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Effects of Whey Protein Supplementation on Hematological Parameters in Healthy Young Resistance Male Athletes

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Abstract: Physical activity changes hematological parameters in athletes which result in cessation of their exercises. There is limited data available about the effects of protein supplements on hematological parameters of resistance exercise. In this clinical trial, changes of hematological parameters were studied after one month resistance training and whey protein supplementation in 32 healthy young subjects (16 in supplement group and 16 in control group). Whey protein supplement (6.6 g day^{-1}) and placebo (starch, in a same dose as the supplement) were consumed between meals. Every one exercised with 80% 1 RM, for 1 h a day, five days a week. Fasting blood specimens were taken before and after beginning of study and WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT and percent of WBCs were investigated separately. Student t-test and paired t-test were used for statistical analyses. WBC, RBC, HGB, HCT, PLT and MCHC levels in two groups significantly decreased ($p<0.05$), without any difference between two groups. MCV increased in the both study groups, but again there was no difference between them. MCH and lymphocytes% remained unchanged in both groups. In the supplement group, neutrophils % and other WBCs showed no noticeable changes, but in the placebo group, they were significantly increased and declined, respectively. However, no important difference was seen between two groups with respect to percentage of WBCs, separately. In conclusion, one month of whey protein supplementation with 6.6 g day^{-1} has no effect on hematological parameters changes after resistance exercise. Further studies with various dosages and longer periods of time are recommended.

Key words: Whey protein, resistance training, hematological tests, athletic, supplement

INTRODUCTION

Physical activity places a wide spectrum of demands on the body, depending on the form, intensity and duration of the required effort. High-intensity exercise causes tissue damage, production of stress hormones (Zarrineh *et al*., 2009) and alterations in the circulating quantity and function of macrophages, neutrophils and lymphocytes; and therefore lead to decrease in immune function and increase in risk of opportunistic infections, especially those of upper respiratory tract (Schumacher *et al*., 2002; Shephard, 1997; Gleeson, 2005; Mackinnon, 1999; Maughan, 2002). Also, induced physiological changes by training can be exhibited in decreasing hemoglobin, hematocrit, WBC, RBC and platelets (Bärtsch, 1998). In other words, when a person begins a training program, a status called sport anemia-temporal decrease in RBC and hemoglobin levels is established. Two hypotheses have been mentioned for sport anemia. First, 2-3 weeks beginning of the training program, blood proteins including erythrocytes (RBCs) are used for increasing concentrations of myoglobin, mitochondrial mass and enzymes which are involved in training adaptation and recovery from training. Second, physical trainings result in rising plasma volume, but increasing red blood cells and hemoglobin are not proportional to it (Maughan, 2002; Bärtsch, 1998; Nemet *et al*., 2005; Williams, 2005; American Dietetic Association, 2000). Although generally trivial in themselves, these cases may be sufficient to interrupt training (Maughan, 2002; Bärtsch, 1998).

People all around the world spend billions of dollars on nutritional supplements, or -as they often called ergogenic aids. These substances are alleged to enhance athletic performance, decrease fatigue, change body composition, improve looks and also response to increased requirement of athlete (Nemet *et al*., 2005). However, side effects of some of them are proved.

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Protein is one of the most popular dietary supplements marketed to athletes and physically-active individuals to enhance nitrogen retention and increase muscle mass, to prevent protein catabolism during prolonged exercise, to promote muscle glycogen resynthesis following exercise and to prevent sports anemia by promoting an increased synthesis of hemoglobin, myoglobin, oxidative enzymes and mitochondria during aerobic training (Nemet *et al*., 2005; Williams, 2005; American Dietetic Association, 2000). Studies about the effects of these supplements on hematological parameters are limited and mostly are focused on amino acids supplements. In these studies, various effects of consumption of proteins supplements have not been considered.

Whey proteins are extracted from the liquid whey that is produced during the manufacture of cheese or casein and consist of complete and rapid effective proteins with high biological value (104-159). These proteins are rich sources of amino acids, vitamins and minerals which are required for athletes and may contain various biologically active components, including growth factors (Williams, 2005). Considering that research about ergogenic effects of whey protein and its influence on hematological parameters are very limited, the aim of this clinical trial was studying the effect of one month whey protein supplementation in resistance athletes while determining of changes of hematological parameters (hemoglobin, hematocrit, red and white blood cell and platelets count, MCV, MCH, MCHC, percent of neutrophils, lymphocytes, total rest of white blood cells (monocytes, basophiles, eusiniphiles).

MATERIALS AND METHODS

Thirty two healthy young men aged 19 to 32 years participated in this double blind clinical trial. This study carried out in 2006. All subjects were healthy according to medical information questionnaire. Exclusion criteria were use for any medication or supplement during the past 6 months, hypertension, cardiovascular diseases, diabetes, dislipidemia, renal or liver diseases, pulmonary diseases and skeletal damages and lactose intolerance. The maximum of their training duration was 6 months and they had no previous regular exercise training and were not under any special diets. The study protocol was approved by the local Ethical Committee.

A written informed consent was obtained from each participant. Before baseline testing and on the last day of experiment, subjects completed a 24 h food record. Total energy, macronutrients and micronutrients intakes during a 24 h period were estimated.

Before the experiment, the subjects participated in a preparatory test. The test was designed to determine one repetition maximum (1RM). During the 1RM test, a leg press was performed at a 90-180° knee angle. The load was progressively increased until the subject could not perform more than one single repetition. The subjects reached 1RM within 5-6 trials. Before the experiment, each subject was initially familiarized with the training program planned by the coaches and researchers. Performance testing included assessment of one repetition maximum (1RM) leg press, leg curl, leg extension, bench press, shoulder press, lateral pull down and arm curl (Mayhew *et al*., 1992, 1993); After a warm-up with a light resistance that allowed 10 repetitions, subjects were given a 1 min rest period. Resistance was then increased to allow the performance of 3-5 repetition with a 2 min rest period. For the final test, resistance was adjusted so that the subject could complete only 1 repetition. These trainings were done for one month, 5 days per week and 1 h per day under surveillance of coach.

Subjects were assigned randomly into one of two groups (whey protein or placebo) which both were provided in the form of powder. Consumed placebo was starch and supplement was whey protein (Ultimate nutrition, Farmington Inc, CT, USA). Fasting blood samples (2 mL) were taken between 07:00 to 09.00 from forearm vein after 10 h fasting before beginning of study, then subjects were weighted.

Subjects were asked to take their daily packages and consume between meals and after training preferably. Placebo and supplement were consumed under coach surveillance. Also, number of consumed packages of placebo and supplement was controlled at the end of each week. Study groups consisted of resistance exercise plus ingestion of: (1) 6.6 g placebo (artificially flavored, colored starch) or (2) 6.6 g of whey protein and were ingested as a solution after resistance exercise. This dose was chosen with respect to study of Ohtani and assistants on 2006 which assessed the effects of 6/6 g of essential amino acids on hematological parameters. One month later, the last blood samples (2 mL from forearm vein) were taken.

The blood samples were collected in evacuated tubes containing EDTA and immediately were transferred to laboratory.

Laboratory tests included measuring hemoglobin, hematocrite, RBC, WBC and platelets counts, percent of

lanphocyte, neutrophils and rest of WBCs (monocytes, eusinophils and basophils) and measuring MCV, MCH, MCHC. These samples were measured by Sysmex K1000 (American Abbott Co).

Results are given as means (±SEM) and analyzed with SPSS 15. The differences between groups were evaluated by Student's t-test. Pre-and post supplement test was carried out by paired samples t-test. Significance level was $p<0.05$.

RESULTS AND DISCUSSION

During the study, all the volunteers were in healthy status and none of them complained about digestive problems. Four subjects were excluded from the study as a result of consuming other supplements. Therefore, 14 subjects in each group (supplement and placebo) continued the study. Subjects' characteristics are presented in Table 1, as it can depict, there was no significant difference between placebo and supplement groups with respect to age, weight, BMI, energy, carbohydrate, protein, fat, Fe, fiber, vitamin C, B6, B12 and folic acid intakes. Also, differences between two groups before intervention with respect to WBC, RBC, HGB, HCT, MCV, MCH, MCHC and PLT were not significant and 2 groups were comparable. Among participants before beginning of the study, on the basis of standard levels (Ohtani *et al*., 2001a, b; Carlson, 2004), 2 subjects had low WBC ($<5\times10^3$ mm^{-3}), 2 had low MCV (<80 Fl), 2 had low MCH (<27 pg). Also, 2 had high WBC ($>10\times10^3$ mm^{-3}), 1 had high RBC ($>5.9\times10^6$ mm^{-6}), 13 had high MCH (>31 pg) and 1 had high MCHC (>36 g day^{-1}).

Table 1: Characteristics of subjects participating in the study (Mean±SE)

Variables	Supplement (n = 14)	Placebo (n = 14)	p-value
Body weight (kg)	71.310±2.65	72.12±3.89	0.961
BMI (kg m^{-2})	23.440±0.84	23.11±0.94	0.849
Age (year)	22.620±0.97	21.56±0.58	0.114
Energy (Kcal)	3915.940±309.41	3781.49±393.99	0.144
Carbohydrate (kcal %)	56.840±1.65	57.19±1.35	0.325
Protein (kcal %)	15.030±0.62	14.75±0.80	0.548
Fat (kcal %)	26.990±1.41	28.44±1.27	0.875
Fiber (g day^{-1})	23.420±2.74	22.99±2.59	0.999
Fe (mg day^{-1})	13.070±3.14	13.21±3.52	0.712
Vitamin C (mg day^{-1})	132.770±30.06	160.95±21.28	0.485
Vitamin B6 (mg day^{-1})	6.070±1.86	7.16±2.80	0.220
Vitamin B12 (μg day^{-1})	5.907±0.89	4.64±0.82	0.857
Folic acid (μg day^{-1})	427.750±60.05	358.55±51.10	0.771

As it is shown in Table 2, WBC, RBC, hemoglobin, hematocrit, MCHC and PLT significantly decreased within groups, but difference between 2 groups was not significant. MCV increased significantly within groups but difference between the two groups was not statistically significant. In placebo group, percent of neutrophils significantly reduced, but in supplement group this reduction was not significant. Also, in placebo group, percent of rest of white blood cells enhanced significantly, but this enhancement was not significant in supplement group and generally, there was no statistical significant difference between two groups with respect to percent of white blood cells, separately. The MCH and percent of lymphocyte had no significant differences in both groups before and after intervention. After intervention, among 28 remained participants, on the basis of standard levels (Ohtani *et al*., 2001a, b; Carlson, 2004), 7 had low WBC, 12 had low RBC, 24 had low HGB (<14 g day^{-1}), 27 had low HCT (<42%), 2 had low MCV, 2 had low MCH and 1 had low MCHC (<32 g day^{-1}). Also, between these participants, 1 had high RBC, 8 had high MCH and 1 had high MCHC.

It is concluded that consuming whey protein supplement for one month during resistance training did not affect individuals, hematological parameters.

As it was expected, exercise training result in changing in hematological parameters, causing anemia, increasing chance of infections caused by changes of hematological parameters (Schumacher *et al*., 2002;

Table 2: Hematological parameters in whey protein supplement group and placebo (Mean±SE, N = 14)

	Whey protein supplement group			Placebo group		
Variables	Before intervention	After intervention	p-value	Before intervention	After intervention	p-value
WBC (×10^3 mm^{-3})	7.22±0.43	6.28±0.37	0.032*	6.84±0.41	5.96±0.30	0.008*
RBC (×10^6 mm^{-3})	5.55±0.13	4.39±0.15	0.000*	5.36±0.08	4.28±0.10	0.000*
Hemoglobin (g dL^{-1})	16.49±0.21	13.05±0.29	0.000*	16.16±0.27	12.83±0.26	0.000*
Hematocrite (%)	47.98±0.58	38.69±0.81	0.000*	46.73±0.61	37.49±0.67	0.000*
MCV (fl)	87.08±2.10	88.93±2.22	0.014*	87.27±1.12	88.37±1.19	0.037*
MCH (pg)	29.97±0.80	30.01±0.80	0.864**	30.16±0.49	30.00±0.47	0.393**
MCHC (g dL^{-1})	34.37±0.23	33.73±0.30	0.013*	34.41±0.28	33.89±0.27	0.005*
Platelet (×10^9 L^{-1})	236.07±11.39	162.14±12.56	0.000*	206.21±13.94	171.28±1.86	0.020*
Lymphocyte (%)	40.86±2.12	40.86±2.12	0.870**	38.54±1.87	40.79±1.63	0.71**
Neutrophil (%)	55.59±2.77	53.65±1.96	0.504**	57.00±2.21	52.43±1.98	0.000*
Mixed (%)	3.94±1.85	5.48±3.95	0.153**	4.46±2.98	6.41±3.85	0.018*

*p<0.005 significant difference. **p>0.005 non-signifant difference

Shephard, 1997; Gleeson, 2005; Mackinnon, 1999; Brenner *et al*., 1999; Wu *et al*., 2004). Declining trend of WBC and changes of percent of lymphocytes, neutrophils and rest of WBCs (monocytes, eusinophils and basophils) in both groups were similar. Prolonged strenuous exercise is associated with a temporary immunodepression that affects macrophages, neutrophils and lymphocytes (Schumacher *et al*., 2002; Shephard, 1997; Gleeson, 2005; Mackinnon, 1999). The mechanism involved is not fully established and appear to be multifactorial, including hormonal actions (e.g., catecholamines and cortisol), inhibition of macrophage and T-cell cytokine production, altered heat shock protein expression and a fall in the plasma concentrations of glutamine. In the study of Wu *et al*. (2004) on ultramarathon runners, reduction of WBC was shown until 9 days after exercise. Inconsistency of findings of this study and most of related studies (Schumacher *et al*., 2002; Pedersen and Bruunsgaard, 1995; Flynn *et al*., 1999) with our findings can be a result of difference in period of study and kind of exercise. It means that, there was one month constant resistance training, while in other researches, subjects were studied immediately and at the most, 9 days after endurance exercise.

In this study, consumption of whey protein supplement had no effect on WBC and percents of separate WBCs. In the study of Bassit *et al*. (2002), influence of supplementation with BCAA (branch chain amino acids: leucine, isoleucine, valine) was shown to improve immune response of runners. But in two other studies on supplementation with amino acids (Ohtani *et al*., 2001a, b), no change of WBC was shown that is consistent with our findings. Regarding the effect of whey protein supplementation on WBC, there is not enough information from other studies and further research is require for convince evidence in this field.

In this study, RBC, HGB, HCT, PLT, MCHC had similar declining trend in both groups. In addition, MCV increased in both groups similarly, MCH did not change and supplementation had no effect on trends of changes of these parameters. In the study of Wu *et al*. (2004) on the ultramarathon runners, RBC, HGB, HCT, three indicators of anemia, were normal before the race and remained decreased until day nine; so-called sports anemia (Wu *et al*., 2004; Weight, 1993), is not only caused by hemolysis owing to mechanical trauma but also by oxidative injuries of the RBCs (Wu *et al*., 2004; Szygula, 1990). Theoretically, the reduction in MCV and MCH can be related to the some disruption in the pathway of RBC synthesis (Al-Sahhaf, 2006). Under normal conditions, red cells with a mean life of 120days are renewed at approximately 1% daily. However, this turnover increases following exercise training. The increased turnover rate is good for the athletes as the young red cells can carry more efficiently than the older cells (Wu *et al*., 2004; Jordan *et al*., 1998; Smith, 1995). These findings are parallel with our study and also a study with a decrease of hemoglobin as a result of long term exercise was seen (Schumacher *et al*., 2002). In the studies of Ohtani *et al*. (2002) related to supplementation with amino acids, increasing of HGB, HCT, RBC were illustrated, but there were no change of platelets count. Differences of our study with these studies can be caused by differences in kind of supplement and exercise. In other researches there are little information about changes of platelets count, MCV, MCH and MCHC following supplementation and concluding in this field needs more research.

In this study, number of participants with sports anemia on the basis of three indicators of low RBC, HGB, HCT (Flynn *et al*., 1999; Weight, 1993), after intervention was increased. Also, numbers of subjects with increasing WBC and platelets counts were enhanced. It shows that resistance exercise may influence hematological parameters and one month supplementation with whey protein has no effect on these changes.

In summery, findings of present study show that one month supplementation with whey protein supplementation does not affect changes of hematological parameters after resistance exercise in healthy young men. Considering that supplementation with amino acids can influence hematological parameters, it is suggested that for findings in this field, more research with different doses of whey protein supplement and with different time periods should be carried out.

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REFERENCES

Al Sahhaf, Z.Y., 2006. Toxicity of sumithion in albino rats: Hematological and biochemical studies. J. Applied Sci., 6: 2959-2962.

American Dietetic Association, 2000. Association, 2000. Position of the American Dietetic Association, Dietitians of Canada and the American College of Sports Medicine Nutrition and Athletic Performance. J. Am. Diet. Assoc., 100: 1543-1546.

Bärtsch, P., H. Mairbäurl and B. Friedmann, 1998. Pseudo-anemia caused by sports. Ther. Umsch., 55: 251-255.

Bassit, R.A., L.A. Sawada, R.F. Bacurau, F. Navarro and E. Martins *et al.*, 2002. Branched-chain amino acid supplementation and the immune response of long-distance athletes. Nutrition, 18: 376-379.

Brenner, I.K., V.M. Natale, P. Vasiliou, A.I. Moldoveanu, P.N. Shek and R.J. Shephard, 1999. Impact of three different types of exercise on components of the inflammatory response. Eur. J. Appl. Physiol. Occup. Physiol., 80: 452-460.

Carlson, T.H., 2004. Laboratory Data in Nutrition Assessment. In: Krause's Food, Nutrition and Diet Therapy, Mahan, L.K. and S. Escott-Stump (Eds.). 11th Edn., W.B. Saunders, Philadelphia, ISBN: 9780721697840, pp: 436-454.

Flynn, M.G., M. Fahlman, W.A. Braun, C.P. Lambert, L.E. Bouillon, P.G. Brolinson and C.W. Armstrong, 1999. Effects of resistance training on selected indexes of immune function in elderly women. J. Appl. Physiol., 86: 1905-1913.

Gleeson, M., 2005. Interrelationship between physical activity and branched-chain amino acids. J. Nutr., 135: 1591S-1595S.

Jordan, J., W. Kiernan, H.J. Merker, M. Wenzel and R. Beneke, 1998. Red cell membrane skeletal changes in marathon runners. Int. J. Sports. Med., 19: 16-19.

Mackinnon, L.T., 1999. Advances in Exercise and Immunology. 1st Edn., Human Kinetics, Champaign, ISBN: 0-88011-562-9 pp3: 363.

Maughan, R., 2002. The athlete's diet: Nutritional goals and dietary strategies. Proc. Nutr. Soc., 61: 87-96.

Mayhew, J.L., J.S. Ware and J.L. Prinster, 1993. Using lift repetitions to predict muscular strength in adolescent males. Strength Condition. J., 15: 35-38.

Mayhew, J.L., T.E. Ball, T.E. Arnold and J.C. Bowen, 1992. Relative muscular endurance as a predictor of bench press strength in college men and women. J. Strength. Condition. Res., 6: 200-206.

Nemet, D., B. Wolach and A. Eliakim, 2005. Proteins and amino acid supplementation in sports: Are they truly necessary? IMAJ., 7: 328-332.

Ohtani, M., K. Maruyama, S. Suzuki, M. Sugita and K. Kobayashi, 2001a. Changes in hematological parameters of athletes after receiving daily dose of a mixture of 12 amino acids for one month during the middle and long-distance running training. Biosci. Biotechnol. Biochem., 65: 348-355.

Ohtani, M., K. Maruyama, M. Sugita and K. Kobayashi, 2001b. Amino acid supplementation affects hematological and biochemical parameters in elite rugby players. Biosci. Biotechnol. Biochem., 65: 1970-1976.

Ohtani, M., M. Sugita, K. Maruyama, 2002. Amino acid mixture improves training efficiency in athletes. J. Nutr., 136: 538S-543S.

Pedersen, B.K. and H. Bruunsgaard, 1995. How physical exercise influences the establishment of infections. Sports Med., 19: 393-400.

Schumacher, Y.O., A. Schmid, D. König and A. Berg, 2002. Effects of exercise on soluble transferrin receptor and other variables of the iron status. Br. J Sports Med., 36: 195-199.

Shephard, R.J., 1997. Physical Activity, Training and the Immune Response. 1st Edn., Cooper Publishing Group, Carmel, ISBN-13: 9781884125652, pp: 463.

Smith, J.A., 1995. Exercise, training and red blood cell turnover. Sports Med., 19: 9-31.

Szygula, Z., 1990. Erythrocytic system under the influence of physical exercise and training. Sports Med., 10: 181-197.

Weight, L.M., 1993. Sports anaemia: Does it exist? Sports Med., 16: 1-4.

Williams, M., 2005. Dietary supplements and sports performance: Amino acids. J. Int. Soci. Sports Nutr., 2: 63-67.

Wu, H.J., K.T. Chen, B.W. Shee, H.C. Chang, Y.J. Huang, R.S. Yang, 2004. Effects of 24 h ultra-marathon on biochemical and hematological parameters. World J. Gastroenterol., 10: 2711-2714.

Zarrineh, F., F. Salami, H.N. Bakht, M. Salavati and M. Hedayati, 2009. Effect of acute and chronic aerobic training on plasma GH isoforms concentration in pubertal and pre-pubertal male athletes. J. Applied Sci., 9: 2469-2474.