Original article

Do High Doses of Protein Supplements Affect Serum Lipid Profiles, Biochemical and Glucose Metabolism Markers?

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Summary. *Aim:* The aim of this study was to investigate the effects of protein supplements (PS) on blood biochemical, haematological, inflammatory parameters and lipid profile. *Methods:* Sixty-nine healthy subjects going to the gym for at least three months were included in the study. The subjects were divided into two groups according to their PS usage status. Fasting blood samples were taken from all of the subjects. *Results:* Among 69 subjects, 30(43%) were using PS, so were included in group 1, and 39 (57%) never used PS, therefore they consisted of group 2. The blood biochemical, haematological, inflammatory, glucose metabolism markers and lipid profiles were not different between the two groups. But, the subjects who used $>$ 55 g PS/day had significantly higher serum creatinine values than subjects who never used PS. Similarly, the subjects who used $\geq$ 30 g PS/day had significantly higher serum sodium levels than subjects who never used PS. Moreover, the subjects using PS for more than 10 months had significantly higher serum sodium levels when compared with subjects who did not use PS. *Conclusion:* Blood lipid profiles were not different in subjects using PS and in subjects who never used PS and they were not related to PS dosage.

Key words: Protein supplement, serum creatinine, serum sodium, glucose metabolism, lipid profile

Introduction

In recent years, protein supplements (PS) are widely used by athletes with the view that they increase muscle gain and strength. There are few studies relating to the effects of these substances on blood glucose metabolism, serum biochemical, haematological, inflammatory markers and lipid profile. Inconsistent results were found relating to the effects of PS on insulin sensitivity and blood glucose metabolism in several human and animal studies (1–4).

There is no consensus in the literature on the effects of these supplements on serum lipid profile because few studies about this issue again have conflicting results (5–7). In a study conducted on elite rugby players, blood low density lipoprotein (LDL) and total cholesterol levels were increased after three months of protein complex use consisting of leucine, isoleucine, valine, arginine, and glutamine amino acids (5). In another survey on overweight and obese people caffeine, conjugated linoleic acid, green tea, and branched-chain amino acid complex did not change the blood lipid profile after 8 weeks (6). On the other hand, whey protein together with exercise improved serum lipid profile parameters in overweight young people (7). There are even fewer studies relating to the effects of PS on blood biochemical, haematological and inflammatory markers. In one of these studies, it was observed that amino acid mixture increased blood haemoglobin, red blood cell and hematocrit values (5). L-carnitine together with exercise decreased high sensitivity C-reactive protein (CRP) values in obese

women, but L-carnitine itself without exercise did not have such an effect (8). Another study on inflammatory markers showed that creatine supplement prevented CRP rise after acute sportive activity (9).

Unfortunately, all of these studies are inconclusive relating to the effects of PS on blood biochemistry, haematology, lipid profile, blood glucose metabolism, and inflammatory markers. But despite this lack of knowledge, they are used widely among sport practitioners. So we decided to lead an investigation on the effects of PS on blood biochemical, haematological, inflammatory markers, lipid profile, and glucose metabolism.

Materials and Methods

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Near East University, Cyprus (2019/66). All of the participants gave written informed consent prior to entering this study.

Sixty-nine subjects without having any kind of chronic or acute illness,e.g.any form of cardiac disease, hypertension, diabetes mellitus, goitre, kidney disease, acute or chronic infection, were recruited to the study. In order to be eligible for the study, these subjects should already be going to the gym for at least three months. They were doing both static and dynamic types of exercises in the gym. Resting electrocardiography (ECG) was taken from all of the participants and subjects having any kind of ECG abnormality were excluded from the study. The exclusion criteria included: athletes who are <18 or >45 years of age, have a body mass index >30 kg/m^2, abnormal ECG, who use any kind of drugs, who have high blood pressure at the time of recruitment, and who have any kind of acute or chronic illness. So sixty-nine healthy subjects were included in this study. All of the participants were male. The height, weight and waist circumference of the subjects were recorded and body mass indexes were calculated. The daily total energy intake of the subjects was assessed by a dietician and the proportions of protein, carbohydrate and fat intake were calculated. The dietician filled a 24-hour food consumption status form for a sports day and a sports-off day and took the average of these two days' results.

For analysis of the laboratory tests, blood samples were collected into serum separator and K_2EDTA tubes by venipuncture (Vacutainer; Becton Dickinson Systems, San Jose, CA, USA) after fasting overnight. Serum specimens were centrifuged, aliquoted and stored at −80 °C until the time of analysis. Whole blood count analysis was immediately carried out on Cell-Dyn Ruby Hematology Analyzer, the biochemical tests and the inflammatory marker (CRP) tests were analyzed on Architect c8000 clinical chemistry system and insulin level was measured on Architect ci4100 (Abbott Laboratories, Abbott Park, IL, USA). The low-density lipoprotein (LDL) concentration of all serum samples was measured by direct method and insulin resistance was calculated by using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

The subjects were divided into two groups. The first group consisted of the ones who used PS for at least three preceding months, and the second group included subjects who never used PS. The subjects in the first group used whey protein as PS. The amount of the PS used and duration of usage were also recorded. Blood test results were compared between the two groups.

Statistical analysis

For the statistical evaluation of the study, Statistical Package For The Social Sciences (SPSS) Version 17.0 was used. For the comparison of the clinical characteristics of the two groups, independent samples t-test was used. Also for the comparison of blood test results between the two groups, independent samples t-test was used. Bivariate correlation analysis was performed using Pearson correlation analysis to search for the correlations between blood test variables and clinical characteristics. Subgroup analyses were made separately after dividing the subjects into two groups according to daily PS dosage and PS usage duration. Independent sample t-test analysis was again used for these subgroup analyses. P-value <0.05 was accepted as significant.

Results

The mean age of the subjects included in the study was 31.4±7.3 years. They were going to the gym for a mean period of 5.2 years. Among 69 subjects, 30 (43%) were using PS, so were included in group 1, and 39 (57%) never used PS, therefore they consisted of group 2. All of the participants in group 1 used whey protein as PS. They were using 44.6 grams (22–70 g, standard deviation (SD) 14.5 g) of PS daily on average (on the days they went to the gym). The dietician assessed the daily dietary energy intake and its distribution in 65 (94%) subjects. The PS dose was included in the daily protein intake of the participants in group 1.

The two group's clinical characteristics were similar when compared for age, height, weight, body mass index, waist circumference, exercise duration, fat or carbohydrate proportion in the daily diet, but subjects who used PS (group 1) had more daily energy intake from diet and also higher protein consumption per weight, as expected when compared with the subjects who did not use PS (group 2) (Table 1).

The haematological markers (serum haemoglobin, platelet, mean platelet volume) were not significantly different between the two groups. Similarly, the serum biochemistry results did not show any significant difference. CRP values, serum lipid profile (total cholesterol, LDL, high-density cholesterol and triglyceride levels) and glucose metabolism markers (fasting blood glucose levels, serum insulin levels, and HOMA-IR) were also not different between the two groups (Table 2).

To search for the correlations between the variables and blood test results, we performed a Pearson correlation analysis. As a result of this analysis, we found that serum creatinine and sodium values were correlated with daily PS dosage ($r=0.378$, $p=0.039$ and $r=0.469$, $p=0.009$ respectively). Duration of PS usage did not affect serum creatinine values, but it affected serum sodium levels. When subgroup analysis was done using independent samples t-test, subjects using PS for more than 10 months had significantly higher serum sodium levels when compared with subjects who did not use PS (139.29 ±1.59 mEq/l vs. 138.08±2.86 mEq/l, %95 CI 0.06–2.358, $p=0.04$). To understand if increased serum sodium levels were related to dietary sodium intake, an additional analysis was done. As a result, we found that serum sodium levels were not associated with dietary

Table 1. Comparison of the clinical characteristics between the two groups

Characteristics	Group 1 (using protein supplement, n=30)	Group 2 (not using protein supplement, n=39)	P value
Age (year)	32.2±7.8	30.8±7.0	0.438
Height (cm)	175.5±5.8	176.8±5.4	0.339
Weight (kg)	79.7±9.3	79.7±8.2	1.000
Body mass index (kg/m²)	25.9±2.4	25.5±2.3	0.538
Waist circumference (cm)	85.7±7.0	87.7±7.0	0.246
Exercise duration (year)	5.5±5.1	4.9±4.8	0.613
Exercise duration per week (hours)	5.4±1.7	5.2±2.8	0.739
Daily dietary energy intake (kcal)	2449±836	2050±589	**0.037**
Protein proportion in diet (%)	26.7±6.6	23.8±5.3	0.067
Fat proportion in diet (%)	39.7±9.4	41.7±6.7	0.338
Carbohydrate proportion in diet (%)	32.0±8.9	33.1±7.1	0.595
Protein intake/weight ratio (g/kg)	2.1±0.8	1.5±0.6	**0.008**

The clinical characteristics were similar between the two groups except for daily dietary energy intake and protein intake per weight.

Table 2. Effects of protein supplements on serum hematological, biochemical, inflammatory, glucose metabolism markers and lipid profile

Serum blood test	Group 1 (using protein supplement, n=30)	Group 2 (not using protein supplement, n=39)	P value
Hemoglobine (g/dl)	15.1±0.9	14.9±0.9	0.537
Mean platelet volume (fL)	8.2±1.4	8.3±1.4	0.714
Platelet (x10³/uL)	228±60	229±59	0.880
Creatinine (mg/dl)	1.02±0.16	0.98±0.14	0.206
Urea (mg/dl)	36.4±9.2	33.5±8.0	0.174
Uric acid (mg/dl)	5.6±1.4	5.8±1.1	0.491
Aspartate aminotransferase (U/L)	21.2±6.4	21.2±7.1	0.999
Alanine aminotransferase (U/L)	30.3±15.5	29.7±16.4	0.882
Gama-glutamile transferase (U/L)	22.8±20.3	19.1±8.8	0.359
Sodium (mmol/L)	138.9±2.4	138.1±2.9	0.220
Potassium (mmol/L)	4.6±0.3	4.5±0.4	0.715
Calcium (mg/dl)	9.4±0.4	9.4±0.4	0.779
Magnesium (mg/dl)	1.96±0.16	1.96±0.16 0.975	
Total cholesterol (mg/dl)	190.1±37.2	185.5±36.3	0.612
High-density lipoprotein (mg/dl)	50.5±9.2	49.9±8.6	0.765
Low-density lipoprotein (mg/dl)	118.3±30.8	115.9±30.2	0.741
Triglyceride (mg/dl)	106.2±57.5	98.9±39.7	0.551
C-reactive protein (mg/dl)	0.24±0.54	0.17±0.24	0.499
Glucose (mg/dl)	89.6±6.0	89.4±7.0	0.904
Insulin (uU/ml)	6.2±2.7	6.2±2.8	0.962
HOMA-IR	1.4±0.6	1.4±0.6	0.955

Serum hematological, biochemical, inflammatory, glucose metabolism markers and lipid profile were not different between the two groups.

sodium intake amounts. Moreover, when an analysis was done between high dose PS users and PS non-user group, dietary sodium intake amounts were not found to be significantly different (3076±2145 g vs. 3001±1143 g, %95 CI 918–1045, p=0.876). The same results were obtained when the analysis was repeated between subjects using PS for more than 10 months and PS non-user group (2730±1112 g vs. 3001±1143 g, %95 CI 371–913, p=0.398). Serum lipid profile values did not correlate with daily PS dosage or duration of PS usage.

To find a cut-off value for PS dose and increased serum creatinine correlation, we performed a subgroup analysis. As a result of this analysis, when we compared the subjects who used > 55 g PS/day with subjects who never used supplements, we found that daily PS dosage > 55 g was associated with increased serum creatinine values (1.16 ± 0.19 mg/dl vs. 0.98 ± 0.14 mg/dl, %95 CI 0.004–0.366, p=0.046). We executed the same analysis for PS dose and serum sodium level association. When we compared the subjects who used ≥ 30 g PS/day with subjects who did not use these supplements, this showed that ≥ 30 g daily PS intake was associated with increased serum sodium levels (139.35 ± 1.65 mmol/l vs. 138.08 ± 2.86, mmol/l %95 CI 0.149–2.390, p=0.027).

Dıscussıon

The main finding of our study is that in the group of subjects who use PS, blood glucose metabolism markers, biochemical, haematological, inflammatory parameters and serum lipid profiles are not significantly different from the group of subjects who do not use PS. In recent years as people are more conscious about the importance and health benefits of doing regular exercise, the number of subjects doing regular exercise in gyms has substantially increased. As a consequence of this, the number of subjects using PS to improve their muscle gain and strength is also increasing. The problem with this issue is that neither health professionals nor the people using these supplements know much about these PS. Because there are very few studies about the effects of PS on blood tests, and maybe half of these studies are animal studies. And many others did not recruit healthy subjects. So this study was planned to put some more information into the literature which was poor regarding this issue and also to supply robust knowledge to people using these supplements so frequently.

Our study showed that in subjects using PS, blood glucose metabolism markers (fasting serum glucose levels, serum insulin levels, and insulin resistance index) were not different from the subjects who did not use PS. Regarding this issue, the previous investigations had shown that whey protein (1) and L-carnitine (10) supplementation improved glucose metabolism, but many others (leucine, arginine, creatine supplements) had negative effects on blood glucose metabolism (4,3,11,12). Two other studies had shown the neutral effects of PS on blood glucose metabolism (2,13). There were also some studies of PS in the diabetic population (14,15). One of these investigated the effects of amino acid supplements in diabetic patients and found that essential amino acid supplementation decreased postprandial serum glucose levels without any change in plasma insulin levels (15). In a more recent study on the diabetic population, neither whey protein supplement is given with a 75-gram oral glucose tolerance test alone, nor when preceded by prior exercise, attenuated postprandial glycemia, although it increased postprandial serum insulin levels (14). So the neutral result of our study may be seen as a positive result in favour of PS, as they did not disrupt insulin sensitivity or harm blood glucose metabolism.

We found that subjects using PS had similar serum biochemical parameters including liver and renal function tests and serum electrolytes with subjects who never used these substances. But when we made a correlation analysis using the Pearson correlation analysis test, we detected significant correlations between serum creatinine, serum sodium levels, and daily PS dosage. This was a new finding for PS. So we tried to make this finding more meaningful although we had a small study population. We made subgroup analyses to find a cut-off value for daily PS dosage and increased serum creatinine and serum sodium level correlations. These analyses showed that $>$ 55 g of daily PS usage was associated with increased serum creatinine levels and $\geq$ 30 g of daily PS usage was associated with increased serum sodium levels. Moreover, using these supplements for more than 10 months was also associated with increased serum sodium levels. In previous studies, the effect of PS on serum creatinine levels was shown in 2 animal studies: one showed that L-arginine supplementation decreased serum urea levels and increased serum creatinine levels in pigs (16), on the other hand, the second study showed a decrease in serum creatinine levels in power-exercised mice after sake protein supplementation, which is a protein-rich in branched-chain amino acids (17). The association between increased serum creatinine levels and high doses of PS may be explained by the harmful effects of high dose protein intake on kidney function (18). There is no study in the literature reporting an association between serum sodium levels and PS. This finding of the present study can be attributed to the salt ingredients of the supplements. Different types of PS, including the ones with whey protein, have different amounts of salt ingredients (0.5–1 g salt/100 g PS). A higher dose and longer duration of usage of these PS may cause increased serum sodium concentrations.So, patients with a history of hypertension or athletes who have first degree relatives with hypertension diagnosis should be careful about starting PS.

As for the effects of PS on lipid profile, we found that they have no significant effect on serum lipid profile parameters. One of the previous studies had shown that a protein complex consisting of leucine, isoleucine, valine, arginine, and glutamine increased serum cholesterol levels (5). Favourable and neutral effects of arginine supplementation on LDL cholesterol was

shown in two animal studies (16,19). On the other hand, whey protein and L-carnitine supplements were shown to improve the lipid profile (7,10,20). L-carnitine supplementation decreased serum cholesterol and triglyceride levels in a study on rats (10). Another study was in older women which showed that whey protein improved total cholesterol/high-density lipoprotein cholesterol ratio and whey protein administered after resistance training was more effective in improving metabolic health Z-score and in reducing body fat compared to the placebo group (20). In our study, PS had a neutral effect on serum lipid profile. This result is consistent with a result observed in one previous study in the literature (6).

Our investigation also showed that in subjects using PS, blood haematological and inflammatory markers are not significantly different from the subjects who do not use PS. As these substances are chemical products with some additives inside them, there may be some concerns that they may cause inflammation in the body in the chronic period. There are not so many studies on this subject. One previous study showed that PS increased serum haemoglobin and hematocrit values (5). A few studies related to the effects of PS on inflammation had shown that L-carnitine and creatine supplements decreased CRP levels (8,9). A different study assessed the effects of high whey protein (50g/day) and omega-3 enriched diet with or without creatinine supplementation on inflammation markers after 5 consecutive days of high volume resistance exercise in females and could not show any improvement in inflammation markers (CRP and interleukin-6) (21). These studies assessed the acute effects of PS on inflammation after sportive activity. But our study assessed the chronic effects of PS on inflammation in healthy subjects. This neutral result can be assessed in favour of these substances.

Limitations

We had a small population of subjects in this study to evaluate the effects of PS on blood tests. A bigger study might have more robust results regarding this issue. We included only male subjects because female subjects had very different clinical variables and different PS usage ratios than male subjects. But this prevented our results to be applicable to the female population. The subjects were doing a mixed type of exercise (i.e.dynamic and static). This is consistent with real-life for these kinds of sportsmen going to the gym, but this may be different from previous studies that assess the effects of PS on participants doing a standardized type of exercise.

Conclusıon

In conclusion, blood haematological, inflammatory, glucose metabolism markers and lipid profiles are not different in subjects using PS and in subjects who never used PS. Higher doses of PS usage is associated with higher serum creatinine and sodium levels. A longer duration of PS usage is also associated with higher serum sodium levels. Sportsmen using high doses of PS or subjects using PS for a long period should be careful about the regular follow-up of their serum creatinine and sodium levels.

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Authors' Contrıbutıons: UY was involved in methodology, investigation, supplying resources, data curation, writing and reviewing the manuscript. LC was involved in the conceptualization, methodology of the study and also in reviewing and editing the manuscript. OHE was involved in studying the blood samples and validating the blood test results. TKBE assessed the dietary habits of the participants and helped in the data curation of the study. HK was involved in formal analysis and native English spelling correction and editing of the manuscript. HD supervised all of the study procedures, was involved in project administration and also made critical review and analysis of the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

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