2.11) when compared with the percentage of the neutrophil cells of the control group that reached (57.35 ± 1.25) . The results also showed a change in the percentage of lymphocytes. The experimental group showed significant decrease ($P < 0.05$) in the percentage of lymphocytes that reached (32.75 ± 0.83) when compared with the control group that reached (40.5 ± 0.91) . An significant increase ($P < 0.05$) was also observed in the percentage of Monocytes cells in the experimental group, which was (9.01 ± 0.52) compared to the percentage of the Monocytes cells of the control group that reached (6.84 ± 0.41) Table-2. The rise in white blood cell count may be due to the efficacy of dietary supplements in increasing protein synthesis and increasing the effectiveness of bone marrow splitting to produce white blood cells, or an indirect change may be caused by the degeneration of cells in different organs, which led to an inflammatory response, which in turn led to stimulate bone marrow cells to divide and generate large numbers of white blood cells for the purpose of defending the body, in other studies by [19], there was a lack of effect on the rate of white blood cells in the consumption of whey protein supplementation. These differences may be due to the type of supplementation, exercise, and the period of time in which dietary supplements are taken. The results of the percentage rates of the differential number of acid cells and alkaline cells in the experimental group did not register any significant differences compared to the percentage rates in the control group. The increase in the percentage of neutrophil cells in the experimental group is an significant increase compared to the control group, which may reflect the effect of Whey Protein dietary supplement in bone marrow stimulation, which led to stimulate neutrophil cell production for the purpose of devouring exotic materials as a defensive measure, this is due to the fact that the neutrophil cells (Microphages) are the first defense line of the body [20], as their numbers increase

because of damage in the tissues of the body [21]. This is supported by the results of biochemical tests of liver and kidney functions where inflammation is obvious. Lymphocytes were significantly decreased ($P < 0.05$) due to migration to the site of degeneration, damage to lymphocytes, and due to misuse or prolonged use of dietary supplements. And what raises the question, and is worth pointing out is the decline in the rate of lymphocytes group experiment ratio, which may be attributed to the negative impact of dietary complement, which led to get damage in some tissues, leading to an increase neutrophil cell production for the purpose of defending the body and then drop lymphocyte rate at experimental group [22].

Phagocytosis Activity Measurement:

The results of the examination of the inflammatory efficacy of the neutrophil cells Table-2 that there are significant differences in the ability of the neutrophil cells to devouring. In the experimental group, there was an significant increase ($P < 0.05$) in the percentage of devouring neutrophil cells which is (41.26 ± 2.17) compared to the percentage of devouring neutrophils in the control group which is (28.01 ± 1.26) . The process of phagocytosis is one of the keys working in inflammation and is represented by the Microphages ,the Monocytes and the Neutrophils that migrate as a result of their impact on the Chemotaxins aspects [23], Neutrophil cells are considered the first of these cells and is described as the first line in devouring operations, this increase can be attributed to the cellular damage resulting from the misuse of the dietary supplement or the time taken to take the protein which leads to the secretion of chemical attractions in the affected area, which in turn leads to the attraction of the cells of the neutrophil to the damaged tissue as it generates free stems neutrophil cells during the process of devouring to defend the body, or may be due to the inflammatory response resulting from strenuous exercise [24].

Table 2- Changes in the means level of WBCc and Differential WBC in experimental group compare with control group.

Group	WBC (Cell/ml ³) M±SE	Differential WBC					Phagocytosis M±SE
		Neutrophil % M±SE	Lymphocyte % M±SE	Monocyte % M±SE	Eosinophil % M±SE	Basophil % M±SE	
Control	8398 ± 392	57.35±1.25	40.5±0.91	6.84±0.4	0.50±0.5	0	28.01±1.26
Experimental	11621±372.4*	64.33±2.4*	32.75±0.83*	9.01±0.82*	0.50±0.5	0	41.26±2.17*

*Significant $P < 0.05$

M ± SE mean ± Standard error

Determination of the effectiveness of AST, ALT, ALP, and Bilirubin:

The results indicated that taking whey protein causes a significant increase in liver enzymes and bilirubin levels. The results showed a significant increase ($P < 0.05$) in (AST) rate in the experimental group that reached (36.27 ± 1.04) compared to the control group that reached (27.11 ± 0.32) , the results also showed a significant increase ($P < 0.05$) in (ALT) rate in the experimental group that reached (39 ± 1.01) compared to the control group that reached (21.28 ± 0.92) . The results showed a significant increase ($P < 0.05$) in the (ALP) enzyme activity in the experimental group that reached (227.02 ± 1.43) when compared to the control group that reached (118.3 ± 0.62) . As for the bilirubin rate, the results showed significant differences in the experimental group that reached (1.060 ± 0.05) compared to the control group that reached (0.430 ± 0.03) Table-3. The increase in the levels of AST, ALT, ALP, and total bilirubin rate indicates a defect in liver metabolism [25], it was found that the increase in the levels of AST, ALT, ALP indicates the susceptibility of some compounds to stimulate the Oxidative Stress of the liver releasing large amounts of free stems that affect the composition and function of the cell [26]. As well as physiological concentrations of free stems are important and when high levels of free stems lead to inflammation, arthritis and kidney inflammation and lead to various diseases such as cancer and high pressure and diabetes and atherosclerosis and early infections [27]. These results came to support [28] findings about the effect of some nutritional supplements for rugby players.

Table 3- Changes in the means of liver enzyme in experimental group compare with control group.

Group	GOT U/L M±SE	GPT U/L M±SE	ALP U/L M±SE	Bilirubin mg/dl M±SE
Control	27.11±0.32	21.28±0.92	118.3±0.62	0.43±0.03
Experimental	36.27±1.04*	39±1.01*	227.02±1.43*	1.06±0.05

*Significant P<0.05

M ± SE mean ± Standard error

References

1. DI pasquale, M. G. **1999**. *Proteins and amino acids in exercise and sport*. In: *Energy-Yielding Macronutrients and Energy Metabolism in Sports Nutrition*. Driskell, J. A. and Wolinsky, I. Eds. Boca Raton, FL: CRC Press. pp. 119-162.
2. Bucci, L. and Unlu, L. **2000**. *Proteins and amino acid supplements in exercise and sport*. In: *Energy-Yielding Macronutrients and Energy Metabolism in Sports Nutrition*. Driskell, J. and Wolinsky, I. (Eds.). Boca Raton, FL: CRC Press, pp. 197-200.
3. Cruzat, V. F.; Krause, M. and Newsholme, P. **2014**. Amino acid supplementation and impact on immune function in the context of exercise. *J Int Soc Sports Nutr.* **11**: 61. doi: 10.1186/s12970-014-0061-8.
4. Reidy, P. T. and Rasmussen, B. B. **2016**. Role of ingested amino acids and protein in the promotion of resistance exercise-induced muscle protein anabolism. *J Nutr.* doi: 10.3945/jn.114.203208.
5. Morifuji, M.; Koga, J.; Kawanaka, K. and Higuchi, M. **2009**. Branched-chain amino acid-containing dipeptides, identified from whey protein hydrolysates, stimulate glucose uptake rate in L6 myotubes and isolated skeletal muscles. *J Nutr Sci Vitaminol.* **55**: 81-86.
6. Haraguchi, F. K.; Silva, M. E.; Neves, L. X.; dos Santos, R. C. and Pedrosa, ML. **2011**. Whey protein precludes lipid and protein oxidation and improves body weight gain in resistance exercised rats. *Eur J Nutr.* **50**(5): 331-9.
7. Maclean, D. A.; Graham, T. E. and Saltin, B. **1994**. Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. *American Journal of Physiology.* **267**: E1010-1022.
8. Antonio, J.; Peacock, C. A.; Ellerbroek, A.; Fromhoff, B. and Silver, T. **2014**. The effects of consuming a high protein diet (4.4 g/kg/day) on body composition in resistance-trained individuals. *J Int Soc Sports Nutr.* **11**: 19.
9. Williams, M. **2005**. Dietary supplements and sports performance: Amino acids. *J. Int. Sports Nutr.* **2**: 63-67.
10. Paul, W. **1984**. Quantitation of Macrophage Phagocytosis *in Vitro*. *Physiology*. 57-71.
11. Furth, R. V.; Thedo, L. V. and Leijilt, P. C. **1985**. *In vitro* determination of phagocytosis and intracellular killing by PMN and Mononuclear of phagocytosis. In: *Hand book of experimental Immunology*, 3rd (vol. 2), *Blackwell Scientific publication, U. K.* pp: 289-298.
12. Reitman, S. and Frankel, S. **1957**. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* **28**: 56-63.
13. Kind, P. and King, E. **1954**. Estimation of plasma phosphate by determination of hydrolysed phenol with amino-antipyrine. *J. Clin. Pathol.* **7**: 322-326.
14. Walters, M. L. and Gerarde, R. W. **1970**. Ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma. *Microchem.*, **15**: 231-36.
15. Gerry, P. Q. and Micheal, J. K. **2002**. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, New York. pp: 556.
16. Eslami, S. Karandish, M.; Marandi, S. M. and Moghaddam, A. **2010**. Effect of Whey Protein Supplementation on Hematological Parameters in Healthy Young Resistance Male Athletes. *Journal of Applied Sciences.* **10**(11): 991-995.

17. Wu, H. J.; Chen, K. T.; Shee, B. W.; Chang, H. C.; Huang, Y. J. and Yang, R. S. **2004**. Effect of 24 h ultra-marathon on biochemical and hematological parameters. *World J. Gastroenterol.* **10**: 2711-2714.
18. Al Sahhaf, Z. Y. **2006**. Toxicity of sumithion in albino rat: Hematological and biochemical studies. *J. Applied Sci.* **6**: 2959-2962.
19. Bassit, R. A., Sawada, L. A., Bacurau, R. F., Navarro, F. and Martins, E. **2002**. Branched-chain amino acid supplementation and the immune response of long-distance athletes. *Nutrition.* **18**: 376-379.
20. Highleyman, L. **2003**. Monitoring test for people with HIV. *Liza @ Blackrose. Com.* pp : 3-14 .
21. Marchi, L. F. **2014**. Interferon-gamma enhances phagocytosis, the production of reactive oxygen species and pro-inflammatory cytokines. Implication for innate and acquired immunity. *Inflammation and Cell Signaling.* **1**(1): 1-3.
22. Naclerio, F., Larumbe-Zabala, E., Ashrafi, N., Seijo, M., Nielsen, B., Allgrove, J. and Earnest, C. P. **2017**. Effects of protein-carbohydrate supplementation on immunity and resistance training outcomes: a double-blind, randomized, controlled clinical trial. *Eur. J. Appl. Physiol.* **117**: 267-277.
23. Kluth, D. C. R. and R. A. J. **1999**. New approaches to modify glomerular inflammation. *J. Nephrol.* **12** (2): 66-75
24. Davison, G., Allgrove, J. and Gleeson, M. **2009**. Salivary antimicrobial peptides (LL-37 and alpha-defensins HNP1-3), antimicrobial and IgA responses to prolonged exercise. *Eur J Appl Physiol.* **106**: 277-284.
25. Jaeger, J. and Hedegaard, H. **2002**. A Review on Liver Function Test: The Danish Hepatitis C Website Available from http://home3.inet.tele.dk/omni/hemochromatosis_iron.htm.
26. Jadhav, V. B., Thakare, V. N., Suralkar, A. A., Deshpande, A. D. and Naik, S. R. **2010**. Hepatoprotective activity of *Luffa acutangula* against CCl₄ and rifampicin induced liver toxicity in rats: A biochemical and histopathological evaluation. *Indian J. Exp. Biol.* **48**: 822-829.
27. Zelko, I. N., Mariani, T. J. and Folz, R. Z. **2002**. Superoxide dismutase multigene family: A comparison of CuZn-SOD, Mn SOD (SOD2) and EC-SOD (SOD3) gene structures, evaluation and expression. *Free radical. Bio. Med.* **33**(3): 337-349.
28. Ohtani, M.; Maruyama, K.; Sugita, M. and Kobayashi, K. **2001**. Amino acid supplementation affects hematological and biochemical parameters in elite rugby players. *Biosci. Biotechnol. Biochem.* **65**: 1970-1976.