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Effect of nutri-bar in the development of stamina building and exercise-performance in young male-athletes

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Abstract

Nutri-bars were prepared (110 g) using dates (64 g), dried apricots (16 g), cheddar cheese (8 g), whey protein isolate (12 g) and roasted-chickpea flour (10 g). Bars were prepared for Pakistani-athletes based on their calories and protein requirement (3500-3925 kcals/day, 1.4-1.8 g/kg body weight). Efficacy trials were performed (1, 15 and 30 days) to evaluate the effect of nutri-bars on blood serum profile, stamina building and body-composition. Results showed that hepatic-indexes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) were decreased ($p > 0.05$) during experimental-period. At day 30, ALT (6.8%), AST (1%) and ALP (0.4%) indicates the inhibitory effect of nutri-bar on the pathological serum-profile of athletes while significant increase ($p > 0.05$) was observed in total-protein (0.52%). Liver injury-indexes lactate dehydrogenase, Creatine kinase, total-cholesterol and total-glycerides showed a significant increase ($p > 0.05$) while low-density-lipoprotein and high-density-lipoprotein were decreased ($p > 0.05$). Effect of nutri-bar on body composition showed increase ($p > 0.05$) in body weight (kg) and body-mass index (BMI (kg/m^2). In the twelve-minutes running test, distance covered by athletes ($p < 0.05$) increased after 30 days as compared to day 1. The study revealed the significant ($p < 0.05$) effect of nutri-bar in developing the stamina building of Pakistani-athletes while the effect on the blood-serum profile was non-significant.

Keywords: nutri-bars; athlete; fitness; whey protein isolate; hepatic-indexes; body composition.

Practical Application: Nutri-bars are healthy snacks for athletes that provides them adequate daily dietary intake of all nutrients and helpful in the development of stamina building as well as maintenance of desired body weight. Major consumers of such products are sports-athletes, arm-force, adolescents and school going children.

1 Introduction

The sports-nutrition is a field of nutrition that deals with body composition of athletes and science of exercise. It covers the practical aspects of nutrition knowledge through dietary plans that provides energy for physical activity, enhances the building and repairing process which induced during heavy workouts and exercises performance. The main concept of athlete's nutrition involves achieving the optimal exercise performance (Indoria and Singh, 2016). Therefore, different factors play an important role in successful sports or exercise training including adequate diet, which depends upon the practical issues of sports, environment and athlete's goals for exercise performance (Beck et al., 2015).

The diet based approaches for athletes often involves to eating a meal before and after exercise/competition that helps to improve the performance by increasing the glycogen-stores. For this purpose, nutri-bars are initially introduced into the market for arm-force and athletes as a quick source of nutrients and energy (Pinto et al., 2019). In the formulation of nutri-bars, the different fruits are being used in order to provide the plenty of supply of nutrients and functional components. Because of social and ethical concerns that involves the changes in dietary habits, an aging population, and enhanced medical expenses, individuals have shown greater interest in health and varied food behaviour (Nadeem et al, 2012).

Athletes required more proteins as compared to others as it is important for optimal cellular-functions and also play the regulatory and developmental roles in the body. Protein should account approximately 10% to 30% of total caloric intake (Purcell, 2013). For resistance-training athletes, the protein requirement is 1.5-1.8 g per kg BW while requirements for endurance-athletes is 1.4-1.6 g per kg BW. Therefore, the latest RDA guidelines for athletes recommended carbohydrates and protein 7-12 g and 1.2-2.0 g/kg body weight respectively and fat intake 20-35% of total calories per day (Jäger et al., 2017).

Cheese whey contained about 15 to 20% of milk proteins with higher biological significance as compared to other proteins (Alves et al., 2019). These are considered as ideal for

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muscle building and several other health benefits due to its high essential and branched-chain amino acid, bioactive peptides and leucine content. Leucine is present about 50 to 75% that is higher than other proteins and it plays a significant role in the synthesis of muscle protein and helps in the regulation of skeleton (Trindade et al., 2019).

Now a day, people are more interested in strenuous exercise and training due to the awareness regarding the positive health effects of physical activity. Different clinical and bio-chemical parameters that considered the biomarkers for studying the physiological functions and status after training and exercise are: creatine-kinase (CK), aspartate-aminotransferase (AST), alanine amino-transferase (ALT) as well as the lactate-dehydrogenase (LDH) (Chiu et al., 2013). Metabolic-index (MI) used to examine the metabolic-status and bio-markers such as blood urea-nitrogen (BUN), AST, CK, ALT and LDH that is useful to evaluate the muscle-recovery, physiological and muscle-fatigue (Lopez et al., 2015). The whey protein (WP) can significantly reduce the production of physiological-biomarkers, which produce due to the exhaustive aerobic exercise and improve the performance during exercise (Chen et al., 2014).

In Pakistan, the least importance is given to the diet of athletes, the only food which is preferred for them by the instructor and nutritionist is WPI. The objective of the present study was to develop the nutri-bars for athletes using dates, dried-apricot, Cheddar cheese (CC) and WPI considering their recommended dietary allowance (RDA) for protein and calories. Dates and apricot contained a significant amount of antioxidants, dietary fiber and some common functional components which helps to promote the immunity, mental ability and enhance the anti-ageing effects (Fatima et al., 2018). Therefore, efficacy study was carried out to evaluate the effect of bars on blood serum profile of Pakistani-athlete. Anthropometric-measurements and the twelve-minutes running test was also performed to evaluate the effect of these bars in the development of stamina building during the whole experimental period. These nutri-bars could be helpful for athletes in the development of muscle-building, to enhance their exercise-performance and have over-all physiological protective-effects against muscular injury.

2 Materials and methods

2.1 Experimental-design

A double-blind, completely randomized study was conducted to evaluate the effects of nutri-bars vs glucose solution (placebo). The glucose solution was used as a reference point which is low glycaemic carbohydrates. These are potentially beneficial for exercise performance due to lower carbohydrate and higher fat oxidation to lower the insulin secretion (Kaviani et al., 2019) that could have a similar effect of nutri-bars. Each group $n = 6$ was consuming nutri-bar (2 bars/day) and placebo with almost equal energy intake for 30 days. Each participant of both groups received 2 nutri-bars/day or glucose drink within 30 minutes after training to evaluate the effect of nutri-bars on the blood profile of selected athletes. Three assessments including the pre, post and end test were examined to investigate the health effects of nutri-bars on clinical trials (blood profile or biochemistry), exercise-performance and body-composition. A-pre-test was conducted at 1st day, post-test at 15th while the end-test at the 30th day to evaluate the physiological status of participants. The prescriptions for exercise/training programme continued for one-month of the experimental period.

2.2 Selection of subjects

Twelve-subjects (Male-athletes) voluntarily joined this study. Subjects, who suffered any metabolic-disorder (such as heart diseases, diabetes, thyroid disease, hepato-renal disorder, muscular-skeletal disorders, hypogonadism, neurological/neuro-muscular diseases, auto-immune diseases, peptic ulcers, anaemia and cancer) were not permitted to participate in this experimental study. Before participation in the experimental study, participants take on a written-consent. The study was approved by the Institute of Bio-ethics/Bio-safety, Office of the Research, Innovation and commercialization (ORIC), University of Agriculture, Faisalabad, Pakistan. Selected subjects advised following their normal diet plan/caloric intake to meet their daily energy requirements during the whole experimental-study. Additional supplements such as creatine, antioxidants, steroids and nutrient-intake like proteins were also not allowed to be taken by the athletes.

2.3 Clinical-biochemical assessments

Blood-serum samples of subjects were collected at a specified time when the subjects had fasted at least 8 hours before bio-chemical assessments. Clinical bio-chemical assessments that were measured including the aspartate amino-transferase (AST), alkaline-phosphatase (ALP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinine-kinase (CK) and total protein (TP), total bilirubin (TBIL), uric acid (UA), blood-urea nitrogen (BUN), low-density lipoprotein (LDL), triacylglycerol (TG), high-density lipoprotein(HDL) and total cholesterol (TC) (Lowe et al., 2013).

2.4 Anthropometric measurements

All participants were arrived at GYM at a specified time for Anthropometric-measurements including height (cm), body weight (BW) in kg, and body mass-index (BMI) in kg/m^2. The body weight was measured by the weighing indicator (XK3188-T20) while the height was measured by standing the subject without socks or shoes, with a height-meter nearest to 0.1 cm attached on the wall (Sebo et al., 2017).

2.5 Twelve-minutes running test

Before starting the running, adequate energetic warmup was prerequisite. Each participant was motivated for their best performance for running in order to cover the distance as much as possible in 12 minutes. Distance covered by each subject was repeatedly measured and noted at 3, 6, 9, and at 12 minutes during the run/walk test to evaluate the maximum running performance (Huang et al., 2017).

2.6 Statistical analysis

The data was statistically analysed using Statistics software 8.1 (Chicago, IL, USA). Data were statistically interpreted (means ± standard deviation of triplicate determinations) by two-way analysis of variance (ANOVA) to compare the clinical (biochemistry) variables, body composition and performance during exercise to evaluate the level of significance at 5%. Multiple-comparisons of means; $\alpha = 0.05$ noted by the Tukey's test when the time-effect was significant.

3 Results and discussion

3.1 Clinical-biochemical assessments

Biochemical tests (blood serum profile) at three experimental points (day 1, 15 and 30) provides the clinical information regarding the physiological status of the subjects. This part categorized the various serum parameters on the basis of physiological functions (Figure 1). In liver function tests (LFT), AST, ALT and ALP were measured to examine the liver function of the participants. Results showed that the hepatic-indexes (AST, ALT, and ALP) were significantly decreased ($p > 0.05$) during nutri-bars supplementation while these indexes were significantly increased ($p > 0.05$) in placebo-group, which represents the positive effect of nutri-bar. End-test values indicate the inhibitory effect of nutri-bar on the pathological values of serum (ALT 6.8%, AST 1% and ALP 0.4%) while the increase in total protein was 0.52%.

The liver injury-indexes (LDH and CK) had not shown the significant ($p > 0.05$) increase and remained within the upper reference limits of these markers while LDH and CK increased in placebo-group. In lipid profile test, TC and TG were increased ($p > 0.05$) while LDL and HDL were decreased in both groups.

The present study demonstrated that the nutri-bar have the potential to lowers the AST, ALT, LDH, ALP, UA, BUN and LDL that can be increased during exercise (after 15 days). It indicates the inhibitory effects of nutri-bars as compared to placebo-group, which provides the muscular cell protection during exercise. The resistance training and endurance exercise, heavy workouts and different sports competitions significantly affects the body haemostasis resulting in the various pathological conditions. The physiological functions of principal organs and the tissues are affected with long-term and heavy exercise that can be helpful to increase the stamina building (Booth et al., 2011). Thus, the nutri-bars showed the bio-activities regarding physiological function as well as muscle-recovery. During long term training/exercise the permeability of cell membranes is changed and different enzymes including CK, AST, LDH and ALT can be spilled-out that is the indicator of muscle damage (Brancaccio et al., 2007). Therefore, the current study showed the potential to decrease these markers that could protects the athletes' muscle damage during exercise.

Nutri-bars increase the total protein about 0.52% during four weeks of exercise/training. Nutri-bars contained the milk-proteins (casein from cheese and whey proteins) and various studies reported that milk-proteins play a significant role in body physiological functions (Cockburn et al., 2013). The current RDA for a healthy person is 1.2-1.6 g/kg/day that is an appreciable amount for active people to adopt the optimize training (Jäger et al., 2017). Therefore, to meet this protein requirements, the consumption rate of protein-supplements is frequently increased. The improvement in the muscle-mass and exercise performance, fat-loss as well as the improvement in recovery-biomarkers are the basic health-claims that influenced the acceptance of protein-supplements (Cintineo et al., 2018). The current study revealed the consumption of two nutri-bars daily in addition to the regular diet, provides the 40% protein of the daily requirements of athletes and helpful in the elevation of muscle-mass and muscular-cell protection.

LDH and CK were increased during nutri-bar supplementation and in placebo-group but it remained within the accepted reference limits of CK and LDH-markers. Elevated serum-LDH and CK levels are the useful markers to assess the stress-injuries in athletes because it shows the muscular-cell damage that can occur during strenuous-exercise (Miyamoto et al., 2018). Daily exercise can result in persistent serum-elevation of CK while the resting CK-levels are greater in athletes. However the significant elevation of CK usually occurred after exercise/training but it remained less in trained-subjects as compared to the untrained-subjects (Cintineo et al., 2018). The time of CK-release into plasma and its clearance depends upon the type, duration, intensity and level of exercise (Baird et al., 2012). Higher CK-serum level in healthy individuals can be correlated with the physical activity while the lower serum-CK level depends upon the resting time after exercise because the short-term rest can reduce the secretion of enzymes from muscular-fibres and lymphatic-CK transport (Havas et al., 1997). Therefore, the supplementation of branched-chain amino acids (BCAA) after prolonged exercise helps to reduce the muscle-damage and serum-CK concentrations (Brancaccio et al., 2007).

Lipid-proteins and total lipid content in athletes who engaged in aerobic/strenuous exercise showed the characteristics of low LDL and TG while a high level of HDL cholesterol as compared to those individuals who have less-activity. Immediately after greatest effort in high-intensity exercise, the TC level will be increased significantly (Sgouraki et al., 2004), and the cholesterol used in bio-synthesis of various steroid-hormones such as aldosterone, adrenal-gland, cortisol and sex-hormones like progesterone, testosterone, estrogens and including their derivatives that are helpful to maintain the physiological-homeostasis (Hanukoglu, 1992). In the present study, the nutri-bar supplementation has slightly increased the level of LDL, TG and TC during exercise/training. Total cholesterol could be more-efficiently converted into other related hormones that can be helpful to improve the physiological-adaptations of athletes during exercise.

Circulating serum-TBIL (total bilirubin) is a by-product of normal heme-catabolism which helps to assess the liver functions and widely used as a biomarker of cholestasis (Kunutsor et al., 2015). In the present study, no significant change in TBIL (Figure 1) was noticed and TBIL level within the accepted reference limit which showed the normal Liver function without any injury of muscular-cells. In normal conditions, the serum-TBIL concentrations appear to be changed due to the regular exercise. The breakdown of red blood cells (RBCs) induced by the

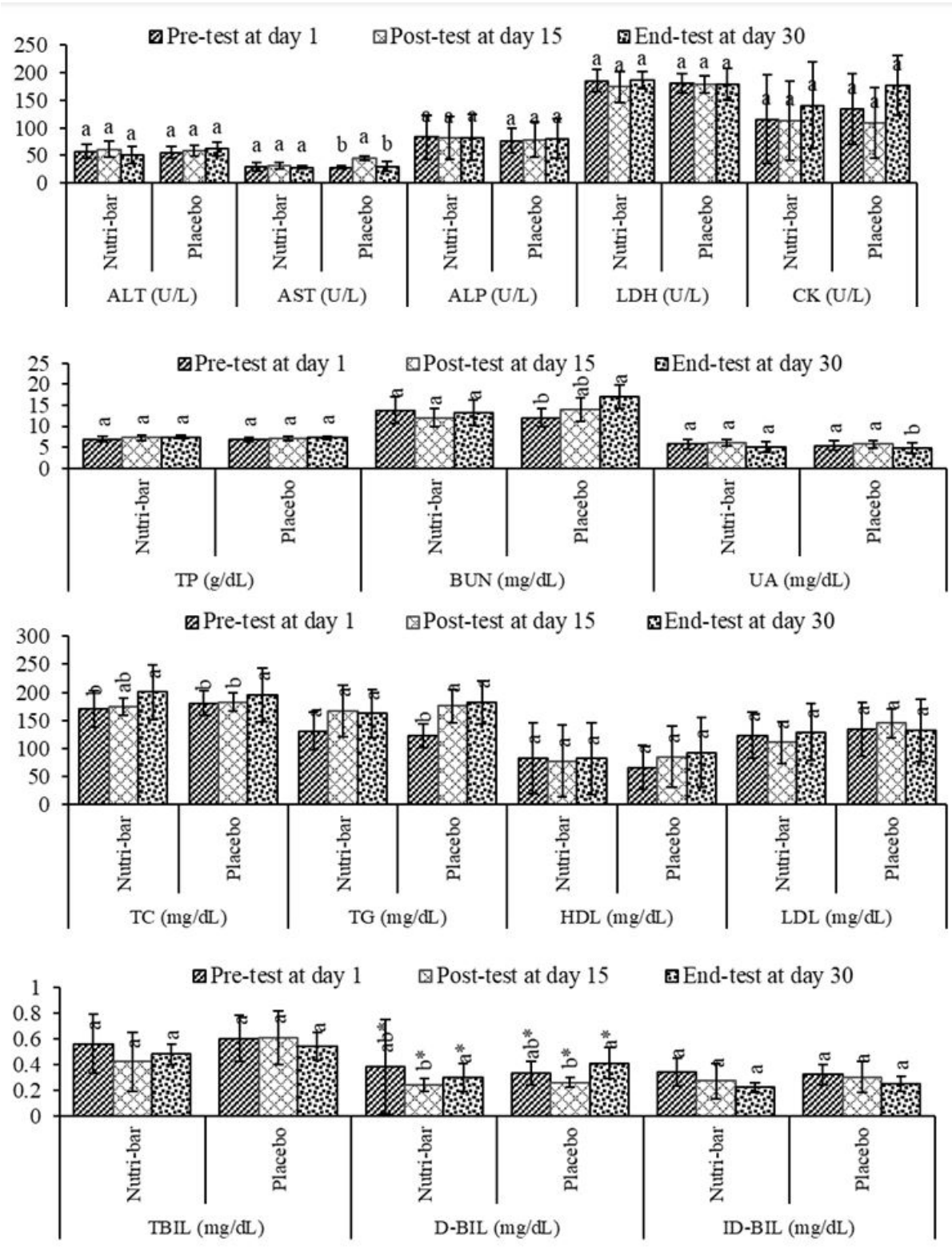

Figure 1. Effect of nutri-bars on blood-serum profile at three times assessments. Pre, post and end-test were measured at 1st day, 15th day and 30th day of the whole experimental study. Pre-test measurements were taken before the supplementation of nutri-bar and placebo. Data showed the readings as Mean ± SD for n = 6 subjects. Data were statistically interpreted by using the two way ANOVA (analysis of variance), Multiple-comparisons of means; α = 0.05 noted by the Tukey's test when the time-effect was significant ($p < 0.05$)$^{a-b}$ Means are non-significantly ($p > 0.05$) if they share a common superscript within the same row. Creatine kinase (CK), Total protein (TP), Total cholesterol (TC), high-density lipoprotein (HDL), lactate dehydrogenase (LDH), low-density lipoprotein (LDL), alanine aminotransferase (ALT), uric acid (UA), aspartate aminotransferase (AST), Indirect Bilirubin (ID-BIL), Direct-Bilirubin (D-BIL), Total Bilirubin (TBIL), Alkaline phosphatase (ALP), Triglycerides (TG), alanine aminotransferase (ALT), Blood urea nitrogen (BUN).

mechanical factors such as marching-haemolysis, injury due to the muscle-workout, RBCs-squeezing through capillaries and due to the destructive effect of free-radicals (Witek et al., 2017). The serum-TBIL concentration also depends upon the dose of exercise, as its concentration increases with increasing the duration and intensity of physical-efforts. Swift et al. (2012) studied the influence of different-volumes of training/exercise on TBIL level and found that a greater physical workout increases the TBIL level. Other studies reported that the bilirubin has the potential to work as an antioxidant and anti-inflammatory agent (Schwertner & Vítek, 2008). Thus, act as an inhibitor of oxidative-changes in lipids and LDL, thereby contributing in neutralizing the free-radical species and reduce the oxidative stress that can also be induced due to the consumption of oxygen during muscle-activity (Witek et al., 2017).

3.2 Effects of nutri-bar on running performance

To study the effect of nutri-bars and placebo-group with exercise training, 12 minutes running test was performed to evaluate the stamina of participants. As mentioned above, the distances were measured four times with three minutes time intervals at 3, 6, 9, and 12 minutes. The measurement of running distance is a time-dependant mechanism, Figure 2. shows that distance readings at post-test significantly increased ($p < 0.05$) in both groups as the time was increased as compared to the pre-test. The inter-group statistical analysis (Table 1) showed that the distance covered by the experimental group and placebo at day 1 after 12 minutes running, was 1.20 km and 1.09 km respectively. After 30 days, the distance covered by the experimental group and placebo, after 12 minutes was 1.27 km and 1.10 km respectively. Which shows the significant ($p < 0.05$) effect of nutri-bar in developing the stamina building of athletes by following the four weeks of exercise training.

Previous studies reported that the long term training exercise can imbalance the antioxidant-status which effect the severe tissue-injury and cause the muscle-fatigue (Dragsted, 2008). This oxidative-stress cause to induce the muscle-damage which ultimately affect the protein metabolism in skeletal-muscles. Nutri-bars contained the antioxidant compounds, bioactive peptides and whey protein that can protect the muscle-proteins from oxidation which induced due to exercise (Rafiq, 2015). Nutri-bars may reduce the physiologic-fatigue due to the long term training-exercise thus contributing to develop the stamina building and improved the exercise performance. The physiological-fatigue induced due to the physical or mental pressure and inadequate rest is generally characterized into peripheral or central fatigue (Zwarts et al., 2008). The serum bio-markers like CK, ALT, BUN, AST, glucose, ammonia and lactate are useful tools to evaluate the peripheral-fatigue that linked to muscle injury and exercise-fatigue (Lin et al., 2015; Antunes et al., 2016). The present study revealed that after four weeks nutri-bar supplementation, participants exhibited lower ALT, AST, BUN, CK, and LDH levels (End-test) that indicating the nutri-bars helps to maintain the lower peripheral-fatigue

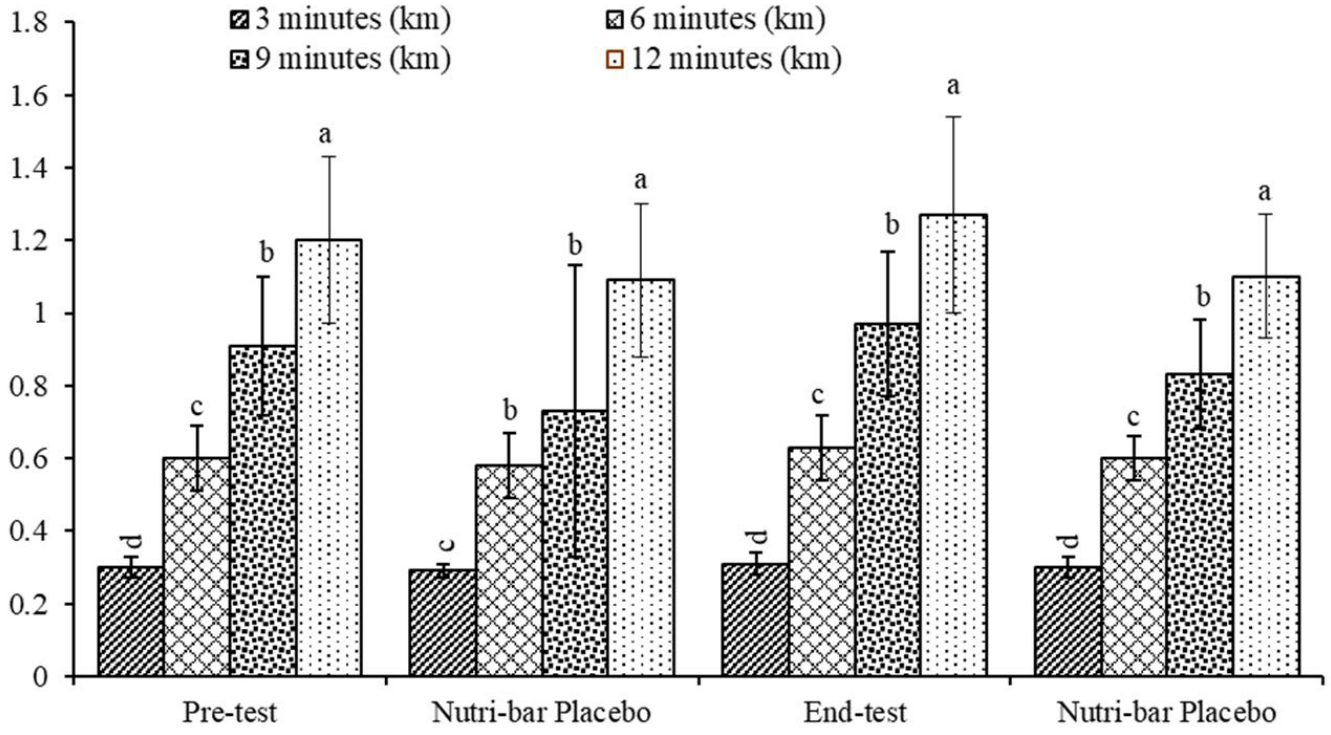

Figure 2. The distance measured during the running of 12 minutes. The pre-test readings were taken at 1st day before starting the supplementation of nutri-bar and placebo while End-test readings were taken after 30 days of the whole experimental period. During the test distance was measured at 4 points (3, 6, 9 & 12 minutes). Data showed the readings as Mean ± SD for n = 6 subjects. Data were statistically interpreted by using the two-way ANOVA (analysis of variance), Multiple-comparisons of means; $\alpha = 0.05$ noted by the Tukey's test when the time-effect was significant ($p < 0.05$). [a-d]Means are non-significantly ($p > 0.05$) if they share a common superscript within the same row.

status. It means that the nutri-bars can reduce the exercise-fatigue and promote the muscle recovery.

3.3 Effects of nutri-bar on the body composition

Data regarding the effect of nutri-bar and placebo on body composition presented in Figure 3. that showed the increase ($p > 0.05$) in body weight (kg) and BMI (kg/m^2) of both group's participants. The study revealed that the four-weeks training exercise with nutri-bar supplementation could help to maintain their body weight as compared to placebo-group. The WP in nutri-bars could play a significant role in regulating the energy-expenditure and the central-appetite. Some previous studies reported that WP strongly suppressed the hunger and decrease the food-intake (Veldhorst et al., 2009) as the WP could be helpful in regulating the energy expenditure. The whey protein increases the grip-strength during exercise which is strongly correlated with muscle-strength (Wu et al., 2013). The improvement in the muscle strength is due to its branched chain amino acids content present in WP (Appuhamy et al., 2012) such as leucine that plays a significant role in the bio-synthesis of protein and increased the glycogen storage in skeletal-muscles (Yoshizawa et al., 2013) which can be helpful in the weight maintenance of the participants of the experimental group.

Another study reported that the high-intensity interval training (HIIT), by following the three weeks whey protein supplementation enhanced the lean muscle-mass and VO_2 max (maximum-oxygen volume) critical-velocity (Smith et al., 2010), which enhanced the muscle-mass and strength. Moreover, a combination of WP supplementation and resistance training exercise is more effective in promoting the muscle-hypertrophy (Farup et al., 2014). Therefore, the total caloric intake, pre-exercise protein-intake for long period, play a dietary-role in facilitating the adaptations to

Table 1. Treatment x time interaction means ± SE.

Treatment	Time				Mean
	3 minutes (km)	6 minutes (km)	9 minutes (km)	12 minutes (km)	
Pre-test	0.30 ± 0.03	0.60 ± 0.09	0.91 ± 0.19	1.20 ± 0.23	0.75 ± 0.12A
Nutri-bar Placebo	0.29 ± 0.02	0.58 ± 0.09	0.73 ± 0.40	1.09 ± 0.21	0.67 ± 0.13A
End-test	0.31 ± 0.03	0.63 ± 0.09	0.97 ± 0.20	1.27 ± 0.27	0.80 ± 0.13A
Nutri-bar Placebo	0.30 ± 0.03	0.60 ± 0.06	0.83 ± 0.15	1.10 ± 0.17	0.71 ± 0.10A
Mean	0.30 ± 0.01D	0.60 ± 0.04C	0.86 ± 0.11B	1.17 ± 0.10A	

Means shares similar letters in a column or in a row are statistically non-significant (P > 0.05).

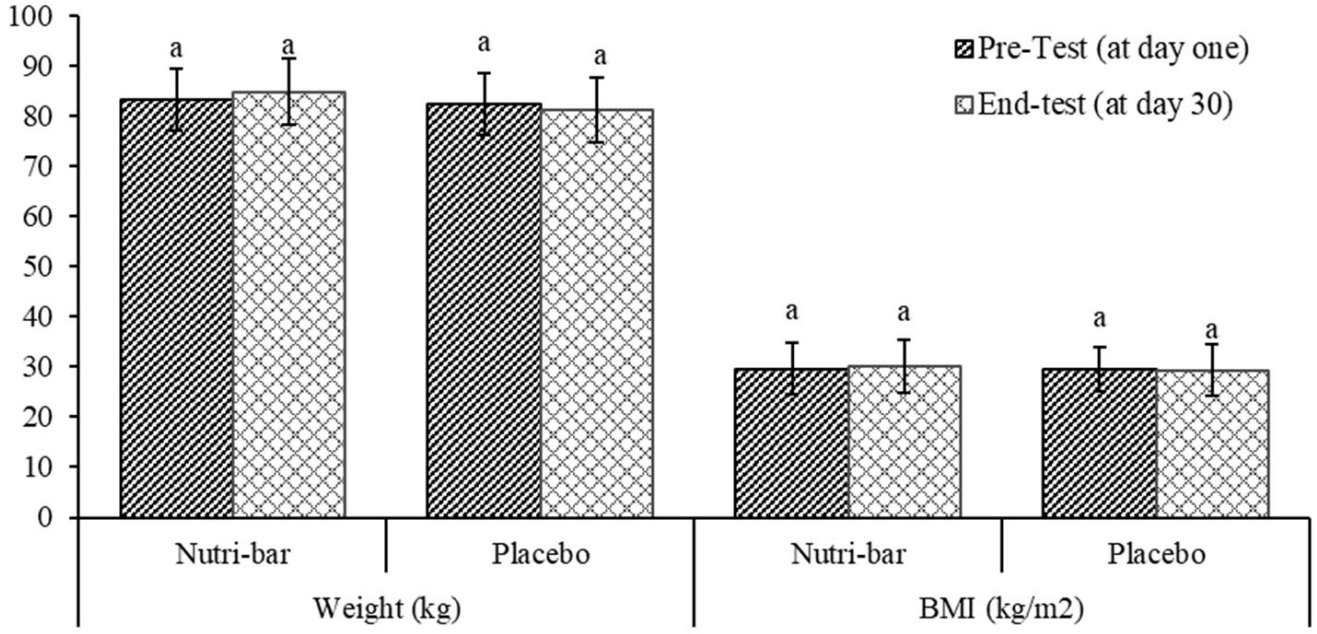

Figure 3. Overall body composition characteristics. Pre-test measurements were taken at 1st day before starting the placebo and nutri-bar supplementation while the End-test measurements were taken after 30 days of the whole experimental period. Data showed the readings as Mean ± SD for n = 6 subjects. Data were statistically interpreted by using the two-way ANOVA (analysis of variance), Multiple-comparisons of means; α = 0.05 noted by the Tukey's test when the time-effect was significant ($p < 0.05$). Means are non-significantly ($p > 0.05$) if they share a common superscript within the same row.

exercise and stamina building. Such factors are helpful to optimize the physical-performance that positively affect the successive recovery processes for endurance exercise as well as a resistance training exercise (Cintineo et al., 2018). It can be concluded that the consumption of nutri-bar before and after training exercise could be helpful in muscle-synthesis and weight maintenance.

4 Conclusion

It is concluded that the nutri-bar could be a healthy snack for athletes. These bars can develop the stamina building and helpful in their weight management. These bars did not show any significant effect on blood serum-profile of athletes. However, if the efficacy trials can be extended up to several months, these bars could be helpful to get a complete effect. Nutri-bars could also be used as a meal replacement for soldiers, in fitness clubs, for school going children and to combat the protein-energy malnutrition (PEM).

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