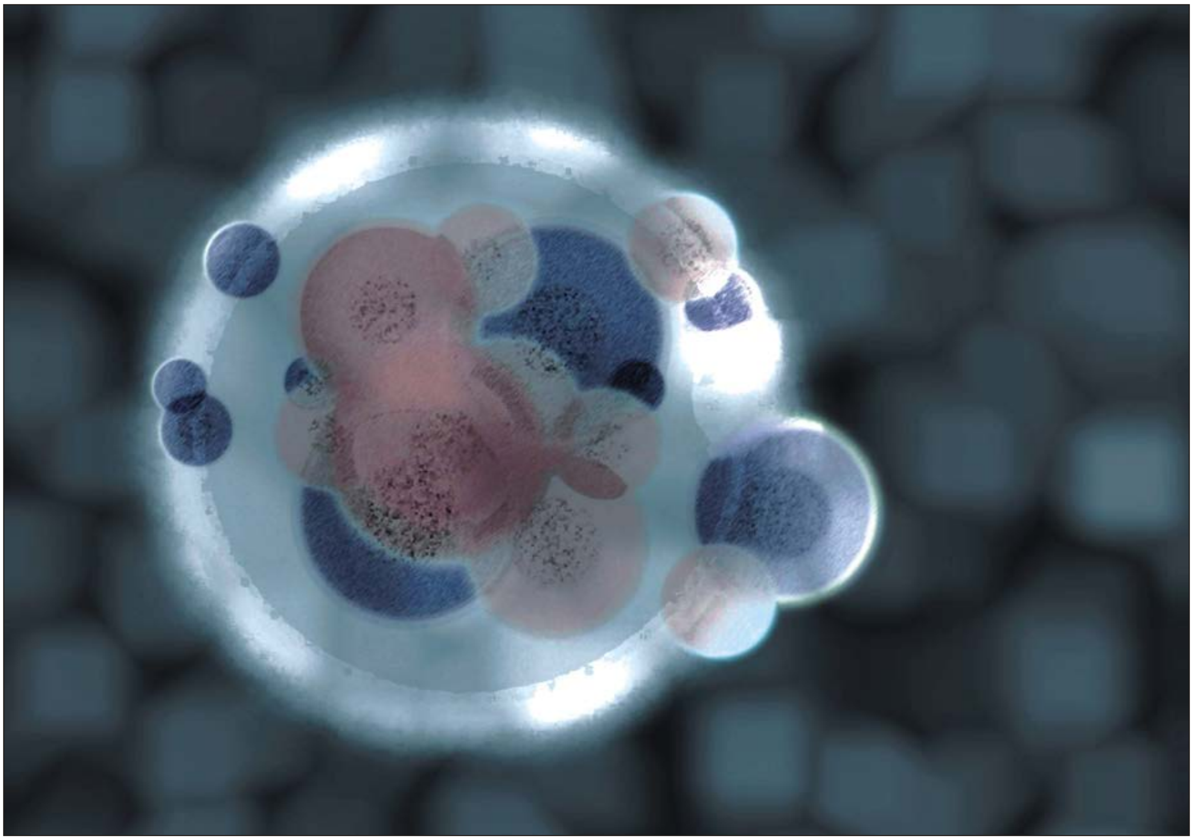

Journal of Biological Sciences

ISSN 1727-3048

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2018.506.513

Research Article
Whey Protein Bar Supplementation Effects Combined with Resistance Training on Athletes

Fawzi Amin El-Shobaki, Shreef Gamal Noub Gabrial, Marie-Christine Raymond Shakib, Magda Soliman Mohamed Abdel Haleem and Gamal Noub Gabrial

Department of Nutrition and Food Science, National Research Centre, El Buhouth St., Dokki, 12622 Giza, Egypt

Abstract
Background and Objective: Resistance training causes damage or tears to the muscle cells, which require supplementation with protein sources that have a rapid rate of digestion and good source of amino acids and other essential nutrients to ensure quick repair and help muscle regeneration and growth. The objective of the present study was to formulate a whey protein bar supplement that satisfies the requirements of the athletes from different nutrients. Also, to investigate its beneficial synergistic effects following resistance training on body measurements. Additional parameters to insure safety of the formula were analyzed. **Materials and Methods:** Eight male athletes, aged 19–25 participated in the present study, which lasted for a period of four months. Bioelectrical impedance analysis, anthropometric and skin fold measurements were registered. Blood hemoglobin concentration, serum activities of AST and ALT enzymes, serum creatinine, uric acid and urea were measured. Serum lipid profile and blood electrolytes were estimated. **Results:** After whey protein bar supplementation, a significant decrease in the activity of AST was recorded. Serum urea level and uric acid were significantly decreased. Concerning electrolytes, results showed a significant increase in serum sodium, phosphorus levels and a significant decrease in serum potassium and magnesium. Moreover, significant reduction in serum lipid profile levels was observed. Whey protein bar supplementation slightly increased body weight and caused an increase in circumference measurements. A regular decrease in body fat, an increase in water percentage and in muscle mass, together with a slight decrease in bone mass and in skin fold measurements occurred. **Conclusion:** Supplementing whey protein bar with other nutrients sources improved athletes' post exercise body composition and other anthropometric measurements. Biochemical assessments proved health effect and safety of this formula; therefore it may be helpful to athletes following resistance training for muscle hypertrophy.

Key words: Whey protein, resistance training, athletes, biochemical parameters, body measurements, bioelectrical impedance analysis

Received: November 06, 2017 Accepted: October 05, 2018 Published: October 15, 2018

Citation: Fawzi Amin El-Shobaki, Shreef Gamal Noub Gabrial, Marie-Christine Raymond Shakib, Magda Soliman Mohamed Abdel Haleem and Gamal Noub Gabrial, 2018. Whey protein bar supplementation effects combined with resistance training on athletes. J. Biol. Sci., 18: 506-513.

Corresponding Author: Fawzi Amin El-Shobaki, Department of Nutrition and Food Science, National Research Centre, El Buhouth St., Dokki, 12622 Giza, Egypt Tel: +201289904866

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Resistance training is exercises that cause an increase in strength, tone, mass and/or endurance of the muscle, by contracting muscles against an external resistance or opposing force. The external resistance can be iron weights, own body weight or any other object[1,2]. Resistance training works by enhancing muscle protein synthesis and incorporation of these proteins into muscle cells causing hypertrophy and thus acquiring a greater strength[3,4].

Milk protein consumed soon after resistance exercise can give rise to enhanced protein metabolism following resistance exercise. Such acute increases in protein net balance could possibly boost the more chronic adjustments that occur with resistance training[5].

There is growing amounts of evidence, to support the use of low-fat milk as a post resistance exercise nutrient. Consumption of milk, which is low in fat, appears to create an anabolic environment following resistance exercise and over the long term with training, it appears that greater gains in lean mass and muscle hypertrophy can be obtained[6].

The composition of milk protein is mostly casein 80% and whey protein[7] (WP) 20%. WP isolates are rich in essential amino acids, including branched chain amino acids; these are needed by the body for tissue synthesis, energy production and maintaining health. The high content of the branched amino acid leucine (50-70% higher than other protein sources) in WP is responsible for its ability to stimulate protein synthesis in muscles[8].

It has been shown that athletes undergoing resistance exercise supplemented with WP resulted in muscle adaptation and hypertrophy[9]. The fast digestion of WP provides a rapid source of amino acids; this insures that they can be taken up by the muscles for repair and rebuilding tissues[10]. The use of WP to improve aerobic exercises and swimming training has only been reported in relation to glycogen storage[11], antioxidant character and lipid metabolism[12]. Studies on moderate exercise and physical training concluded that these are beneficial to heart and lung functions; also, they enhance performance and in the field of preventive medicine are helpful in reducing the incidence and complications of chronic disease[13].

Physical exercise affects the biochemical homeostasis within the muscle cells. Muscle metabolites are produced and accumulate in the internal environment, putting stress on different body systems. High intensity exercise compared to resting conditions put a heavy load on body's endurance. Thus much needed nutrients must be supplied to the muscles and to replenish body stores[14,15]. In a previous report, WP supplementation was found to reduce weight improve glucose intolerance, increase insulin sensitivity and reduce plasma cholesterol in rats fed high fat diet to induce obesity[16]. In another study, a combination of resistance training and WP benefitted the lipid profile of rats, especially plasma triglycerides and cholesterol[17].

The purpose of this study was to formulate a dietary supplement for athletes in the form of a whey protein bar that contains most nutrients that are essential for muscle growth, hypertrophy and repair following resistance training, also to investigate its effect on the body measurements and some biochemical parameters.

MATERIALS AND METHODS

Subjects: Eight male subjects, aged 19-25 were recruited from the Sport and Fitness centers, Cairo, Egypt (from 2016-2017) to participate in the present study. All subjects were considered experienced weightlifters with at least 1 year experience in strength training. Subjects were non-smokers, not on a vegetarian diet, not currently taking supplements of any kind, with no major health problems (i.e., diabetes, cardiovascular disease, etc.). Body mass index (BMI) of all subjects was <26.

The volunteers were enrolled for this study at the National Research Centre (NRC), Giza, Egypt, after a written informed consent was obtained from each one of them. The study was carried out according to the Medical Research Ethics Committee, National Research Centre, Giza, Egypt. At the start of the study, each subject was put on a common workout-training program to closely follow for the duration of the study. Preliminary measurements were performed to each person. Bioelectrical impedance and skin fold measurements were done. Blood analysis, including hemoglobin concentration made according to the method of Van Kampen and Zijlstra[18], serum AST and ALT enzymes following the method of Reitman and Frankel[19]. Serum creatinine[20], uric acid[21] and urea[22] were determined to monitor kidney function. Lipid parameters including serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were determined as described by Allain *et al.*[23], Lopes-Virella *et al.*[24] and Buccolo and David[25], respectively. Serum low-density lipoprotein cholesterol (LDL-C) calculated using Friedewald Eq.[26]:

$$LDL = TC\text{-}HDL\text{-}TG/5 \text{ (mg dL}^{-1}\text{)}$$

Electrolytes including Na, K, Ionized Ca, Ph and Mg were also measured as described by Tietz[27].

Each subject was informed to consume the whey protein bar post workout as the body is able to repair muscle tissue and stimulate muscle protein synthesis to a greater degree.

Methods: Whey protein bars were made from:

- Hydrolyzed whey protein isolate 37 g
- Dry oat 15 g
- Natural peanut butter 6 g
- Natural honey 9 g
- Almond milk 18 mL
- Crushed almonds 15 g

Each protein bar contains 26 g of protein and was supplied to the study personnel in plain wrappers. The relative fatty acids content in the formula were determined using gas liquid chromatography (GLC)[28]. The antioxidant capacity was determined following methods such as, percentage DPPH radical scavenging activity, EC50 (concentration of the substrate required to decrease the initial DPPH radical concentration by 50%)[29] and tannins concentration[30].

Each subject was instructed to consume one bar per day post workout, 4 times per week for the whole training period which lasted for 4 months.

Body circumferences, body composition analysis and skin fold measurements were documented every 15 days until the end of the study.

Statistical analysis: Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software for windows (SPSS Inc., Chicago, IL, version 17.0). Data were presented as Mean±SEM. Paired t-test was used to identify differences between subjects before and after supplementation with whey protein bars. The p-value <0.05 indicated a statistically significant difference for all tests.

RESULTS

Analysis of the formula

Relative fatty acids: The fatty acids pattern shown in Table 1 indicated that the unsaturated fatty acids are dominant 74%. The total saturated fatty acids amounted to 19.2%. The most dominant fatty acid was C18 (1) oleic acid (n-9) which amounts to 38.1%. This was followed by C18 (2) linoleic acid (n-6) 28.1% then C18 (3) linolenic acid (n-3) 4.7%.

Antioxidant power: The protein bar formula showed relatively high antioxidant power as evidenced from the values of DPPH%, EC50 and tannin content as reported in Table 2.

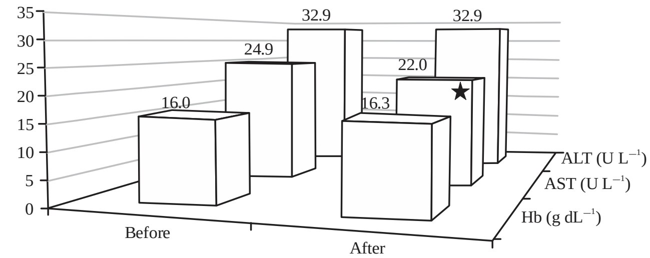

Fig. 1: Mean blood Hb level, activities of AST and ALT before and after supplementation with whey protein bar (*p<0.05)

Table 1: Relative fatty acids percentage in whey protein bar

Fatty acid	Percentage
C16 (0) palmitic acid*	5.2
C18(0) stearic acid*	7.1
C18(1) oleic acid (n-9)	38.1
C18(2)linoleic acid(n-6)	28.1
C18(3) linolenic acid(n-3)	4.7
C20(0) arachidonic acid	2.3
C22(0) behenic acid*	6.6
C22(1) erucic acid (n-9)	0.4
C24(0) lignoceric acid*	0.3
C24(1) nervonic acid (n-9)	0.4

*Saturated fatty acids

Table 2: Percentage DPPH radical scavenging activity, EC50 (half maximal effective concentration) and tannins in whey protein bar

Whey protein bar	Concentration
DPPH radical scavenging activity (%)	54
EC50 (concentration required to obtain a 50% radical scavenging activity)	6.1 mg mL^{-1}
Tannins	4.1 mg gm^{-1}

Blood analysis and chemistry of subjects: Blood analysis and chemistry of subjects enrolled in the study were made to ensure that those individuals did not suffer from any health complications that may have an influence on the study outcome and to detect any change that is caused by whey protein bar. In Fig. 1, blood hemoglobin concentration showed a non-significant increase in its value (p>0.05) after supplementation with protein bar. The AST enzyme activity significantly decreased (p<0.05), while ALT enzyme activity showed no significant change in its value (p>0.05). Kidney function parameters were shown in Fig. 2 and blood electrolytes can be shown in Fig. 3. Protein bar supplementation did not affect the concentration of serum creatinine, while it resulted in a significant decrease in serum urea and serum uric acid level (p<0.05). Concerning electrolytes, results showed a significant increase in mean serum sodium level (p<0.05) and a significant decrease in mean serum potassium (p<0.05). No significant change was shown in ionized calcium (p>0.05), a significant increase

Table 3: Anthropometric measurements of athletes before and after supplementation with whey protein bar

Athletes (No.)	Height (cm)	Weight (kg)	Circumferences (cm) Forearm	Neck	Chest	Shoulder	Biceps	Abdomen	Hip	Thigh	Calf
1a	180.00	83.3	29.0	39.0	105.0	120.0	35.5	88.0	100.0	54.0	39.0
1b	180.00	87.1	28.0	39.0	106.5	125.0	36.5	85.0	97.0	57.5	40.0
2a	185.40	85.8	29.9	40.0	108.2	123.6	36.6	88.1	103.0	55.6	40.2
2b	185.40	89.7	28.8	40.1	109.7	128.8	37.6	89.6	99.9	59.2	41.2
3a	175.90	81.4	27.4	38.1	102.6	117.0	35.7	85.6	94.8	52.8	38.1
3b	175.90	85.1	28.0	38.3	104.1	122.1	34.7	83.5	97.7	56.2	39.1
4a	184.10	85.2	28.6	39.3	107.4	122.8	37.3	90.0	99.2	55.2	39.9
4b	184.10	89.1	29.7	39.4	108.9	127.9	36.3	87.0	102.3	58.8	40.9
5a	183.00	80.6	28.0	38.7	102.0	111.0	34.5	86.5	98.0	54.0	39.0
5b	183.00	83.4	29.0	38.8	107.0	119.0	36.5	88.0	100.0	56.0	38.0
6a	179.30	78.4	27.4	38.2	100.0	108.8	33.8	87.2	98.0	52.9	38.2
6b	179.30	81.7	28.4	38.2	104.9	116.6	36.3	85.3	96.0	54.9	37.2
7a	173.85	76.6	26.6	37.3	96.9	105.5	32.8	84.6	95.0	51.3	37.1
7b	173.85	78.6	27.6	37.1	101.7	113.1	35.2	82.2	93.1	51.3	36.1
8a	184.30	83.0	28.8	40.2	105.1	114.3	35.5	90.6	103.0	55.6	40.2
8b	184.30	85.2	29.9	40.3	110.2	122.6	38.1	89.1	100.9	55.6	39.1

a: Before, b: After

Table 4: Bioelectrical impedance analysis of athletes before and after supplementation with whey protein bar

Athletes (No.)	Bioelectrical impedance analysis (%) Body fat	Water	Muscle	Bone mass
1a	13.3	56.6	43.0	14.2
1b	11.9	58.0	45.4	13.7
2a	13.3	58.3	44.7	14.5
2b	12.7	59.7	46.4	14.0
3a	13.0	55.3	42.0	13.7
3b	11.6	56.7	44.3	13.2
4a	13.6	57.9	44.0	14.3
4b	12.2	59.3	46.4	14.0
5a	9.8	59.0	44.9	13.7
5b	11.3	61.7	47.0	13.5
6a	10.6	57.8	44.5	13.4
6b	9.6	57.8	45.8	13.3
7a	10.2	56.0	43.1	13.0
7b	10.4	58.6	44.3	12.9
8a	11.1	60.8	46.8	14.1
8b	10.1	60.8	47.5	14.0

a: Before, b: After

in mean serum phosphorus (p<0.05), while a significant decrease in mean serum magnesium was shown (p<0.05).

The lipid patterns including serum total cholesterol, HDL, LDL and triglycerides are shown in Fig. 4. A significant reduction in serum TC, HDL-C, LDL-C and TG concentrations was observed (p<0.05).

Body measurements: Anthropometric measurements were made for the individuals included in the study every 15 days till the end of the experiment. Results at the start and the end of the study were shown in Table 3. It can be noticed that most of the individuals showed an increase in body weight and circumference measurements including in particular the forearm, chest, shoulder, biceps, thigh and calf. This increase was regular starting from the first to the last measurement.

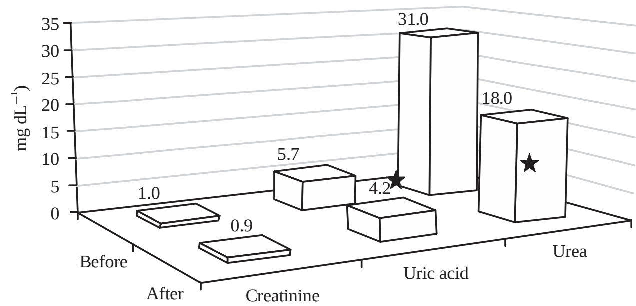

Fig. 2: Kidney functions before and after supplementation with whey protein bar (*p<0.05)

However, the increase was sometimes not appreciable in some subjects while in others, it was noticeable. The most appreciable difference in body circumference measurements of all cases was the increase in the shoulder circumference.

Bioelectrical impedance analysis including body fat, water, muscle and bone mass were measured and the data are given in Table 4. In most cases it can be noticed that a regular decrease in body fat, an increase in water percentage and in muscle mass and a slight decrease in bone mass occurred. The change in these parameters were calculated as percentage. There was a drop in the body fat, with an increase in water and muscle mass, accompanied by a decrease in bone mass.

Skin fold measurements including triceps, subscapular, thigh, abdominal, suprailiac were made to all subjects and the results are shown in Table 5. The most noticeable change was a decrease in most of these measurements.

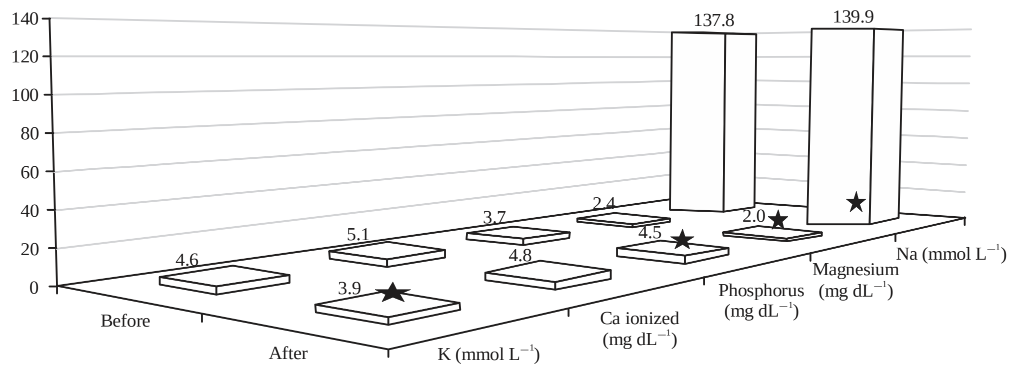

Fig. 3: Mean values of blood electrolytes before and after supplementation with whey protein bar (*p<0.05)

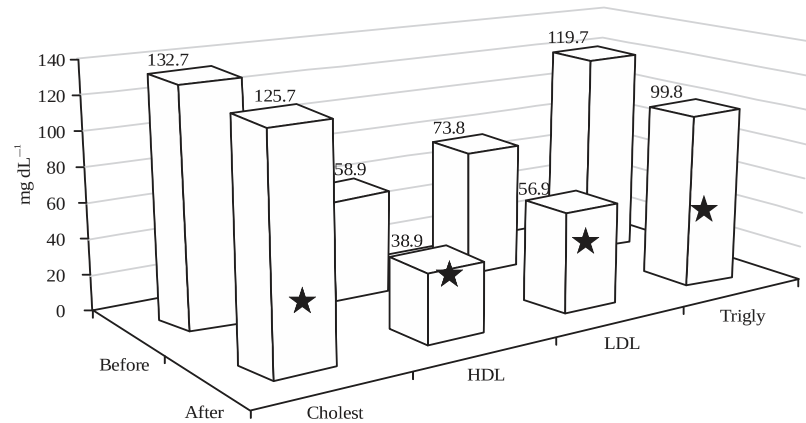

Fig. 4: Mean serum lipid levels before and after supplementation with whey protein bar (*p<0.05)

Table 5: Skinfold measurements of athletes before and after supplementation with whey protein bar

	Skinfold measurements				
Athletes No.	Triceps	Subscapular	Thigh	Abdominal	Supra-iliac
1a	2.3	1.3	2.0	1.3	2.1
1b	2.0	1.0	2.0	1.1	1.9
2a	2.4	1.3	2.1	1.2	2.2
2b	2.2	1.0	2.1	1.1	2.0
3a	2.3	1.3	2.0	1.2	1.9
3b	2.0	1.0	1.9	1.1	2.0
4a	2.4	1.4	2.2	1.4	1.8
4b	2.1	1.0	2.0	1.2	2.3
5a	2.3	1.3	2.0	1.3	2.0
5b	2.0	1.0	1.9	1.0	1.8
6a	2.3	1.3	2.0	1.3	2.0
6b	2.0	1.0	2.0	1.0	1.9
7a	2.1	1.2	1.9	1.2	1.9
7b	1.9	0.9	1.9	0.9	1.7
8a	2.4	1.3	2.1	1.3	2.1
8b	2.0	1.0	2.1	1.0	2.0

a: Before, b: After

DISCUSSION

Results of the anthropometric measurements made to the volunteers were shown in Table 3-5. After 4 months, subjects who consumed the hydrolyzed whey protein isolate bars 4 times per week in combination with resistance training exercise, showed a loss in body fat (Table 4). Furthermore, consuming whey protein bar was shown to have benefits on strength and muscle mass demonstrated by the increase in muscle percentage and therefore in body weight. These results were in accordance with previous studies made by Hayes and Cribb[31]. A slight decrease in bone mass was also shown. Triceps skin fold thickness showed a decrease in its value.

Hydrolyzed whey protein bar enhances muscle protein synthesis. It also increases body fat loss by increasing stimulated fat oxidation[32] which would promote decreased fat mass[33]. A 12 week study on Brazilian elite soccer players showed that hydrolyzed whey protein reduces muscle damage markers compared with whey protein and maltodextrin[34]. As it has been mentioned before, heavy physical exercise lead to oxidative stress and muscle damage[35]. A condition where supplementation with high nutritional value proteins are necessary, hydrolyzed whey protein isolate can be the best choice for this state since it is rich in the essential amino acids l-leucine, l-valine and l-isoleucine levels[36]. In addition, Pimenta *et al.*[37], have shown that it contains a high content of short peptides, which is assumed to be better absorbed than free amino acids and exerts antioxidant characters. The second component of the formula was dry oat. Xu *et al.*[38] found that rats fed on dietary oat β-glucan reduced the body weight and increased the maximum running time compared with normal control. Those authors conclude that dietary oat β-glucan can enhance the endurance capacity of rats while facilitating their recovery from fatigue. Bodybuilder's subject their bodies to physical hardships thus need adequate amounts of calories, protein, carbohydrates and healthy fats that can facilitate better recuperation and growth. Peanut butter contains oligopeptides and polysaccharides in addition to dietary fibers[39]. All these naturally occurring compounds have good impact on health and are expected to improve muscle performance[40]. Almond contains a high percentage of fat, approximately 50% and in turn a high energy value. The protein content is also high (about 25%) making it a good and beneficial high nutritious food for those who are in need to extra supply of different nutrients such as body builders. Almond milk contains good percentage of unsaturated fatty acids and some antioxidant compounds such as polyphenols which makes it a good source of beneficial compounds which has effects on oxidation and inflammatory conditions[41]. Bee's honey is characterized by containing a complex mixture of carbohydrates, proteins, enzymes, amino acids, lipids, vitamins, volatile chemicals, phenolic acids, flavonoids and minerals. This makes it a nutritious food of worldwide economic importance and widely used and preferred by many people[42].

Excessive consumption of high protein diets exerts negative influence on renal health. Martin *et al.*[43] detected a significant increment in blood urea, serum uric acid and urea excretion among healthy young men who consumed high protein diets. In the present study, protein bar supplementation combined with resistance training showed potential positive renal effects manifested by the decrease in urea (the major end product of protein metabolism), uric acid level and normal creatinine level. These results could be interpreted by the fact that consumption of protein bar combined with resistance training lead to a significant reduction in urea and uric acid levels as resistance training reduces inflammation. The present results were in accordance with those reported by Aparicio *et al.*[44]. Data from this study also indicated an increase in Na level and a decrease in K level. Reduction in K level increases Na retention. When consumed, sodium is absorbed into the body's cells, bringing water along with it (to hydrate the body). When excess water is retained due to extra sodium consumption, the body will naturally store water explaining the increase of water percentage in present study subjects. Some authors concluded that whey protein was an effective beverage for promoting rehydration following exercise induced dehydration[5,45].

The body naturally labors to create a balance of electrolytes, so when potassium is consumed in the proper dosages, it pulls excess Na and H_2O out of cells through the electrolyte pumps and excretes them via urination, thus forming an electrolyte balance within the body[46].

Moreover, excessive protein consumption may affect bone health due to its acidogenic effect that may result in bone resorption and ultimate bone loss[44]. Results of this study revealed a decrease in ionized Ca^{2+} and significant decrease in Mg and a significant increase in P. It was proposed that inorganic phosphate (P_i), which increases substantially during fatigue, may enter the sarcoplasmic reticulum (SR), combine with Ca^{2+} and form an insoluble precipitate of calcium phosphate (CaP_i), leading to reduced SR Ca^{2+} release and a consequent decline in muscle performance as originally suggested by Fryer *et al.*[47].

CONCLUSION AND FUTURE RECOMMENDATION

It could be therefore concluded that the formulated whey protein bar used by athletes following resistance training improved their body composition and other anthropometric measurements and could be used to enhance muscle hypertrophy. It is also clear from this study that whey protein bars have no deleterious effect on any of the liver or kidney functions or any of the lipid components in the body, which makes it safe to be consumed. It is recommended that the whey protein bar should be consumed post exercise together with an adequate source of K, Ca and Mg, so that athletes could have its maximum benefits.

SIGNIFICANCE STATEMENTS

This study discovered that the formulated whey protein bar can be beneficial for athletes following resistance training, it improves body composition and other anthropometric measurements. This study also will help the researcher to uncover the critical areas of developing muscle hypertrophy that many researchers were not able to explore. Thus a new theory on hypertrophic gains may be reached by consuming the formulated whey protein bar following resistance training for achieving maximum gain in muscle hypertrophy.

REFERENCES

1. Thomas, M.H. and S.P. Burns, 2016. Increasing lean mass and strength: A comparison of high frequency strength training to lower frequency strength training. Int. J. Exerc. Sci., 9: 159-167.
2. Westcott, W.L., 2012. Resistance training is medicine: Effects of strength training on health. Curr. Sports Med. Rep., 11: 209-216.
3. Sundell, J., 2011. Resistance training is an effective tool against metabolic and frailty syndromes. Adv. Prev. Med., Vol. 2011. 10.4061/2011/984683.
4. Schoenfeld, B.J., A.A. Aragon and J.W. Krieger, 2013. The effect of protein timing on muscle strength and hypertrophy: A meta-analysis. J. Int. Soc. Sports Nutr., Vol. 10. 10.1186/1550-2783-10-53.
5. Roy, B.D., 2008. Milk: The new sports drink? A review. J. Int. Soc. Sports Nutr., Vol. 5. 10.1186/1550-2783-5-15
6. Hartman, J.W., J.E. Tang, S.B. Wilkinson, M.A. Tarnopolsky, R.L. Lawrence, A.V. Fullerton and S.M. Phillips, 2007. Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. Am. J. Clin. Nutr., 86: 373-381.
7. Krissansen, G.W., 2007. Emerging health properties of whey proteins and their clinical implications. J. Am. Coll. Nutr., 26: 713-723.
8. Garlick, P.J., 2005. The role of leucine in the regulation of protein metabolism. J. Nutr., 135: 1553S-1556S.
9. Farup, J., S.K. Rahbek, M.H. Vendelbo, A. Matzon and J. Hindhede *et al*., 2014. Whey protein hydrolysate augments tendon and muscle hypertrophy independent of resistance exercise contraction mode. Scand. J. Med. Sci. Sports, 24: 788-798.
10. Chen, W.C., W.C. Huang, C.C. Chiu, Y.K. Chang and C.C. Huang, 2014. Whey protein improves exercise performance and biochemical profiles in trained mice. Med. Sci. Sports Exerc., 46: 1517-1524.
11. Morifuji, M., A. Kanda, J. Koga, K. Kawanaka and M. Higuchi, 2010. Post-exercise carbohydrate plus whey protein hydrolysates supplementation increases skeletal muscle glycogen level in rats. Amino Acids, 38: 1109-1115.
12. Elia, D., K. Stadler, V. Horvath and J. Jakus, 2006. Effect of soy- and whey protein-isolate supplemented diet on the redox parameters of trained mice. Eur. J. Nutr., 45: 259-266.
13. Pedersen, B.K. and B. Saltin, 2006. Evidence for prescribing exercise as therapy in chronic disease. Scand. J. Med. Sci. Sports, 16: 3-63.
14. Huang, C.C., W.C. Huang, S.C. Chan, C.C. Yang and W.T. Lin, 2013. *Ganoderma tsugae* hepatoprotection against exhaustive exercise-induced liver injury in rats. Molecules, 18: 1741-1754.
15. Ament, W. and G.J. Verkerke, 2009. Exercise and fatigue. Sports Med., 39: 389-422.
16. Tranberg, B., L.I. Hellgren and J. Lykkesfeldt, 2013. Whey protein reduces early life weight gain in mice fed a high-fat diet. Plos One, Vol. 8. 10.1371/journal.pone.0071439.
17. Aparicio, V.A., C. Sanchez, F.B. Ortega, E. Nebot, G. Kapravelou, J.M. Porres and P. Aranda, 2013. Effects of the dietary amount and source of protein, resistance training and anabolic-androgenic steroids on body weight and lipid profile of rats. Nutr. Hospital., 28: 127-136.
18. Van Kampen, E.J. and W.G. Zijlstra, 1961. Standardization of hemoglobinometry II. The hemiglobincyanide method. Clin. Chim. Acta, 6: 538-544.
19. Reitman, S. and S. Frankel, 1957. Colorimetric methods for aspartate and alanine aminotransferase. Am. J. Clin. Pathol., 28: 55-60.
20. Szasz, G., U. Borner, E.W. Busch and W. Bablok, 1979. Enzymatic assay of creatinine in serum: Comparison with Jaffe methods (author's transl). J. Clin. Chem. Clin. Biochem., 17: 683-687.
21. Fossati, P., L. Prencipe and G. Berti, 1980. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin. Chem., 26: 227-231.
22. Fawcett, J.K. and J.E. Scott, 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol., 13: 156-159.
23. Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

24. Lopes-Virella, M.F., P. Stone, S. Ellis and J.A. Colwell, 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem., 23: 882-884.
25. Bucolo, G. and H. David, 1973. Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem., 19: 476-482.
26. Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.
27. Tietz, N.W., 1986. Textbook of Clinical Chemistry. W.B. Saunders, Philadelphia, PA.
28. Firestone, D., 2009. Official methods and recommended practices of the AOCS. American Oil Chemists' Society, Boulder, Urbana, IL. USA., pp: 2710.
29. Choi, Y., H.S. Jeong and J. Lee, 2007. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chem., 103: 130-138.
30. AOAC., 1990. Official Methods of Analysis of the Association of Analytical. 15th Edn., AOAC, Washington, DC., USA.
31. Hayes, A. and P.J. Cribb, 2008. Effect of whey protein isolate on strength, body composition and muscle hypertrophy during resistance training. Curr. Opin. Clin. Nutr. Metab. Care., 11: 40-44.
32. Lu, H., D. Huang, R.M. Ransohoff and L. Zhou, 2011. Acute skeletal muscle injury: CCL2 expression by both monocytes and injured muscle is required for repair. FASEB J., 25: 3344-3355.
33. Soenen, S., G. Plasqui, A.J. Smeets and M.S. Westerterp-Plantenga, 2010. Protein intake induced an increase in exercise stimulated fat oxidation during stable body weight. Physiol. Behav., 101: 770-774.
34. Lollo, P.C.B., J. Amaya-Farfan, I.C. Faria, J.V.V. Salgado and M.P.T. Chacon-Mikahil *et al*., 2014. Hydrolysed whey protein reduces muscle damage markers in Brazilian elite soccer players compared with whey protein and maltodextrin. A twelve-week in-championship intervention. Int. Dairy J., 34: 19-24.
35. Zoppi, C.C., R. Hohl, F.C. Silva, F.L. Lazarim, J.A. Neto, M. Stancanneli and D.V. Macedo, 2006. Vitamin C and e supplementation effects in professional soccer players under regular training. J. Int. Soc. Sports Nutr., 3: 37-44.
36. Yuan, J., B. Jiang, K. Li, W. Shen and J.L.Tang, 2017. Beneficial effects of protein hydrolysates in exercise and sports nutrition. J. Biol. Regul. Homeost. Agents, 31: 183-188.
37. Pimenta, F.M.V., M.I. Abecia-Soria, F. Auler and J. Amaya-Farfan, 2006. Physical performance of exercising young rats fed hydrolysed whey protein at a sub-optimal level. Int. Dairy J., 16: 984-991.
38. Xu, C., J. Lv, Y.M. Lo, S.W. Cui, X. Hu and M. Fan, 2013. Effects of oat β-glucan on endurance exercise and its anti-fatigue properties in trained rats. Carbohydr. Polym., 92: 1159-1165.
39. Apong, P.E., 2013. Nutrition and Dietary Recommendations for Bodybuilders. In: Nutrition and Enhanced Sports Performance: Muscle Building, Endurance and Strength, Bagchi, D., S. Nair and C.K. Sen (Eds.)., Academic Press, UK., pp: 509-521.
40. Wang, Q., A. Shi, H. Liu, L. Liu and L. Zheng *et al*., 2016. Peanut by-Products Utilization Technology. In: Peanuts: Processing Technology and Product Development, Wang, Q. (Ed.)., Academic Press, UK., pp: 211-325.
41. Torres, L.R.D.O., F.C. de Santana, F.L., Torres-Leal, I.L.P. de Melo and L.T. Yoshime *et al*., 2016. Pequi (*Caryocar brasiliense* Camb.) almond oil attenuates carbon tetrachloride-induced acute hepatic injury in rats: Antioxidant and anti-inflammatory effects. Food Chem. Toxicol., 97: 205-216.
42. Ball, D.W., 2007. The chemical composition of honey. J. Chem. Educ., 84: 1643-1646.
43. Martin, W.F., L.E. Armstrong and N.R. Rodriguez, 2005. Dietary protein intake and renal function. Nutr. Metab., Vol. 2. 10.1186/1743-7075-2-25.
44. Aparicio, V.A., E. Nebot, J.M. Porres, F.B. Ortega, J.M. Heredia, M. Lopez-Jurado and P.A. Ramirez, 2011. Effects of high-whey-protein intake and resistance training on renal, bone and metabolic parameters in rats. Br. J. Nutr., 105: 836-845.
45. Phillips, S.M. and L.J.C. van Loon, 2011. Dietary protein for athletes: From requirements to optimum adaptation. J. Sports Sci., 29: S29-S38.
46. Palmer, B.F., 2015. Regulation of potassium homeostasis. Clin. J. Am. Soc. Nephrol., 10: 1050-1060.
47. Fryer, M.W., V.J. Owen, C.D. Lamb and D.G. Stephenson, 1995. Effects of creatine phosphate and P (i) on Ca^{2+} movements and tension development in rat skinned skeletal muscle fibres. J. Physiol., 482: 123-140.