

# Effect of Whey Protein Supplementation and Exercise on Body Composition and Biochemical Indices among Overweight and Obese Adults

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## Abstract

THJPH 2022; 52(1): 57-68

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Received: April 17 2020;

Revised: February 28,

August 30,

December 17, 29 2021;

Accepted: January 14 2022

This study aimed to determine the effects of whey protein supplementation (WPS) and exercise on body composition, lipid, glucose and renal function among overweight and obese individuals. A parallel-design comparison study was conducted among 80 overweight or obese adults. Subjects with similar characteristics were matched and randomly assigned to 1 of the 4 groups: control, exercise, WPS or WPS plus exercise group for 12 weeks. Two groups (1. WPS group and 2. WPS + exercise group) consumed 29g whey protein mixed in 300mL water 30 minutes before breakfast and dinner twice daily. The two groups which included exercise (1. exercise group and 2. WPS + exercise group) increased exercise 30 minutes daily, 5 days weekly. Body composition, lipids, glucose, and renal function were assessed at weeks 0, 6 and 12. Between-group mean changes in parameters after six and 12 weeks were assessed using one-way ANOVA. Time points were compared within groups using one-way repeated measures ANOVA and Duncan post hoc comparison. 62 subjects completed the study. The WPS + exercise group exhibited significantly improved body composition, blood glucose and total cholesterol at 12 weeks compared with baseline ( $p < 0.05$ ). Triglyceride, LDL cholesterol, HDL cholesterol, blood urea nitrogen, creatinine, and uric acid did not significantly differ in all groups. Supplementation using whey protein and exercise may improve body composition, plasma glucose and total cholesterol among overweight and obese adults. However, a decrease in dietary intake was observed in the intervention group that could have changed body composition. Consequently, the relationship between supplementation, exercise, dietary intake and body composition needs to be clarified in a future study.

**Keywords:** Whey protein, Obesity and body composition, Lipids, Glucose, Renal function

**What was Known**

- Whey protein contains high concentrations of beneficial essential and branched-chain amino acids
- Whey protein and exercise can promote weight loss and improve body composition

**What's New and Next**

- Whey protein twice daily for 12 weeks and exercise improved body composition, blood glucose and cholesterol
- Outcomes for whey protein with exercise, whey protein only, or exercise were similar
- Relationships between whey protein, exercise and dietary intake, or effects of whey protein with diet restriction should be clarified

## Introduction

Since 1975, the prevalence of overweight and obesity has nearly tripled and remains a continuing and serious health problem worldwide. In 2016, 1.9 billion adults were overweight and more than 650 million adults were obese<sup>1</sup>. It has been well recognized that obesity and overweight are important risk factors for many health complications. A high body mass index (BMI) is directly associated with developing metabolic syndrome, insulin resistance, hypertension, dyslipidemia, and atherosclerosis, which significantly increases the risk of diabetes mellitus, cardiovascular disease, stroke, and cancer<sup>2,3</sup>.

Obesity is the result of an imbalance between energy intake and energy expenditure over an extended period<sup>4</sup>. The treatment for overweight and obesity includes lifestyle intervention, pharmacotherapy and bariatric surgery<sup>5</sup>. Dietary modification and increased physical activity are effective for promoting weight loss<sup>4</sup>. Some studies showed that a high protein diet maintains weight and fat loss benefits long term<sup>6</sup>. Protein has the greatest effect on satiety, influences appetite control, increases dietary thermogenesis and decreases energy intake<sup>7,8</sup>.

Whey protein, one of several significant milk proteins, contains all the essential amino acids and constitutes a rich

source of branched chain amino acids (BCAA) with a high nutritional quality value<sup>9,10</sup>. The bioactive components in whey protein such as beta-lactoglobulin, alpha-lactalbumin, lactoferrin, glycomacropeptide and immunoglobulins have a beneficial impact on human health<sup>11</sup>. Several studies have found that whey protein can promote weight loss, reduce fat mass, and improve glucose level and lipid profile<sup>12-16</sup>. Furthermore, related studies demonstrated that exercise can induce energy expenditure and improve body composition especially among overweight and obese individuals<sup>17</sup>. However, the results of related studies have been inconsistent and comparative investigations between exercise and without exercise among overweight and obese participants remain limited. Thus, this study aimed to determine the effects of whey protein supplementation (WPS) with or without exercise or exercise alone on body composition, lipid, glucose, and renal function among individuals with overweight and obese status.

## Materials and Methods

**Ethical approval**

The study was approved by the Ethics Review Committee for Human Research, Faculty of Public Health, Mahidol University (COA. MUPH 2017-221), and all participants provided written informed consent.

**Participants**

Overweight and obese individuals (BMI  $\geq 23 \text{ kg/m}^2$ ) aged 18 to 60 years were recruited via advertisements in social media and via local poster exposure. 80 participants were enrolled in this study following screening by telephone and online devices, according to the inclusion criteria: not taking any medication or supplements causing weight loss, no history of serious clinical problems in the last six months, no allergy to dairy products, no cigarette smoking or alcohol consumption in the previous three months and not pregnant or lactating. Subjects were also excluded if they had major illnesses and less than 70% compliance to the treatment. The required sample size was calculated from the formula used for a clinical trial comparing two means<sup>18</sup>. In all, 15 participants per group, accounting for dropout rate, was

calculated to provide 80% study power for detecting a mean change in total cholesterol of 0.23 mg/dL (SD 0.16 mg/dL) based on a similar study by Pal et al<sup>15</sup>.

#### Study design and method

This study employed a parallel-comparison design over a 12 week period. Subjects with similar characteristics in terms of sex, age, body weight and BMI were grouped for four matched members and then each member was randomly assigned to one of the four groups: 1) control 2) exercise 3) WPS or 4) WPS plus exercise. After randomization, subjects in all groups were instructed to maintain a regular lifestyle, physical activity, and dietary intake during the study. The two groups which included exercise (exercise group, and WPS plus exercise group) were asked to increase their exercise 30 minutes/day with moderate to intense or vigorous to intense activities or walking 10,000 steps a day for five days weekly. Examples included brisk walking, jogging, dancing, bicycling, and swimming, running or playing sports. The two groups that included whey protein (WPS group, and WPS plus exercise group) were requested to consume WPS (29 g protein per sachet) mixed in 300 mL water and to drink it within 30 minutes, twice daily, before breakfast and dinner. The composition of the supplement is shown in Table 1.

**Table 1** Composition of whey protein supplement per sachet

Composition	
Ingredient (mg)	
Whey Protein Concentrate	25,000
Whey Protein Isolate	5,490
Flavour powder	2,000
Fructooligosaccharides	1,000
Calories (kcal)	190
Protein (g)	29
Fat (g)	1
Carbohydrate (g)	15
Dietary fiber (g)	2
Sugars (g)	1

Data source: Taokaenoi Food and Marketing Public Co., Ltd, Nonthaburi, Thailand

The total amount of protein (58 g daily) served in the supplement was based on similar studies which showed the effective daily dose for improving body composition and biochemical indices was 40 to 65 g whey protein<sup>14, 15, 19, 20</sup>. To ensure compliance with the study, participants in all groups were asked to monitor exercise or physical activity by recording type and duration of exercise or physical activity in a diary. Moreover, participants in the intervention group were asked to monitor daily supplement intake by marking and ticking boxes in a calendar as well as returning emptied sachets and a handbook at their appointment every three weeks. Food consumption among all participants was assessed using a 3-day dietary record, to estimate total energy and macronutrient intake.

#### Assessments

Participants were assessed every six weeks. Personal data were evaluated using a general questionnaire. Body weight and composition were measured using the Tanita SC-330. BMI was calculated accordingly. Waist circumference was measured around the body in a horizontal plane at the midpoint between the lowest rib and iliac crest, and hip circumference was measured around the widest portion of the buttocks using a standard cloth tape. Biochemical blood samples were collected after 10 to 12 hours of overnight fast by a medical laboratory technologist using a venipuncture procedure. All biochemical samples were collected and analyzed at the Health Sciences Service Unit, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand, at a standard medical technology laboratory.

#### Statistical analysis

Data analysis included data from all participants who completed the study. The results were reported as mean  $\pm$  standard error of the mean (SEM) and were assessed for normality. Participant characteristics and baseline parameters between each group were compared using one-way ANOVA. Mean changes of parameters after the 12-week intervention were assessed using one-way ANOVA. Time points were compared within groups using one-way

repeated measure ANOVA and Duncan post hoc comparison, and Shapiro-Wilks test for normality. A *p* value <0.05 was considered to be statistically significant.

## Results

### Study population

62 participants completed the study, as 18 could

not be followed up and they discontinued the study for personal reasons. The characteristics of the participants at baseline did not significantly differ in all groups (Table 2). No adverse events after consuming the beverages were observed. Body composition and biochemical indices did not significantly differ at baseline between groups.

**Table 2** Characteristics of participants completing the study at baseline

	Control (n = 15)	Exercise (n = 17)	WPS (n = 14)	WPS + exercise (n = 16)	<i>p</i>
Male (%)	7 (46.67)	7 (41.18)	5 (35.71)	7 (43.75)	0.39
Female (%)	8 (53.33)	10 (58.82)	9 (64.29)	9 (56.25)	0.09
Age (years)	33.63 ± 3.56	35.75 ± 4.10	32.38 ± 4.03	35.75 ± 3.22	0.90
Body weight (kg)	79.12 ± 4.40	74.49 ± 3.64	73.27 ± 3.83	67.88 ± 2.21	0.20
BMI (kg/m <sup>2</sup> )	29.99 ± 2.22	27.69 ± 0.83	28.40 ± 1.09	26.08 ± 0.69	0.25

WPS, whey protein supplement; BMI, body mass index; Values are presented as n (percentage) and mean ± standard error of the mean (SEM)

### Body composition

After 12 weeks, the WPS plus exercise group exhibited significantly decreased percentage body fat and fat mass, while the control group significantly increased body weight, BMI, and percentage body fat compared with baseline

(*p* < 0.05). Mean changes in body weight, BMI, and percentage fat in the control group significantly differed from the intervention groups (*p* < 0.05). No significant difference was found regarding muscle mass between weeks 0 and 12 for all groups (*p* > 0.05). Data are shown in Table 3.

**Table 3** Changes in body composition

	Mean ± SEM			Change from baseline
	Week 0	Week 6	Week 12	
<b>Body weight (kg)</b>				
Control	79.12 ± 4.40	79.65 ± 4.36	80.27 ± 4.21 <sup>d</sup>	1.15 ± 0.22 <sup>a</sup>
Exercise	74.49 ± 3.64	74.77 ± 3.64	74.40 ± 3.67	-0.09 ± 0.17 <sup>b</sup>
WPS	73.27 ± 3.83	73.71 ± 3.92	73.15 ± 3.83	-0.13 ± 0.25 <sup>b</sup>
WPS + exercise	67.88 ± 2.21	68.19 ± 2.33	67.71 ± 2.24	-0.16 ± 0.30 <sup>b</sup>
<b>BMI (kg/m<sup>2</sup>)</b>				
Control	29.99 ± 2.22	30.17 ± 2.18	30.43 ± 2.19 <sup>d</sup>	0.44 ± 0.09 <sup>a</sup>
Exercise	27.69 ± 0.83	27.81 ± 0.83	27.66 ± 0.85	-0.03 ± 0.06 <sup>b</sup>
WPS	28.40 ± 1.09	28.43 ± 1.10	28.36 ± 1.11	-0.04 ± 0.10 <sup>b</sup>
WPS + exercise	26.08 ± 0.69	26.20 ± 0.70	26.03 ± 0.64	-0.05 ± 0.10 <sup>b</sup>

**Table 3** Changes in body composition (cont.)

	Mean $\pm$ SEM			
	Week 0	Week 6	Week 12	Change from baseline
<b>Fat percentage</b>				
Control	32.01 $\pm$ 3.94	31.80 $\pm$ 3.95	32.38 $\pm$ 3.98 <sup>d,e</sup>	0.36 $\pm$ 0.10 <sup>a</sup>
Exercise	32.81 $\pm$ 3.08	32.42 $\pm$ 3.31	32.61 $\pm$ 3.15	-0.20 $\pm$ 0.17 <sup>b</sup>
WPS	34.48 $\pm$ 3.33	34.28 $\pm$ 3.29	34.34 $\pm$ 3.31	-0.07 $\pm$ 0.11 <sup>b</sup>
WPS + exercise	34.59 $\pm$ 1.96	34.36 $\pm$ 1.90	34.20 $\pm$ 1.92 <sup>d</sup>	-0.39 $\pm$ 0.14 <sup>b</sup>
<b>Fat mass (kg)</b>				
Control	27.21 $\pm$ 3.49	27.00 $\pm$ 3.47	27.66 $\pm$ 3.60	0.45 $\pm$ 0.16 <sup>a</sup>
Exercise	26.93 $\pm$ 3.06	26.55 $\pm$ 3.13	26.64 $\pm$ 2.97	-0.29 $\pm$ 0.26 <sup>b</sup>
WPS	24.33 $\pm$ 2.42	24.14 $\pm$ 2.31	24.29 $\pm$ 2.40	-0.04 $\pm$ 0.16 <sup>a,b</sup>
WPS + exercise	23.46 $\pm$ 1.79	23.41 $\pm$ 1.78	23.09 $\pm$ 1.73 <sup>e</sup>	-0.38 $\pm$ 0.22 <sup>b</sup>
<b>Muscle mass (kg)</b>				
Control	45.50 $\pm$ 2.17	45.55 $\pm$ 2.29	45.39 $\pm$ 2.21	-0.11 $\pm$ 0.12 <sup>a</sup>
Exercise	50.26 $\pm$ 3.57	50.26 $\pm$ 3.63	50.26 $\pm$ 3.58	0.00 $\pm$ 0.07 <sup>a,b</sup>
WPS	41.27 $\pm$ 2.12	41.35 $\pm$ 2.22	41.26 $\pm$ 2.10	-0.01 $\pm$ 0.07 <sup>a,b</sup>
WPS + exercise	46.56 $\pm$ 3.06	46.92 $\pm$ 3.21	46.83 $\pm$ 3.08	0.26 $\pm$ 0.15 <sup>b</sup>
<b>Waist circumference (cm)</b>				
Control	98.31 $\pm$ 4.18	97.69 $\pm$ 3.83	99.19 $\pm$ 4.27 <sup>d</sup>	0.88 $\pm$ 0.23 <sup>a</sup>
Exercise	89.81 $\pm$ 3.63	89.50 $\pm$ 3.75	89.38 $\pm$ 3.75	-0.44 $\pm$ 0.41 <sup>b</sup>
WPS	89.94 $\pm$ 2.87	88.63 $\pm$ 3.23	89.40 $\pm$ 2.99	-0.54 $\pm$ 0.35 <sup>b,c</sup>
WPS + exercise	92.00 $\pm$ 4.32	91.56 $\pm$ 4.38	90.44 $\pm$ 4.52 <sup>d</sup>	-1.56 $\pm$ 0.43 <sup>c</sup>
<b>Hip circumference (cm)</b>				
Control	110.25 $\pm$ 2.82	109.75 $\pm$ 3.01	110.69 $\pm$ 2.84	0.44 $\pm$ 0.42 <sup>a</sup>
Exercise	106.75 $\pm$ 2.44	106.19 $\pm$ 2.55	106.50 $\pm$ 2.49	-0.25 $\pm$ 0.65 <sup>a,b</sup>
WPS	110.00 $\pm$ 4.90	108.75 $\pm$ 4.89	108.69 $\pm$ 4.89	-1.31 $\pm$ 0.55 <sup>b</sup>
WPS + exercise	105.50 $\pm$ 3.11	104.00 $\pm$ 2.90	104.19 $\pm$ 2.91	-1.31 $\pm$ 0.45 <sup>b</sup>

Control n = 15, Exercise n = 17, WPS n = 14, WPS plus exercise n = 16; WPS, whey protein supplementation; BMI, body mass index; <sup>a,b,c</sup> Mean values with unlike superscript letters were significantly different between groups at 12 weeks ( $p<0.05$ ); <sup>d</sup> Mean values were significantly different within group (compared to week 0,  $p<0.05$ ); <sup>e</sup> Mean values were significantly different within group (compared to week 6,  $p<0.05$ )

**Anthropometry**

The WPS plus exercise group significantly decreased waist circumference from baseline, while the control group significantly increased waist circumference at 12 weeks ( $p < 0.05$ ). Mean changes in waist circumference did not differ in the three intervention groups, but exhibited a significant difference from control groups ( $p < 0.05$ ). No decreased hip circumference was observed at week 12 compared with week 0 for all groups (Table 3).

**Glucose**

A significant decrease in fasting blood glucose was observed in the WPS plus exercise group at 12 weeks compared with baseline ( $p < 0.05$ ). At the end of the study, three intervention groups exhibited a significant difference in the mean change in blood glucose from the control group (Table 4).

**Table 4** Changes in biochemical parameters

	Mean $\pm$ SEM			
	Week 0	Week 6	Week 12	Change from baseline
<b>Glucose (mg/dL)</b>				
Control	89.38 $\pm$ 2.58	90.50 $\pm$ 2.17	92.63 $\pm$ 2.56 <sup>d</sup>	3.25 $\pm$ 1.01 <sup>a</sup>
Exercise	91.50 $\pm$ 2.92	94.50 $\pm$ 2.60	90.38 $\pm$ 2.94	-1.13 $\pm$ 0.61 <sup>b</sup>
WPS	90.75 $\pm$ 3.05	92.88 $\pm$ 2.92	87.13 $\pm$ 2.25	-3.63 $\pm$ 1.87 <sup>b</sup>
WPS plus exercise	92.75 $\pm$ 2.05	91.63 $\pm$ 2.24	89.88 $\pm$ 2.11 <sup>d</sup>	-2.88 $\pm$ 0.61 <sup>b</sup>
<b>Total cholesterol (mg/dL)</b>				
Control	197.75 $\pm$ 9.56	211.75 $\pm$ 13.59	205.63 $\pm$ 8.63 <sup>d</sup>	7.88 $\pm$ 3.39 <sup>a</sup>
Exercise	220.63 $\pm$ 15.49	217.00 $\pm$ 11.28	216.75 $\pm$ 12.64	-3.88 $\pm$ 3.47 <sup>b</sup>
WPS	197.00 $\pm$ 12.86	201.50 $\pm$ 11.24	197.25 $\pm$ 12.50	0.25 $\pm$ 4.11 <sup>a,b</sup>
WPS plus exercise	193.88 $\pm$ 15.86	188.38 $\pm$ 15.79	186.63 $\pm$ 18.02 <sup>d</sup>	-7.25 $\pm$ 3.51 <sup>b</sup>
<b>Triglyceride (mg/dL)</b>				
Control	122.13 $\pm$ 24.36	122.75 $\pm$ 26.99	122.50 $\pm$ 25.38	0.38 $\pm$ 4.80 <sup>a</sup>
Exercise	88.75 $\pm$ 12.73	93.88 $\pm$ 15.93	82.75 $\pm$ 10.57	-6.00 $\pm$ 4.41 <sup>a</sup>
WPS	106.88 $\pm$ 19.36	108.38 $\pm$ 12.62	91.50 $\pm$ 14.78	-15.38 $\pm$ 8.17 <sup>a</sup>
WPS plus exercise	106.63 $\pm$ 14.58	110.75 $\pm$ 15.11	95.13 $\pm$ 14.63	-11.50 $\pm$ 4.91 <sup>a</sup>
<b>LDL cholesterol (mg/dL)</b>				
Control	132.25 $\pm$ 7.07	145.50 $\pm$ 10.54	133.13 $\pm$ 6.87	0.88 $\pm$ 1.95 <sup>a</sup>
Exercise	142.13 $\pm$ 14.44	142.50 $\pm$ 14.43	141.38 $\pm$ 15.84	-0.75 $\pm$ 2.87 <sup>a</sup>
WPS	121.00 $\pm$ 7.96	127.00 $\pm$ 11.24	119.63 $\pm$ 7.76	-1.38 $\pm$ 3.10 <sup>a</sup>
WPS plus exercise	134.00 $\pm$ 10.19	137.63 $\pm$ 9.54	131.38 $\pm$ 9.99	-2.63 $\pm$ 2.49 <sup>a</sup>

**Table 4** Changes in biochemical parameters (cont.)

	Mean $\pm$ SEM			
	Week 0	Week 6	Week 12	Change from baseline
<b>HDL cholesterol (mg/dL)</b>				
Control	48.88 $\pm$ 3.31	52.63 $\pm$ 3.01	50.88 $\pm$ 3.18	2.00 $\pm$ 1.27 <sup>a</sup>
Exercise	43.63 $\pm$ 4.42	44.88 $\pm$ 4.66	46.88 $\pm$ 4.49	3.25 $\pm$ 0.82 <sup>a</sup>
WPS	41.63 $\pm$ 2.27	44.88 $\pm$ 2.79	46.25 $\pm$ 1.86	4.63 $\pm$ 0.82 <sup>a</sup>
WPS plus exercise	48.63 $\pm$ 2.46	51.00 $\pm$ 2.72	52.25 $\pm$ 2.91	3.63 $\pm$ 0.79 <sup>a</sup>
<b>BUN (mg/dL)</b>				
Control	9.75 $\pm$ 0.88	10.00 $\pm$ 0.73	9.88 $\pm$ 0.69	0.13 $\pm$ 0.40 <sup>a</sup>
Exercise	12.38 $\pm$ 0.78	11.00 $\pm$ 0.71	12.25 $\pm$ 0.65	-0.13 $\pm$ 0.35 <sup>a</sup>
WPS	10.38 $\pm$ 0.86	12.38 $\pm$ 1.05 <sup>d</sup>	11.13 $\pm$ 0.74	0.75 $\pm$ 0.62 <sup>a</sup>
WPS plus exercise	10.63 $\pm$ 0.92	14.00 $\pm$ 1.21 <sup>d</sup>	10.75 $\pm$ 1.10 <sup>e</sup>	0.13 $\pm$ 0.40 <sup>a</sup>
<b>Creatinine (mg/dL)</b>				
Control	0.69 $\pm$ 0.08	0.71 $\pm$ 0.08	0.70 $\pm$ 0.07	0.01 $\pm$ 0.03 <sup>a</sup>
Exercise	0.80 $\pm$ 0.06	0.75 $\pm$ 0.04	0.83 $\pm$ 0.05 <sup>e</sup>	0.02 $\pm$ 0.02 <sup>a</sup>
WPS	0.72 $\pm$ 0.05	0.70 $\pm$ 0.06	0.73 $\pm$ 0.07	0.01 $\pm$ 0.02 <sup>a</sup>
WPS plus exercise	0.78 $\pm$ 0.04	0.80 $\pm$ 0.05	0.82 $\pm$ 0.05	0.04 $\pm$ 0.02 <sup>a</sup>
<b>Uric acid (mg/dL)</b>				
Control	5.75 $\pm$ 0.64	6.00 $\pm$ 0.66	6.04 $\pm$ 0.58	0.29 $\pm$ 0.19 <sup>a</sup>
Exercise	7.36 $\pm$ 0.74	7.45 $\pm$ 0.69	7.41 $\pm$ 0.75	0.05 $\pm$ 0.15 <sup>a</sup>
WPS	5.99 $\pm$ 0.30	5.61 $\pm$ 0.28	6.01 $\pm$ 0.39	0.02 $\pm$ 0.20 <sup>a</sup>
WPS plus exercise	6.65 $\pm$ 0.46	6.34 $\pm$ 0.52	6.64 $\pm$ 0.55	-0.01 $\pm$ 0.19 <sup>a</sup>

Control n = 15, Exercise n = 17, WPS n = 14, WPS plus exercise n = 16; WPS, whey protein supplementation; LDL, low density lipoprotein; HDL, high density lipoprotein; BUN, blood urea nitrogen; <sup>a,b,c</sup>Mean values with unlike superscript letters were significantly different between groups at 12 weeks ( $p < 0.05$ ); <sup>d</sup>Mean values were significantly different within group (compared to week 0,  $p < 0.05$ ); <sup>e</sup>Mean values were significantly different within group (compared to week 6,  $p < 0.05$ )

#### Lipids

At 12 weeks, mean change in total cholesterol in the WPS plus exercise group was significantly decreased and significantly differed from the control group ( $p < 0.05$ ). Mean change in triglyceride, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) did not significantly differ between all groups at 12 weeks and

demonstrated no significant difference within groups from baseline ( $p > 0.05$ ) (Table 4).

#### Renal function

Mean change in blood urea nitrogen (BUN), creatinine, and uric acid did not significantly differ between all groups at 12 weeks and no significant difference was observed within groups from baseline ( $p > 0.05$ ) (Table 4).

#### Total energy intake

Based on the 3-day dietary record, at the end of the study, the exercise group showed significantly decreased total energy and carbohydrate intake when compared with week 6 ( $p < 0.05$ ). The WPS group showed significantly decreased total energy, carbohydrate, fat and protein intake when compared with week 0 ( $p < 0.05$ ). The WPS plus exercise group exhibited significantly decreased total energy intake when compared with weeks 0 and 6 and decreased carbohydrate and fat intake when compared with baseline ( $p < 0.05$ ). There was no significant change in energy and macronutrient intake in the control group throughout the study. When comparing mean change in total energy and macronutrient intake, no significant differences were found between the three intervention groups ( $p > 0.05$ ) (data not shown).

## Discussion

The WPS and exercise groups significantly improved body composition, waist circumference, and blood glucose and total cholesterol concentrations. No significant differences were found between the WPS, exercise, and WPS plus exercise groups regarding body weight and body fat mass at the end of study, but all differed from the control group. The effect of physical activity or exercise is well known to improve body composition by promoting a negative energy balance and increasing energy expenditure<sup>21</sup>. Furthermore, whey protein increases total protein in the diet, which might affect postprandial satiety and thermogenesis<sup>7, 22</sup>.

Whey protein, a dairy industry byproduct, offers high biological value and contains all essential amino acids<sup>13</sup>. Most studies have suggested that whey protein has a greater effect in suppressing appetite, enhancing satiety and reducing energy intake compared with casein or dietary carbohydrate or fat, which could improve body composition and weight loss<sup>8, 23-25</sup>. Amino acids from whey protein may reduce food intake by increasing the release of anorectic hormones such as cholecystokinin (CCK), peptide tyrosine tyrosine (PYY), glucagon-like peptide-1 (GLP-1) and leptin, and by decreasing the release of orexigenic hormones such as ghrelin and neuropeptide Y10. Two recent meta-analyses

of randomized controlled trials by Mollahosseini et al. and Miller et al. indicated that whey protein consumption can reduce appetite and lead to improving body composition parameters<sup>26, 27</sup>. Moreover, Baer et al. found that overweight and obese individuals, after consuming WPS for six months, exhibited lower body weight, body fat mass, and waist circumference when compared with similar individuals consuming carbohydrates<sup>19</sup>. In contrast, Pal et al. demonstrated that overweight and obese individuals, after consuming WPS for 12 weeks, showed no significant change in body composition compared with control or casein groups<sup>15</sup>. The appetite-suppressing and satiety-increasing mechanism of whey protein may result in significantly decreased energy, carbohydrate and fat intakes. All participants recorded their food consumption using a 3-day dietary record. The result showed WPS, exercise, and WPS plus exercise groups significantly decreased dietary intake over the 12 weeks, while the control group revealed no significant change in total energy and macronutrient intake at the end of the study. However, the possibility that body composition was improved by mechanisms of whey protein, exercise or decreasing dietary intake needs to be clarified in a future study.

Muscle damage can be induced by high intensity exercises or long duration exercises and BCAA have a beneficial effect to reduce muscle damage and encourage muscle recovery<sup>28, 29</sup>. Whey protein, and its high proportion of BCAA, especially leucine, can be quickly digested and absorbed rapidly, thereby leading to increased levels of plasma amino acid and effectively stimulating muscle protein synthesis and suppressing exercise-associated muscle breakdown<sup>16, 27</sup>. The 12-week intervention by Tahavorgar et al. demonstrated that consuming WPS significantly increased lean mass when compared with soy protein among overweight and obese men<sup>24</sup>. Although Hulmi et al. found that WPS after exercise did not affect muscle size or strength compared with carbohydrates in healthy subjects<sup>30</sup>, they also concluded that whey protein preserves muscle mass during exercise and it proved essential for muscle recovery after damage among overweight and obese individuals.

Additionally, whey protein produces an insulinotropic and glucose-lowering effect through increased incretin and insulin secretion<sup>12,31</sup>. It seems to generate these effects via bioactive peptides and amino acids in whey protein that stimulate the release of several gut hormones such as CCK, PYY, GLP-1 and insulin secretion from  $\beta$ -cells to control food intake and glucose metabolism<sup>32</sup>. Related studies demonstrated that whey protein decreased postprandial plasma glucose and glycosylated hemoglobin (HbA1C) at 12 weeks among subjects with type 2 diabetes<sup>33</sup> and reduced glycemic symptoms after a test meal among young females<sup>22</sup>. Similarly, Tahavorgar et al. reported a stronger effect of whey protein on fasting blood sugar in free-living overweight and obese men<sup>14</sup> and the study by Pal et al., which involved supplementing with whey protein, reported improved insulin levels among overweight and obese individuals for 12 weeks<sup>15</sup>.

WPS could have potential effects on blood lipid profiles, although the findings remain inconsistent. The meta-analyses results of 13 trials by Zhang et al. showed that WPS had a significant effect in reducing triglyceride (TG) level but the study did not demonstrate any lipid lowering effect on LDL-C, HDL-C, and total cholesterol (TC)<sup>34</sup>. Fluegel et al. observed that consuming whey protein beverage significantly decreased both TC and LDL-C concentrations but no change was observed over six weeks in TG and HDL-C levels among young men and women<sup>35</sup>. Similarly, a randomized control trial by Pal et al. indicated that whey protein decreased TC and LDL-C among overweight and obese individuals when compared with casein and control groups over 12 weeks<sup>15</sup>. In addition, Tahavorgar et al. suggested that free-living overweight and obese men, after consuming WPS for 12 weeks, displayed a greater decrease in TC, TG and LDL-C and increase HDL-C<sup>14</sup>. Nonetheless, the present study found that TC was significantly decreased in the WPS plus exercise group, despite TG, LDL-C and HDL-C remaining unchanged. The possible mechanism of whey protein on the lipid-lowering effect may result from reduced cholesterol

synthesis in the liver by inhibiting the effect of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity<sup>12</sup>. Other mechanisms may include beta-lactoglobulin inhibiting gene expression involved in both absorption and synthesis of cholesterol<sup>14</sup>. Moreover, the ingredients of WPS in this study included fructooligosaccharides and dietary fiber that exhibit prebiotic properties, and are considered to produce prebiotic-lowering effects on lipid profile<sup>36</sup>. Also, decreased TC seemed to occur in both exercise groups that may be explained by evidence suggesting that exercise can promote the transport of cholesterol from the bloodstream to the liver, so it then will be removed from the body by the gallbladder<sup>37</sup>.

In this study, free-living overweight and obese adults consumed whey beverages containing 29g of protein twice daily for 12 weeks. No serious adverse effects were observed regarding WPS, revealing no significant change in BUN, creatinine, and uric acid levels.

Nonetheless, the results of this study showed no significant difference in participants consuming WPS with exercise, WPS only, or exercise only. However, combining the supplement with exercise tended to present more positive effects. Consequently, the period of intervention in a future study needs to be extended to investigate any significant difference between groups. Furthermore, following up the effects of the intervention four to six weeks after the end of the study would also be interesting to evaluate any long-term effect.

The strengths of this study included the large number of participants and the study design that divided subjects among four parallel groups. However, the limitation of this study was reporting the effects of consuming whey protein among free-living overweight and obese subjects without energy restriction and exercise programs. The present study monitored the physical activity or exercises in all groups and found that the control group had no changes in physical activity.

## Conclusion

The results of the study show that consuming WPS and doing exercise for 12 weeks may improve body composition, and blood glucose and total cholesterol concentrations among overweight and obese individuals. Our findings may be used as an intervention for managing the chronic disease or dietary plans involving functional food to promote health or loss of weight. However, future studies should clarify the relationship between WPS, exercise and dietary intake or investigate the effects of WPS with dietary restriction. Moreover, future research should investigate the positive effects of WPS including among specific types of patients such as those with diabetes, hypertension, cancers or infectious diseases.

## Author Contributions

All authors conceived and designed the study, contributed to the experiments, analysed the data, and wrote the manuscript. All authors discussed the results and approved the final version of the manuscript.

## Acknowledgements

The authors would like to thank all volunteers for their participation and Taokaenoi Food and Marketing Public Company limited for providing financial support for this study. The sponsor defined the objectives of the study, but played no role in analyzing or interpreting data or in composing the manuscript.

## Source of Funding

This study was supported by grants from Taokaenoi Food and Marketing Public Co., Ltd. All researchers independently collected, analyzed, and interpreted the results from this study and have no financial interests concerning the outcome of this investigation.

## Conflicts of Interest

The authors have no conflicts of interest.

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