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Effects of biscuit fortified with whey protein isolate and wheat bran on weight loss, energy intake, appetite score, and appetite regulating hormones among overweight or obese adults

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ABSTRACT

This study assessed the impact of biscuit fortified with whey protein isolate and wheat bran on body composition, energy intake, appetite score, and appetite regulating hormones among overweight or obese adults. Ninety-six participants were randomly allocated to receive biscuit fortified with whey protein isolate and wheat bran (Whey + WB), whey protein (Whey), wheat bran (WB), and control, for 8 weeks. Weight, BMI, and waist circumference decreased more in Whey group compared to WB and control, also energy intake decreased more in Whey group compared to the control group. Moreover, composite appetite score decreased more in both Whey + WB and Whey groups compared to the control group. GLP-1 increased more in Whey group compared to the control group, and high-density lipoprotein decreased less in Whey + WB and whey groups compared to WB. Consumption of biscuit fortified with whey protein isolate, with or without wheat bran reduce appetite, energy intake, and body weight in overweight or obese persons.

1. Introduction

Increasing prevalence of obesity and its related consequences has been known as a public health problem in recent years (WHO, 2014). The Global Burden of Disease study reported that obesity rate has doubled between 1980 and 2015, in more than 70 countries (Afshin et al., 2017). In addition, it is predicted that 1.35 billion and 537 million people will be overweight and obese, up to 2030, respectively (Kelly, Yang, Chen, Reynolds, & He, 2008).

Some physiologic factors are responsible for maintaining body weight via short term and long term regulatory systems. Peripheral hormones including leptin and insulin, also gastrointestinal hormones; ghrelin, gastric inhibitory polypeptide (GIP), glucagon like peptide-1 (GLP-1), peptide YY (PYY), and cholecystokinin (CCK), are secreted in response to food intake and regulate body weight. These hormones control appetite and metabolism thorough signaling the hypothalamus nd activating or deactivating the neurons of hypothalamic nuclei (Bojanowska & Ciosek, 2016; Kairupan et al., 2016). Furthermore, behavioral and psychological factors affected food intake by acting on reward system in the hypothalamus. Psychological and emotional disorders may increase food intake beyond the adequate amount, and consequently may also disturb hemostasis control of food intake (Blundell et al., 2010; Bojanowska & Ciosek, 2016). Serotonin is a neurotransmitter that regulate appetite, mood, sleep, and cognitive function (Berger, Gray, & Roth, 2009). It has been reported that overeating were normalized after increasing serotonin by dietary and pharmacological factors (Wurtman, 1993).

A possible strategy to prevent or treat obesity is consuming food items which have the potential to control appetite and food intake via modulation of hypothalamic neurotransmitters. Foods with similar calorie contents and different macronutrient compositions represent different satiety responses (Johnstone, Stubbs, & Harbron, 1996). Proteins induce the highest satiety feeling while fats cause the lowest. High fat diets and diets containing high amounts of simple sugars suppress reward system, increase expression of neuropeptide Y (NPY), and decrease Pro-opiomelanocortin (POMC) in hypothalamic arcuate nucleus. Whereas, proteins induce satiety thorough increasing POMC and activating melanocortin pathway (Tulloch, Murray, Vaicekonyte, & Avena, 2015).

Type and quality of protein may determine its ability to control appetite. It has been shown that diets high in milk and dairy products could prevent weight gain (Drapeau et al., 2004). Furthermore, whey protein is more effective than casein, soy, and egg albumin in suppressing hunger and reducing food intake (Hall, Millward, Long, &

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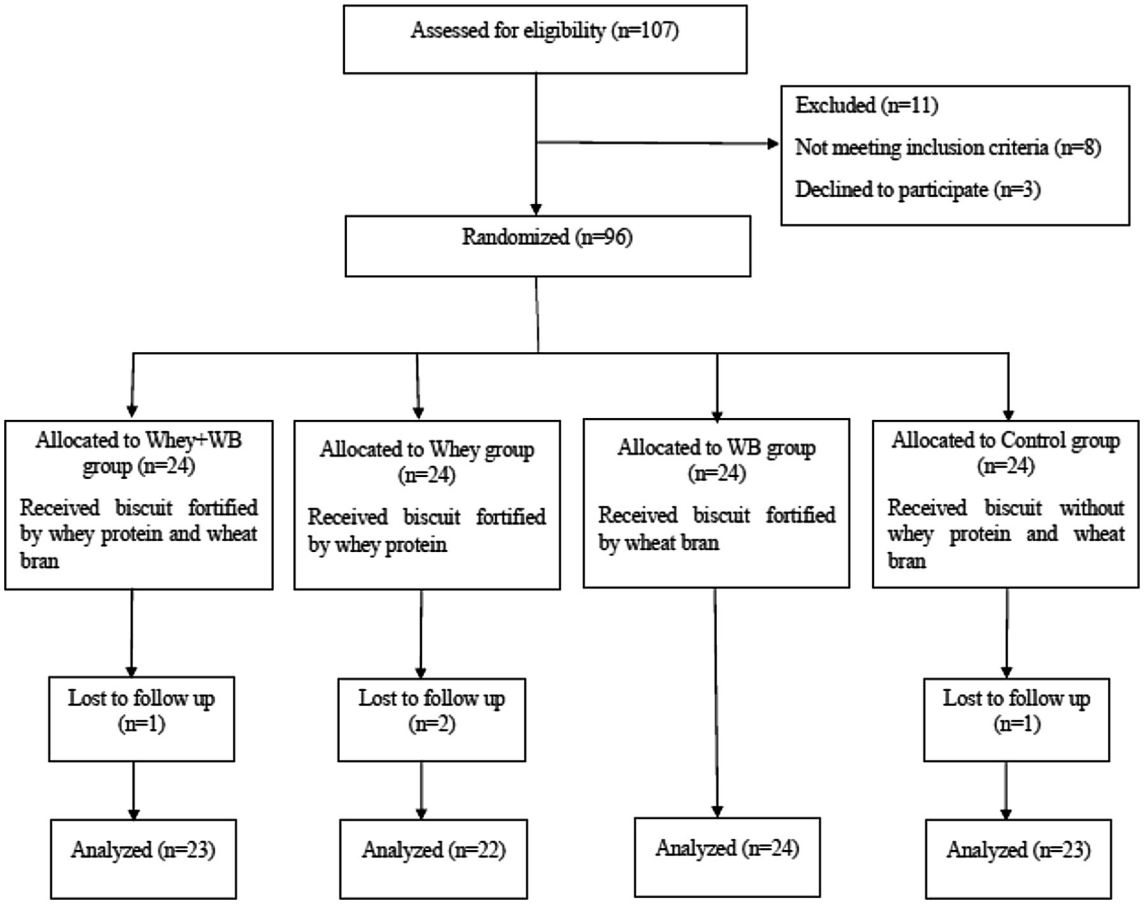

Fig. 1. Flow diagram of the study.

Morgan, 2003; Pal & Ellis, 2010; Veldhorst et al., 2009). Whey protein may regulate food intake through the following mechanisms: releasing CCK, GLP-1, and PYY, suppressing ghrelin (Luhovyy, Akhavan, & Anderson, 2007), and preserving GIP and GLP-1 (Tulipano, Sibilia, Caroli, & Cocchi, 2011).

Moreover, dietary fibers intake is inversely associated to food intake, body weight and abdominal obesity (Du et al., 2010; Koh-Banerjee et al., 2004; Liu et al., 2003). It has been proposed that dietary fibers decrease energy density of foods, need more time to be chewed, and delay gastric emptying. In addition, foods containing dietary fibers have lower glycemic index that leads to a more balanced insulineamic response. Dietary fibers also control food intake by affecting gut hormones secretion (Howarth, Saltzman, & Roberts, 2001; Slavin & Green, 2007).

The most available evidences are trials conducted to assess changes in dietary macronutrient composition (Leidy, Tang, Armstrong, Martin, & Campbell, 2011; Li, Armstrong, & Campbell, 2016). Whereas, food fortification is a practical strategy to add specific nutrients with therapeutic effects on diet, as a functional food. Regarding its satiety-induced properties, whey protein is a proper choice to be added to foods. In addition, previous studies mostly assessed acute effect of whey protein enriched foods, during one to three intervention days, and their study population were almost healthy and normal weight persons (Chungchunlam, Henare, Ganesh, & Moughan, 2015; Doyon et al., 2015; Martinelli et al., 2017). Besides, inconsistent results were found in previous trials regarding the impact of whey protein and/or dietary fiber on weight loss (Aldrich et al., 2011; Bodinham, Hitchen, Youngman, Frost, & Robertson, 2011; Pal, Radavelli-Bagatini, Hagger, & Ellis, 2014; Reimer et al., 2017; Tahavorgar, Vafa, Shidfar, Gohari, & Heydari, 2014). Thereby, this study was conducted to assess the effect of biscuit fortified with whey protein isolate and wheat bran on anthropometric parameters, body composition, energy intake, appetite score, and appetite regulating hormones among overweight and obese adults.

2. Material and methods

2.1. Study design and sampling

Our study was a single-blind, randomized controlled clinical trial (RCT) with 4 parallel groups, conducted between July and December 2018. The protocol of the study was approved by ethics committee of Shiraz University of Medical Sciences with ID number of 96-01-84-15484, and registered at IRCT with ID number of IRCT20180509039596N2. The study procedures followed the ethical standards of guidelines of declaration of Helsinki.

Based on a previous study (Mobley et al., 2017) and considering energy intake as main variable, the sample size was computed as 24 persons per group with a significant level of 0.05, power of 90%, d equal to 195, and considering a drop-out rate of 15%. Primary outcomes were energy intake, composite appetite score, body weight, body mass index (BMI), waist circumference (WC), body fat, and muscle mass. Secondary outcome were appetite regulating hormones, and blood lipids. Participants were recruited via announcements in Imam Reza clinic, Shiraz University of Medical Sciences, Shiraz, Iran. Inclusion criteria were as follows: males and females aged 20–50 years old, and BMI $\geq$ 25 kg divided by square meters (kg/m^2). Persons who had any weight loss diet during last 6 months; eating disorders; food allergies chronic disease such as diabetes, hyperlipidemia, hypertension, cancer, renal failure, hepatic failure, etc.; use of multivitamin and mineral supplements or herbal drugs; use of medications with any effect on appetite, body composition or metabolism; smokers; pregnant or

lactating women were not included the study. Experiencing any adverse effect during the intervention; protocol violation; and unwilling to continue were the study exclusion criteria.

2.2. Intervention

Protocol of the study was explained to all participants, then informed consents were singed by them. After 2 weeks of run-in period, 96 eligible participants were randomly allocated to 4 study groups, as follow: (1) received whey protein plus wheat bran (Whey + WB group); (2) received whey protein (Whey group); (3) received wheat bran (WB group); and (4) control, for a period of 8 weeks (Fig. 1). Stratified randomization was done according to age and sex, and balanced block randomization method was used in each stratum. To conduct balanced block randomization, different forms of A, B, C and D letters (e.g. ABCD, ABDC, ADBC, …) were written in different cards and put in a box. Then, a card was randomly picked up to allocate subjects of each stratum in a group, according to the A, B, C and D arrangement of the cards.

All participants were instructed to follow a 2092 kJ (~500 kcal) deficit balanced-macronutrients diets (54, 17, and 29% of energy from carbohydrate, protein and fat, respectively). We used estimated energy expenditure prediction equations recommended for overweight and obese subjects to estimate total energy expenditure for each subject, considering his/her age, sex, weight, height, and physical activity level (Mahan & Raymond, 2016). Furthermore, we asked the subjects to follow their routine physical activity and not get moderate to severe exercises.

Energy and nutrients composition of biscuits are shown in Table 1. The samples were analyzed for protein (Kjeldahl digestion), fat (Soxhlet method), crude fiber, saturated fatty acid, carbohydrate and sugar content (Fehling method) using AOAC procedures (AOAC, 1984).

Each participant received 50 g biscuit per day. The amount of whey protein in 50 g of biscuits, fortified with whey protein plus wheat bran and those fortified with only whey protein were 12.05 g. The amount of wheat bran in 50 g of biscuits, fortified with whey protein plus wheat bran and those fortified with only wheat bran were 5.47 g. Forty percentage of sugar were replaced by date syrup in all biscuits. The composition of date syrup were as follow; protein (1.43%), fat (0.005%), reducing sugar (52.91%), moisture (24.07%), and ash (2.18%) (Farahnaky, Mardani, Mesbahi, & Majzoobi, 2016). The appropriate percentage of date syrup in biscuits was obtained from a previous study (Majzoobi, Mansouri, Mesbahi, Farahnaky, & Golmakani, 2016). Whey protein isolate were provided by Karen Pharma and Food Supplement Co. Wheat bran, date syrup, and other raw materials were bought from local sellers.

Participants were blinded to treatment, thus four types of biscuits were prepared in the same size, shape, and flavor; and packed in the similar packages with identical labels. Participants were asked to eat 2 biscuits (25 g) as mid-morning snack and 2 biscuits (25 g) as mid-afternoon snack, per day. We visited participants every 2 weeks, delivered them 14 packages of biscuits for next 14 days, and monitored their compliance by counting unused biscuits. We asked the participants to notify any adverse effects by phone call or at regular visits.

2.3. Outcome measurements

Anthropometric characteristics including body weight, height, BMI, WC, and body composition were measured at baseline and after 8 weeks of intervention. Body weight was recorded to the nearest 100 g, using a scale (Seca, Germany), barefoot in light clothing. Height was measured barefoot on a flat surface, using wall mountable height rod, to the nearest 0.1 cm. WC was measured at the narrowest part of the body between the lowest rib and the iliac crest using an unstrechable tape to the nearest 0.1 cm. BMI was calculated as weight (kilogram) divided by the square of height (square metres (m^2)). Body fat mass (% and kilogram), visceral fat area (kilogram), and skeletal muscle (kilogram) were measured using bioelectrical impedance analysis (In Body s10, Korea). Participants were in normal hydration state, did not use caffeine in last 6 h, and did not have vigorous activity during last 2 h.

Physical activity level was estimated pre- and post-intervention using international physical activity questionnaire (IPAQ) and reported as metabolic equivalent hours per week (METh/W).

Participants filled a 3-day food record, including two weekdays and one weekend, at baseline and after 8 weeks of intervention. We used NUTRITIONIST IV software, modified for Iranian foods, to estimate energy and nutrients intakes.

Subjective appetite perception were measured prior to lunch using validated 100 mm visual analogue scale (VAS) including 4 questions as follow; How much do you feel hunger?, How much do you feel fullness?, How much do you desire to eat?, and How much food do you prospect you could eat?. (Flint, Raben, Blundell, & Astrup, 2000). We asked the participants to point a 100-mm horizontal lines which anchored with opposite statement at each end, considering the intensity of their feeling for each question. Then, a composite appetite score (CAS) was calculated using the following formula: CAS = [hunger + (100 − fullness) + desire to eat + prospective food consumption]/4. Higher CAS represent higher appetite feeling (Sloth et al., 2009).

After an overnight fast (8–12 h), 5 cc blood samples were collected, then serums were separated and stored at −80 °C. Biochemical parameters were measured for all participants at baseline and after 8 weeks of intervention. Fasting blood sugar (FBS) and serum blood lipids including triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) were measured by commercial kits (Pars Azmoon Inc., Tehran, Iran) and Biosystem auto analyzer bt1500 device (Blotecnica instruments,

Table 1
Nutritional composition of biscuits per serving (50 g).

	Biscuit fortified with whey protein isolate and wheat bran	Biscuit fortified with whey protein isolate	Biscuit fortified with wheat bran	Control
Energy (KJ)	940.90	971.94	921.10	952.44
Total Carbohydrate (g)	22.59	25.38	32.49	35.31
Total Carbohydrate (% of energy)	40.18	43.70	59.03	62.04
Sugar (g)	9.87	10.13	10.80	11.07
Fiber (g)	1.28	0.15	1.97	0.17
Total Protein (g)	14.96	14.80	4.28	4.12
Total Protein (% of energy)	26.60	25.48	7.77	7.23
Total Fat (g)	7.50	6.75	7.75	7.50
Total Fat (% of energy)	30.01	26.15	31.68	29.65
Saturated fatty acid (g)	2.88	2.84	2.90	2.87
Saturated fatty acid (% of energy)	1.28	1.22	1.32	1.26

Biscuit fortified with whey protein isolate and wheat bran and biscuit fortified with whey protein isolate contained 12.05 g whey protein.
Biscuit fortified with whey protein isolate and wheat bran and biscuit fortified with wheat bran contained 5.47 g wheat bran.

Italy), using standard colorimetric methods. Serum insulin level was determined by Enzyme Linked Immunosorbent Assay (ELISA) kits (infinitum biotech, IBT; Netherland). Serum ghrelin, leptin, serotonin, and GLP-1 level were measured using ELISA kit (Bioassay Technology Laboratory, BT lab; China).

2.4. Statistical analyses

We analyzed data using SPSS software (ver. 22 for Windows; SPSS Inc., Chicago, USA). Normal distribution of variables were assessed by one sample *Shapiro-Wilk* test. Quantitative data were reported as mean ± standard deviation (SD) and categorical variables were expressed as number (N) and percentage (%). We used Pearson chi-square test or Fisher's exact test to compare categorical variables among the study groups. Within group analysis was analyzed by Paired sample *t*-test and Wilcoxon signed-rank test for normal and abnormal distributed data, respectively. Between group analyses were done by one way ANOVA test, and for two by two comparisons Tukey post hoc test was used. We also used Kruskal–Wallis test for between group analysis and Mann–Whitney *U* test for post hoc comparisons adjusted with Bonferroni method, when data distributed abnormal. Furthermore, ANCOVA test was applied for adjusting some baseline values as confounders. P values lower than 0.05 were considered statistically significant in all statistical tests.

3. Results

Flow diagram of the study is shown in Fig. 1. Ninety-two subjects completed the study and 4 were dropped out due to unwilling to continue. Table 2 shows the baseline characteristics of the participants. As shown, no significant differences were seen in demographic variables, and physical activity level among the study groups.

Table 3 shows CAS parameters during intervention. CAS were the same in all study groups, at baseline (P = 0.21). After week 2, no differences were seen among the groups (P = 0.13). But after week 8, it decreased more in whey + WB group compared to WB (P = 0.03) and control (P = 0.01) groups, and decreased more in whey group compared to the control group (P = 0.03).

Table 4 represents changes in anthropometric parameters, dietary intakes, and physical activity level during intervention. Anthropometric parameters and dietary intakes were not significantly different among the participants at baseline, except for fat and fiber intakes. Weight, BMI, and WC decreased significantly in Whey group compared to WB and control groups. No significant change was seen in kilograms of muscle mass, and visceral fat among the study groups. However, percentage and kilograms of total body fat more decreased in Whey group compared to the others (P = 0.05). We found no significant change in physical activity level among study groups.

Mean differences of energy intake was significantly different among the groups (P = 0.01). Post hoc analysis showed that energy intake decreased more in Whey group compared to the control group. Moreover, grams of carbohydrate intake decreased in Whey group was more than WB and control groups. However, changes in carbohydrate intake as percentage of energy intake was not statistically significant after adjusting for baseline values of carbohydrate percentage. Although it was found that changes in grams of fat intake was different among the groups (P = 0.005), it was not statistically significant after adjusting for baseline values of fat intake. Changes in protein intake as percentage of energy intake in Whey + WB and Whey groups was more than WB and control groups (P < 0.001). Fiber intake increased more in Whey + WB and WB groups compared to Whey and control groups that was just statistically significant between WB group and both Whey and control groups (P = 0.003).

Changes in biochemical parameters of appetite regulating hormones during intervention are shown in Table 5. Appetite-regulating hormones were not significantly different among the participants at baseline. No significant differences were observed in mean differences of grehlin, leptin, and serotonin from baseline to endpoint among the study groups. However, within group analysis showed that serotonin decreased significantly only in control group (P = 0.004). Moreover, change in GLP-1 was statistically significant among the study groups, and post hoc analysis revealed that it increased more in Whey group compared to the control group. Also, within group analysis showed that insulin decreased significantly in Whey + WB (P = 0.03) and Whey

Table 2
Baseline Characteristics of the Participants.

	Whey + WB group (n = 23)	Whey group (n = 22)	WB group (n = 24)	Control (n = 23)	P value
Sex					0.98*
Male	3(13.0)†	4(18.2)	4(16.7)	4(17.4)	
Female	20(87.0)	18(81.8)	20(83.3)	19(82.6)	
Age (years)	38.26 ± 8.44‡	37.09 ± 8.07	39.04 ± 7.37	37.34 ± 6.88	0.81**
Education					0.66*
Lower than diploma	1(4.3)	1(4.5)	4(16.7)	2(8.7)	
Diploma	10(43.5)	9(40.9)	4(16.7)	8(34.8)	
Academic education (BS.)	10(43.5)	9(40.9)	12(50.0)	9(39.1)	
Academic education (MSc and higher)	2(8.7)	3(13.6)	4(16.7)	4(17.4)	
Job					0.99*
Housewife	13(56.5)	11(50.0)	12(50.0)	13(56.5)	
Unemployed	1(4.3)	1(4.5)	0(0)	1(4.3)	
Employee	7(30.4)	8(36.4)	10(41.7)	7(30.4)	
Worker	1(4.3)	1(4.5)	0(0)	1(4.3)	
Self-employed	1(4.3)	1(4.5)	2(8.3)	1(4.3)	
Intervention duration (day)	61.08 ± 8.52	58.90 ± 7.51	58.57 ± 4.57	60.60 ± 7.22	0.53**
Height (cm)	162.91 ± 7.46	163.70 ± 9.88	161.50 ± 8.01	165.65 ± 8.36	0.39**
Physical activity level (MET.h/week)	460.30 ± 742.18	635.72 ± 972.35	652.32 ± 1137.58	319.52 ± 498.47	0.93***

† Number (%).
‡ Mean ± SD.
* P value were resulted from Fisher's Exact Test.
** P value were resulted from One-way ANOVA Test.
*** P value were resulted from Kruskal Wallis Test.

Table 3
Comparison of subjective appetite score changes after study.

	Whey + WB group (n = 23)	Whey group (n = 22)	WB group (n = 24)	Control (n = 23)	P value*
CAS at baseline	6.58 ± 1.62	7.08 ± 1.51	6.47 ± 1.78	7.38 ± 1.70	0.21
CAS after weeks 2	4.26 ± 2.24	4.68 ± 1.49	5.22 ± 2.20	5.68 ± 2.12	0.13
CAS after weeks 8	3.46 ± 2.03[a]	3.58 ± 2.11[ab]	5.11 ± 2.12[bc]	5.29 ± 1.91[c]	0.003

CAS, Composite appetite score.
* P value were resulted from One-way ANOVA Test; Different superscript letters show statistical significant differences at $P < 0.05$, by using Tukey post-hoc test.

(P = 0.06) groups, but the mean differences of insulin was not different among the 4 groups (P = 0.32). We found no differences in FBS and blood lipids of all study groups during intervention, except for HDL which decreased less in Whey + WB and Whey groups compared to WB group.

4. Discussion

Our results revealed that consumption of biscuits fortified with whey protein isolate, with or without wheat bran, could reduce CAS. Consistent to our results that showed whey protein could strongly reduce CAS, other studies on participants with overweight or obesity reported that whey protein supplementation controlled appetite better than casein, soy, or placebo intake, during 12 weeks intervention (Pal et al., 2014; Reimer et al., 2017; Tahavorgar et al., 2014). In contrast, some other studies found that whey protein could not acutely control appetite. For instance, Doyon et al. concluded that yogurt with 1.5–1 proportion of casein to whey did not improve satiety, fullness, or hunger compared to 2.8–1 proportion (Doyon et al., 2015). Moreover, in another study 12.9 g whey protein plus 6 g polydextrose delivered as pudding or snack bar could not change fullness and hunger (Astbury, Taylor, French, & Macdonald, 2014; Martinelli et al., 2017). In this line, a meta-analysis showed that whey protein supplementation decreased CAS during long-term but not in short-term (Mollahosseini, Shab-Bidar, Rahimi, & Djafarian, 2017). Other than intervention duration, type of foods and their components may also affect appetite sensation. Whey protein could induce satiety via increasing POMC and decreasing NPY in hypothalamus, and affecting energy hemostasis. Besides, fast peak in plasma amino acids after whey protein ingestion; high content of branch chain amino acids (BCAAs), and tryptophan are underlying mechanisms by which whey protein could regulate appetite. In addition, whey protein could control appetite thorough secreting neurotransmitters that are metabolites of amino acids and contribute in satiety feeling such as serotonin, dopamine, and norepinephrine. Whey protein could also regulate gastrointestinal hormones (Chungchunlam et al., 2015; Luscombe-Marsh et al., 2016; Sousa et al., 2012; Ullrich et al., 2015; Zhou et al., 2011).

We found that consumption of biscuits enriched by wheat bran could not control the participants' appetite. Similarly, previous studies showed that whole-wheat bread had no further effect on satiety compared to refined bread (Bodinham et al., 2011; Gonzalez-Anton et al., 2015). In contrast, adding beta-glucan to bread, biscuit and juice acutely induced satiety (Pentikainen et al., 2014; Vitaglione et al., 2010), and long-term consumption of snack bar contained inulin with or without whey protein decreased hunger, desire to eat, and prospective food consumption (Reimer et al., 2017). However, a systematic review concluded that high fiber diets/meals with any type or dose of fibers did not reduce appetite (Clark & Slavin, 2013). In addition, other factors such as energy density, food palatability, volume, and particle size could also affect satiety, which are different in various studies (Gerstein, Woodward-Lopez, Evans, Kelsey, & Drewnowski, 2004). It seems that further studies need to determine chronic effect of wheat bran as a single fiber source on appetite and food intake.

We found that energy intake, body weight, BMI, and WC decreased more following consumption of whey protein. It has been reported that 65 g whey protein supplements decreased energy intake compared to soy protein, during 12 weeks (Tahavorgar et al., 2014), but it could not be effective at dose of 54 g, compared to casein and glucose (Pal et al., 2014) and as snack bar enriched whey protein at dose of 5 g compared to control (Reimer et al., 2017). Moreover, ad libitum energy intake at lunch was lower after consumption of snack bar enriched by 12.5 g whey protein plus 6 g polydextrose, in both first day of intervention and after 2 weeks (Astbury et al., 2014). Whey protein effectiveness may be affected by a group of factors including dose of whey protein, intervention duration, and type of whey protein intake (supplement or food fortification). Other than decreasing energy intake, whey protein could increase energy expenditure through inducing thermogenesis resulted in weight reduction (Halton & Hu, 2004). In this line, Tahavorgar et al. showed that daily intake of 65 g whey protein reduced body fat and weight (Tahavorgar et al., 2014). Our study showed that body fat decreased more in Whey group compared to the others. Consistently, Zhou et al. found that body fat reduced more following a high protein diet contained whey protein compared to soy protein that was attributed to whey protein potential to lower food intake and increase fat oxidation (Zhou et al., 2011). In addition, this study showed that grams of carbohydrate reduced more in Whey group compared to WB and control groups. Decreasing carbohydrate intake lead to lower postprandial insulin. Thus, the inhibitory effect of insulin on lipolysis in adipose tissue would be removed (Pesta & Samuel, 2014). Change in metabolic rate may be another cause of fat reduction in subjects consuming high protein diet. A study showed that high whey protein meal had higher thermic effect compared to high soy- casein- or carbohydrate-meal (Acheson et al., 2011). A few studies assessed the effect of whey protein on body weight, and none of them evaluated whey protein effect in the context of food fortification. Further studies are needed to clarify this effect.

Despite the inconsistent results regarding the impact of dietary fibers on energy intake, the most of them concluded that whole grains could not decrease energy intake (Bodinham et al., 2011; Kristensen et al., 2010), which was similar to our results. The most potent effects of dietary fibers on appetite, energy intake, and body weight are related to their physicochemical properties such as viscosity, fermentability, and solubility (Howarth et al., 2001; Slavin & Green, 2007). However, other factors such as insulin response, growth of gut microbiota, and physiological adaptation to a changed dietary pattern probably mediated the fiber effects in long-term (Wanders et al., 2011). Ways of fiber consumption such as consuming fiber from its natural sources, as an isolated fiber supplements, or as an isolated fiber in processed foods may also affect the outcomes (Wanders et al., 2011).

We found that whey protein could not enhance muscle mass compared to control, but within group analysis showed slight increase in muscle mass in 3 intervention groups, and higher increase in Whey group. In this study, participants followed a low calorie diet beside the intervention protocol and did not take exercise program, which could maintain or enhance lean body mass. Similarly, Aldrich et al reported that lean mass did not improve in subjects who followed a diet with 15% of energy from mixed protein plus 15% from whey protein, and did not increase their physical activity (Aldrich et al., 2011). Thereby, it seems that simultaneous dietary change and exercise may be more successful in retention of muscle mass (Layman et al., 2005).

Table 4
Comparison of anthropometric indices, dietary intakes, and physical activity changes after study.

	Whey + WB group (n = 23)	Whey group (n = 22)	WB group (n = 24)	Control (n = 23)	P value*
Weight (kg)					
Before	76.51 ± 12.81	84.75 ± 16.16	80.23 ± 12.34	81.58 ± 12.19	0.23
After	73.60 ± 12.03	81.02 ± 14.92	78.44 ± 12.89	79.71 ± 13.09	0.26
P value**	< 0.001	< 0.001	< 0.001	< 0.001	
Mean differences	–2.90 ± 1.57[ab]	–3.73 ± 2.63[a]	–1.79 ± 1.65[b]	–1.87 ± 1.79[b]	0.002
BMI (kg/m^2)					
Before	28.72 ± 3.81	31.49 ± 4.34	30.63 ± 3.69	29.55 ± 2.58	0.06
After	27.77 ± 3.68	30.02 ± 3.92	29.95 ± 3.87	28.99 ± 2.55	0.11
P value	< 0.001	< 0.001	< 0.001	< 0.001	
Mean differences	–0.94 ± 0.61[ab]	–1.46 ± 0.99[a]	–0.68 ± 0.68[b]	–0.56 ± 0.61[b]	< 0.001 ‡‡
Waist circumference (cm)					
Before	98.50 ± 10.20	102.63 ± 12.55	101.19 ± 7.97	102.08 ± 7.87	0.50
After	94.06 ± 9.48	96.68 ± 12.24	98.71 ± 8.18	98.73 ± 8.40	0.31
P value	< 0.001	< 0.001	0.001	< 0.001	
Mean differences	–4.43 ± 2.42[ab]	–5.95 ± 3.10[a]	–2.47 ± 3.25[b]	–3.34 ± 3.16[b]	0.001
Total body fat (% of body weight)					
Before	35.64 ± 6.74	39.55 ± 7.00	38.56 ± 6.45	36.10 ± 5.33	0.41
After	31.50 ± 6.21	34.17 ± 7.95	34.67 ± 6.16	33.37 ± 4.96	0.69
P value	< 0.001	< 0.001	0.001	0.002	
Mean differences	–4.13 ± 1.79	–5.37 ± 2.42	–3.88 ± 2.32	–2.73 ± 2.19	0.05
Total body fat (kg of body weight)					
Before	25.97 ± 8.92	30.65 ± 7.47	29.83 ± 6.50	26.94 ± 5.19	0.36
After	22.18 ± 8.17	25.60 ± 7.86	26.25 ± 6.80	24.23 ± 4.57	0.56
P value	< 0.001	< 0.001	0.001	0.001	
Mean differences	–3.79 ± 1.64	–5.05 ± 2.13	–3.57 ± 2.14	–2.70 ± 1.99	0.05
Visceral fat area (kg of body fat)					
Before	94.11 ± 30.55	108.91 ± 24.36	108.08 ± 18.05	100.79 ± 16.45	0.41
After	85.20 ± 29.61	97.58 ± 23.63	98.48 ± 18.03	93.65 ± 15.20	0.51
P value	< 0.001	0.008	0.004	0.01	
Mean differences	–8.91 ± 3.64	–11.33 ± 12.21	–9.60 ± 7.32	–7.13 ± 8.36	0.70
Muscle mass (kg of body weight)					
Before	24.60 ± 3.00	25.70 ± 5.58	26.47 ± 5.44	26.16 ± 1.91	0.76
After	25.50 ± 2.95	26.79 ± 5.43	27.43 ± 5.06	26.67 ± 1.83	0.75
P value	0.003	< 0.001	0.009	0.06	
Mean differences	0.90 ± 0.79	1.09 ± 0.76	0.95 ± 0.83	0.50 ± 0.81	0.36
Energy (kJ/day)					
Before	7417.89 ± 1582.47	7721.36 ± 2176.34	6787.24 ± 1604.77	7252.16 ± 1921.87	0.36
After	5886.76 ± 1161.68	5995.00 ± 1372.05	6184.16 ± 1178.13	6801.76 ± 1648.41	0.11
P value	< 0.001	< 0.001	0.05	0.07	
Mean differences	−1531.09 ± 1628.62[ab]	−1726.31 ± 1766.15[a]	−603.03 ± 1544.81[ab]	−450.36 ± 1137.92[b]	0.01
Carbohydrate (g/day)					
Before	260.73 ± 68.05	309.67 ± 112.95	264.65 ± 66.58	286.95 ± 92.37	0.21
After	198.10 ± 46.74[a]	207.57 ± 49.09[ab]	225.66 ± 48.77[ab]	245.74 ± 75.09[b]	0.03
P value	< 0.001	< 0.001	0.002	0.003	
Mean differences	–62.63 ± 63.09[ab]	–102.10 ± 106.86[a]	–38.99 ± 57.85[b]	–41.20 ± 59.02[b]	0.01
Carbohydrate (% of energy)					
Before	58.37 ± 7.39[a]	66.55 ± 8.57[b]	65.24 ± 5.33[b]	65.41 ± 6.19[b]	0.001
After	56.16 ± 5.36	58.03 ± 4.69	60.91 ± 3.65	59.73 ± 5.92	0.01‡
P value	0.07	< 0.001	0.001	< 0.001	
Mean differences	−2.21 ± 5.31	−8.51 ± 8.47	−4.33 ± 6.04	−5.68 ± 6.53	0.02‡
Fat (g/day)					
Before	55.76 ± 18.59[a]	43.81 ± 16.99[ab]	40.83 ± 13.98[b]	41.05 ± 9.80[b]	0.004
After	42.40 ± 9.08	39.57 ± 14.73	40.27 ± 9.31	42.66 ± 10.07	0.72
P value	0.003	0.12	0.86	0.43	
Mean differences	–13.36 ± 18.18	–4.24 ± 12.01	–0.55 ± 16.47	1.60 ± 9.63	0.005‡
Fat (% of energy)					
Before	28.53 ± 7.61[a]	21.47 ± 6.65[b]	22.78 ± 5.43[b]	22.07 ± 5.44[b]	0.001
After	27.31 ± 4.10	24.60 ± 5.28	24.52 ± 2.97	24.18 ± 5.22	0.08
P value	0.36	0.06	0.18	0.10	
Mean differences	−1.22 ± 6.04	3.13 ± 7.26	1.73 ± 6.42	2.11 ± 6.06	0.15
Protein (g/day)					
Before	63.21 ± 17.75	60.85 ± 12.75	56.70 ± 17.42	64.55 ± 13.79	0.32
After	65.38 ± 20.12	64.25 ± 14.41	55.06 ± 11.51	63.19 ± 14.68	0.08
P value	0.64	0.14	0.65	0.61	
Mean differences	2.17 ± 21.12	3.39 ± 10.25	–1.64 ± 18.31	–1.36 ± 12.86	0.65
Protein (% of energy)					
Before	14.48 ± 3.43	13.64 ± 2.83	13.94 ± 2.45	15.38 ± 3.07	0.21

(*continued on next page*)

Table 4 (*continued*)

	Whey + WB group (n = 23)	Whey group (n = 22)	WB group (n = 24)	Control (n = 23)	P value*
After	18.42 ± 3.48[a]	18.23 ± 3.10[a]	15.00 ± 2.20[b]	15.86 ± 3.30[b]	< 0.001
P value	< 0.001	< 0.001	0.05	0.54	
Mean differences	3.93 ± 3.34[a]	4.58 ± 3.36[a]	1.06 ± 2.68[b]	0.47 ± 3.66[b]	< 0.001
Fiber (g/day)					
Before	10.51 ± 3.89[a]	14.01 ± 4.36[b]	12.43 ± 4.41[ab]	14.29 ± 4.38[b]	0.01
After	12.05 ± 4.20[ab]	11.36 ± 4.24[a]	14.54 ± 3.59[b]	11.83 ± 4.25[ab]	0.03
P value	0.14	0.05	0.03	0.05	
Mean differences	1.53 ± 4.69[ab]	–2.65 ± 5.96[a]	2.10 ± 4.91[b]	–2.45 ± 5.82[a]	0.003 ‡‡
Physical activity level (MET.h/week)					
Before	460.30 ± 742.18	635.72 ± 972.35	652.32 ± 1137.58	319.52 ± 498.47	0.93
After	517.41 ± 728.04	501.95 ± 890.15	592.57 ± 1111.45	385.26 ± 571.40	0.79
P value	0.14	0.27	0.61	0.55	
Mean differences	57.10 ± 205.39	−133.77 ± 622.07	−59.75 ± 1045.30	65.73 ± 384.42	0.52

All data are presented as mean ± SD.

* P value were resulted from One-way ANOVA Test, except physical activity level that was resulted from Kruskal Wallis Test.; Different superscript letters show statistical significant differences at $P < 0.05$, by using Tukey post-hoc test.

** P value were resulted from Paired sample *t*-test, except physical activity level that was resulted from Wilcoxon Signed Ranks test.

‡ $P > 0.05$ after analysis of covariance adjusted for baseline value.

‡‡ $P < 0.05$ after analysis of covariance adjusted for baseline value.

Biochemical analysis showed some beneficial effects of both whey protein and wheat bran consumption on GLP-1, and whey protein consumption on HDL-c.

No change in ghrelin was found in this study. Opposite to our result, Astbury et al. revealed that ghrelin decreased after consumption of snack bar containing whey protein and polydextrose (Astbury et al., 2014). However, different results were reported by other studies, which assessed acute effects of whey protein (Bowen, Noakes, & Clifton, 2007; Chungchunlam et al., 2015; Doyon et al., 2015). Daily changes in ghrelin level depends on hunger and satiation status, and it increases before meal and decreases after meal (Badman & Flier, 2005), so it is complicated to compare fasting level of ghrelin in our study with postprandial ghrelin level in previous short-term studies.

This study showed that whey protein could not change serum leptin level. No significant results were also reported by some other studies (Ullrich et al., 2015; Zhou et al., 2011). Similarly, regardless of decreasing body weight and body fat, high protein diet containing whey protein did not change serum leptin level in rats (Zhou et al., 2011). In addition, it has been reported that adding whey protein to high fat diet prevented gene expression of leptin receptor in hypothalamus, however serum leptin level was still higher than low fat diet (McAllan et al., 2013). Thus, type of diet and amount of fat probably are more related to leptin than adding protein.

Although, change in serotonin level was not different among the groups, it decreased more in the control group. Alpha-lactalbumin (LAC), a main component of whey protein, has the highest ratio of tryptophan to large neutral amino acid (LNAAs) that facilitate transport of tryptophan to the brain (Layman, Lonnerdal, & Fernstrom, 2018). Tryptophan is a precursor of serotonin which could control food intake and mood. Fernstorm et al. reported that juice contained LAC increased plasma tryptophan at three fold levels, while juices contained starch, gluten, or Zein did not change or reduced plasma tryptophan (Fernstrom et al., 2013). In our study, although whey protein did not increase serotonin, prevented its reduction. Moreover, wheat bran induces a non-significant and slight increase in serotonin. It has been demonstrated that short chain fatty acids which are produced by colonic fermentation of dietary fibers bind free fatty acid receptors on enterochromaffin cells, stimulate tryptophan hydroxylase and finally enhances serotonin biosynthesis and secretion (Martin et al., 2017).

We found that GLP-1 increased within all three intervention groups which was more in Whey group compared to the control group. Consistent with our results, in 2 other studies intake of whey drinks (Bowen et al., 2007; Chungchunlam et al., 2015), and snack bars contained whey protein and polydextrose increased GLP-1 (Astbury et al., 2014). GLP-1 increases gastric emptying time and suppresses appetite by affecting central nervous system (Harrold, Dovey, Blundell, & Halford, 2012). Rapid increase in plasma BCAAs after whey consumption induce GLP-1 secretion (Chungchunlam et al., 2015). In addition, bioactive peptides protect GLP-1 thorough inhibiting DPP-4 and preventing incretins degradation (Tulipano et al., 2011).

This study showed that serum insulin was dropped after whey protein intake, decreased within Whey + WB and whey groups. The most studies revealed no changes in serum insulin following whey protein consumption and high fiber diets (Astbury et al., 2014; Doyon et al., 2015; Gonzalez-Anton et al., 2015; Santaliestra-Pasias, Garcia-Lacarte, Rico, Aguilera, & Moreno, 2015). A component of whey protein, beta-lactoglobulin, may contributes to reduce insulin (Pichon et al., 2008). On the other hand, it has been reported that insulin decreased following weight loss (Zeyda & Stulnig, 2009). A study also reported that whey protein decreased insulin that was in accordance with reducing visceral fat, which could be increase insulin sensitivity (Belobrajdic, McIntosh, & Owens, 2004). Therefore, it seems that in our study higher insulin reduction in Whey + WB and whey groups could be due to their higher weight loss.

FBS more decreased within WB and control groups in this study, and whey protein could not change FBS. Controversial results were found regarding change in FBS from previous studies, following whey protein consumption (Bowen et al., 2007; Chungchunlam et al., 2015; Bowen, Noakes, & Clifton, 2006). Whey protein may prevent hypoglycemia between meals and during night time, due to decreasing hyperinsulinemia and enhancing gluconeogenesis.

We found lower decreasing effects of whey protein on HDL-C. Besides, TC/HDL decreased just within Whey + WB group. Similar improving effects of whey protein on HDL-C were also reported by some other studies (Tahavorgar et al., 2014; Teixeira, Silva, Neves, & Santos, 2012). The mechanisms by which, whey protein improves HDL-C is unclear. It is supposed that whey protein could affect paraxonase activity and protect HDL-C from oxidation (Haraguchi, Pedrosa, Paula, Santos, & Silva, 2010). As another finding, our study showed that TG decreased more after Whey + WB, Whey, and WB intake. Beta-lactoglobulin, the major component of whey protein, and sphingolipids could decrease intestinal lipid absorption (Ohlsson et al., 2010; Sawyer & Kontopidis, 2000). In addition an *in vitro* study showed that whey protein and BCAAs down-regulated gene expression related to lipogenesis (Chen & Reimer, 2009). Dietary fibers also regulate lipid metabolism via colonic fermentation and SCFAs production, and decrease

Table 5
Comparison of biochemical parameters changes after study.

	Whey + WB group (n = 22)	Whey group (n = 22)	WB group (n = 24)	Control (n = 22)	P value*
Ghrelin					
Before	608.69 ± 491.27	483.93 ± 406.52	366.28 ± 388.55	415.32 ± 313.37	0.21
After	573.54 ± 482.39	458.63 ± 434.31	330.50 ± 378.36	315.31 ± 251.74	0.08
P value**	0.26	0.29	0.13	0.001	
Mean differences	–35.15 ± 156.40	–25.30 ± 132.44	–35.78 ± 122.21	–100.01 ± 112.97	0.15
Leptin					
Before	156.53 ± 173.64	118.11 ± 157.44	65.77 ± 111.14	78.77 ± 89.97	0.21
After	154.51 ± 170.14	118.66 ± 156.48	65.84 ± 109.54	78.85 ± 92.37	0.22
P value	0.93	0.68	0.68	0.93	
Mean differences	–2.01 ± 11.73	0.54 ± 5.23	0.06 ± 8.78	0.07 ± 23.89	0.98
Serotonin					
Before	0.99 ± 1.10	0.88 ± 1.07	0.55 ± 0.69	0.66 ± 0.61	0.76
After	0.93 ± 1.01	0.82 ± 1.04	0.58 ± 0.83	0.48 ± 0.38	0.61
P value	0.59	0.16	0.59	0.004	
Mean differences	–0.05 ± 0.22	–0.06 ± 0.18	0.03 ± 0.17	–0.17 ± 0.35	0.06
GLP-1					
Before	1.29 ± 1.25	0.99 ± 1.11	0.61 ± 0.72	0.81 ± 0.80	0.48
After	1.34 ± 1.28	1.12 ± 1.18	0.66 ± 0.73	0.78 ± 0.79	0.12
P value	0.03	0.002	0.05	0.28	
Mean differences	0.04 ± 0.11[ab]	0.13 ± 0.24[a]	0.04 ± 0.11[ab]	–0.02 ± 0.08[b]	0.008
Insulin (mIU/L)					
Before	14.15 ± 8.10	16.46 ± 9.43	13.39 ± 5.08	15.36 ± 7.99	0.56
After	11.84 ± 6.91	14.20 ± 8.93	12.99 ± 6.47	14.83 ± 6.91	0.54
P value	0.03	0.06	0.64	0.55	
Mean differences	–2.30 ± 4.86	–2.25 ± 5.34	–0.39 ± 4.06	–0.52 ± 4.11	0.32
Fasting blood sugar (mg/dl)					
Before	94.18 ± 12.92	91.68 ± 9.95	92.79 ± 9.01	97.86 ± 9.38	0.22
After	91.22 ± 13.24	91.04 ± 9.32	85.95 ± 11.76	92.22 ± 11.38	0.24
P value**	0.12	0.71	0.004	0.004	
Mean differences	–2.95 ± 8.64	–0.63 ± 8.11	–6.83 ± 10.45	–5.63 ± 8.15	0.09
Triglyceride (mg/dl)					
Before	122.27 ± 67.61	142.18 ± 66.74	127.37 ± 55.86	130.04 ± 55.13	0.74
After	105.22 ± 66.38	126.66 ± 51.71	105.00 ± 46.57	118.45 ± 39.47	0.42
P value	< 0.001	0.03	0.03	0.19	
Mean differences	–17.04 ± 18.55	–15.52 ± 31.90	–22.37 ± 49.39	–11.59 ± 40.89	0.80
Total cholesterol (mg/dl)					
Before	192.40 ± 34.94	185.59 ± 35.45	207.66 ± 33.06	195.77 ± 30.26	0.16
After	181.09 ± 38.32	176.32 ± 40.40	180.95 ± 41.20	177.36 ± 36.01	0.96
P value	0.03	0.08	< 0.001	0.001	
Mean differences	–11.31 ± 23.34	–9.26 ± 23.79	–26.70 ± 27.52	–18.40 ± 22.34	0.07
Low density lipoprotein (mg/dl)					
Before	105.09 ± 24.11	101.77 ± 29.29	115.00 ± 23.40	107.00 ± 22.96	0.32
After	100.09 ± 25.56	96.36 ± 29.90	101.62 ± 27.03	100.90 ± 24.66	0.91
P value	0.16	0.29	0.001	0.07	
Mean differences	–5.00 ± 16.43	–5.40 ± 23.48	–13.37 ± 17.29	–6.09 ± 15.10	0.35
High density lipoprotein (mg/dl)					
Before	47.27 ± 8.11	44.18 ± 9.57	52.41 ± 15.33	45.45 ± 7.39	0.05
After	46.09 ± 7.88	42.40 ± 8.42	46.41 ± 16.55	42.31 ± 9.56	0.44
P value	0.31	0.16	< 0.001	0.008	
Mean differences	–1.18 ± 5.39[a]	–1.77 ± 5.74[a]	–6.00 ± 5.91[b]	–3.13 ± 4.98[ab]	0.01 ‡
TC/HDL ratio					
Before	4.16 ± 0.96	4.29 ± 0.88	4.13 ± 0.87	4.37 ± 0.70	0.77
After	4.00 ± 0.91	4.22 ± 1.00	4.13 ± 0.97	4.28 ± 0.71	0.75
P value	0.04	0.66	0.96	0.36	
Mean differences	–0.15 ± 0.33	–0.06 ± 0.73	–0.002 ± 0.35	–0.08 ± 0.41	0.76

All data are presented as mean ± SD.
Different superscript letters show statistical significant differences at $P < 0.008$, by using Mann-Whitney *U* test adjusted with the Bonferroni method for GLP-1, and at $P < 0.05$, by using Tukey post-hoc test for high density lipoprotein.

* P value were resulted from One-way ANOVA Test; P values of leptin, grehlin, serotonin, and GLP-1 were resulted from Npar tests including Kruskal Wallis test (for between group analysis) and Wilcoxon Signed Ranks test (for within group analysis).

** P value were resulted from Paired sample *t*-test.

‡ $P = 0.05$ after analysis of covariance adjusted for baseline value.

bile acid absorption (G. Liu et al., 2014), thereby could exert hypolipidemic responses.

4.1. Strengths and limitations

To the best of our knowledge this is the first study which introduced a functional food to control appetite and food intake and be substituted for high calorie snacks in the diet of people with overweight; and assess the effects of a whey protein enriched-food in long-term. Moreover, the dosage of whey protein prescribed in our study was not higher than recommended daily allowance (RDA), whilst the most long-term studies evaluated the effects of whey protein supplements on appetite and body weight, used mega doses of whey protein that mostly exceeds RDA. However, as a limitation, we could not conduct our study on a larger sample size due to financial problems.

5. Conclusion

We concluded that adding whey protein isolate to the biscuits could control appetite, decrease energy intake, body weight, WC, and serum insulin, also increased GLP-1 and attenuate reduction of HDL-C level. However, adding wheat bran in biscuits could not suppress appetite and decrease energy intake in overweight or obese subjects.

So whey protein isolate fortified biscuits could be an appropriate choice to be substituted for the available snacks which are energy-densed and have high content of fat, sugar, and salt. Therefore, besides providing essential nutrients and adequate protein, adding this functional food to a low energy diet could control appetite, decrease food intake, and exert some health promoting effects.

Ethics statement

This study was approved by ethics committee of Shiraz University of Medical Sciences with ID number of 96-01-84-15484. The study procedures followed the ethical standards of guidelines of declaration of Helsinki. All participants were informed about the study protocol and they signed consent form before onset the study. They were free to leave the study, if they did not want to continue.

CRediT authorship contribution statement

Zahra Hassanzadeh-Rostami: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Azam Abbasi:** Conceptualization, supervision, writing - review & editing. **Shiva Faghih:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing - review & editing.

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Declaration of Competing Interest

The authors have no conflict of interest to declare.

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