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Whey protein positively alters inflammatory markers and metabolic parameters of overweight and obese adults

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ABSTRACT

Introduction: The effects of prolonged consumption of whey protein on health are controversial. This study aimed to determine whether whey protein positively alters health parameters of overweight and obese adults. **Methods:** Randomised controlled trial was conducted. Fifty-eight participants, aged 30-50 years, were randomly allocated into four groups and supplemented with 50 g protein for eight weeks (group 1: plant-based protein (PBP), group 2: whey protein isolate (WPI) with cocoa powder, group 3: PBP with whey protein concentrate (WPC), and group 4: WPI with milk powder). Body composition and biochemical parameters (kidney and liver functions, inflammation, oxidative stress, and antioxidant capacity) were evaluated at pre-intervention and 8 weeks after intervention. **Results:** At Week 8, group 3 had lower diastolic blood pressure, waist circumference, visceral fat, and risk of insulin resistance ($p<0.05$ for all). Group 2 had decreased levels of total cholesterol and low-density lipoprotein cholesterol ($p<0.05$ for all). A drop in triglyceride was seen in group 4 ($p=0.026$). Whey protein decreased alanine aminotransferase level ($p=0.028$), while PBP increased aspartate aminotransferase level ($p=0.034$). PBP or WPI with milk powder increased blood urea nitrogen level ($p>0.05$ for all). Interleukin-6 and lactoferrin levels fell in all groups ($p<0.05$), while hs-CRP increased in the PBP group ($p=0.043$). Group 2 experienced increased antioxidant capacity. However, levels of oxidative stress markers were significantly decreased in the PBP group and WPI with milk powder group. **Conclusion:** Whey protein revealed positive effects on anthropometric parameters and biochemical markers of overweight and obese adults. Therefore, proper supplementation of whey protein can potentially promote health.

Keywords: inflammatory marker, obesity, overweight, plant-based protein, whey protein

INTRODUCTION

Overweight and obesity is a substantial public health problem, and its global prevalence has continually increased (World Obesity Federation, 2021). The latest Thai national survey reported that the prevalence of obesity among those aged over 15 years was 42.0%

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in females and 33.0% in males, and these figures will likely increase every year (Aekplakorn & Thai National Health Examination Survey Office, 2016). Obesity is reportedly associated with inflammation. Accumulation of fat cells in obesity can stimulate the secretion of acute phase reactants and pro-inflammatory cytokines, such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α), consequently triggering oxidative stress and increasing the risk of non-communicable diseases (de Heredia, Gómez-Martínez & Marcos, 2012; Brimelow *et al.*, 2017).

Proper dietary intake plays a crucial role in the prevention and reduction of the severity of obesity and its related diseases. Presently, the role of functional food in the prevention and mitigation of chronic diseases has been widely studied (Pal & Radavelli-Bagatini, 2013). Whey protein is rich in branched-chain amino acids, which are essential for building muscle, reducing muscle injury and muscle fatigue (Witard *et al.*, 2014; Jackman *et al.*, 2017; Shimomura *et al.*, 2010). It has also been found to improve antioxidant capacity and reduce oxidative stress in the body (Zhenyukh *et al.*, 2017). A previous study revealed that consumption of 0.5 g whey protein/kg body weight/day for 16 weeks, versus no whey protein, decreased the body weight and fat mass of obese individuals who had gastric surgery. However, levels of blood glucose and inflammatory indicators, such as IL-6 and adiponectin, did not change (Gomes *et al.*, 2017). Likewise, fat mass and uric acid concentration in diabetes and pre-diabetes patients were remarkably reduced after intake of whey protein, while blood pressure, inflammatory markers, antioxidant capacity, and oxidative stress were not altered (Flaim *et al.*, 2017). Additionally, a recent study has found that consumption of whey protein is associated with the secretion of satiety hormones (Chungchunlam *et al.*, 2015).

However, based on the outcomes of previous studies, the efficacy of whey protein on the health of individuals who are at risk of obesity and non-communicable diseases is not entirely clear. Therefore, this study aimed to evaluate the effect of whey protein on the clinical health of adults. We specifically investigated whether consumption of whey protein resulted in alterations of body composition and biochemical parameters, including blood sugar, lipid profiles, liver and kidney functions, inflammation, antioxidant capacity, and oxidative stress.

MATERIALS AND METHODS

Study subjects

Sixty overweight and obese Thai adults [body mass index (BMI) 23-30 kg/m^2, classified by the Steering Committee of the Regional Office for the Western Pacific Region of WHO, 2000], aged 30-50 years old, were enrolled into this single-blind randomised controlled trial. Exclusion criteria included having chronic diseases, any infection or inflammation six months prior to the study, currently taking medication or nutritional supplements, smoking, regularly drinking alcohol, pregnant or lactating. The subjects were asked to complete an online screening questionnaire and present their annual medical check-up report before participating in the study. Study participants were informed of the risks, discomforts, and benefits associated with the study before providing their signed informed consent. The study procedure was approved by the Ethics Committee of the Faculty of Public Health, Mahidol University, Thailand (Certificate of Approval No. MUPH 2020-215) and was registered with the Thai Clinical Trials Registry (Registration

number TCTR20210721004). Stratified and block randomisation was utilised to allocate the study subjects into four groups.

Supplement characteristics and study intervention

Each study group received different supplements contained in an aluminium foil sachet. Group 1 received plant-based protein (PBP), group 2 received whey protein isolate (WPI) with cocoa powder, group 3 received PBP with whey protein concentrate (WPC), and group 4 received WPI with milk powder. The PBP supplement mainly contained 80.6% isolated soy protein, 10.0% isolated wheat protein, and 7.5% isolated pea protein; the WPI with cocoa powder supplement consisted of 84.9% WPI, 8.5% cocoa powder, and 4.8% mixed amino acids. The PBP with WPC supplement mainly contained 38.9% isolated soy protein, 16.7% fish collagen peptide, 5.6% WPC, and 5.6% malt extract powder. The main composition of the WPI with milk powder supplement was 82.4% WPI, 8.2% milk powder, and 2.7% premixed vitamins and amino acids. The total protein content of the supplements given to the participants was 50 g/day. All participants were asked to continually consume the received supplement for eight weeks.

Study parameters assessment

Dietary intake was recorded three times a week (two weekdays and one weekend) using a food record. To monitor the intakes of study supplements and diet, subjects were asked to take photographs of their food items before and after intake. Additionally, trained staffs randomly called the subjects once a week to inquire about their dietary intake. Energy and macronutrient intakes were estimated by the NutriSurvey programme (Copyright© 2007, SEAMEO TROPMED RCCN-University of Indonesia, Indonesia). Participants underwent anthropometric assessment and biochemical evaluation at pre-intervention, and after the 8-week intervention. Body weight, body mass index (BMI), body fat mass, percentage visceral fat, and muscle mass were assessed by a body composition analyser (DC-360, Tanita Corporation, Japan). Waist circumference (WC) was measured at the umbilical level.

Participants were requested to fast at least 12 hours before blood sampling. A Cobas® 6000 analyser (Roche Diagnostics Ltd., Switzerland) was utilised to evaluate levels of fasting blood glucose (FBG), lipid profiles (total cholesterol, high-density lipoprotein cholesterol: HDL-C, low-density lipoprotein cholesterol: LDL-C, triglyceride: TG), and kidney and liver function markers (aspartate aminotransferase: AST, alanine aminotransferase: ALT, blood urea nitrogen: BUN, creatinine, uric acid). Fasting insulin was examined using a human insulin ELISA kit (ab200011, Abcam, Cambridge, UK). To determine insulin resistance, the homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR = [Fasting insulin (μIU/mL) × FBG (mmol/L)] / 22.5. High-sensitivity CRP (hs-CRP) concentration was determined by the nephelometry method. Concentrations of lactoferrin, IL-6, and TNF-α were measured using the enzyme-linked immunosorbent assay technique. Antioxidant capacity was evaluated by using an oxygen radical absorbance capacity (ORAC) assay kit (ab233473, Abcam, Cambridge, UK). To determine oxidative stress, a lipid peroxidation assay kit (ab118970, Abcam, Cambridge, UK) was used.

Statistical analysis

Sample size was calculated using G*power programme. To detect the

difference of -1.4±0.9 kg in body fat and -1.7±1.5 cm in waist circumference, with 80% power and α=0.05, the minimum number of participants in each study group was 10. The calculated sample size was increased by 20% to prevent missing data, subject withdrawals, etc. Statistical analysis was performed using Statistical Package for Social Science version 18 (SPSS, SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) and Tukey's post-hoc test was conducted to determine differences among the four study groups. The differences between pre- and post-intervention within each study group were evaluated using paired-sample *t*-test. Data were presented as mean ± standard deviation (SD). A $p<0.05$ was considered to be statistically significant.

RESULTS

Baseline characteristics of study subjects

Seventy overweight and obese adults were screened according to the inclusion and exclusion criteria, of which 60 participants were recruited into the study and allocated into four study groups. During the study, two participants declined to participate due to personal reasons. Thus, a total of 58 screened participants completed the 8-week intervention (97% retention rate) (Figure 1). The remaining participants at the end of the study were 93% for group 1 and group 3, and 100% for group 2 and group 4. Group 1 consisted of 14 participants (7 males and 7 females), group 2 consisted of 15 participants

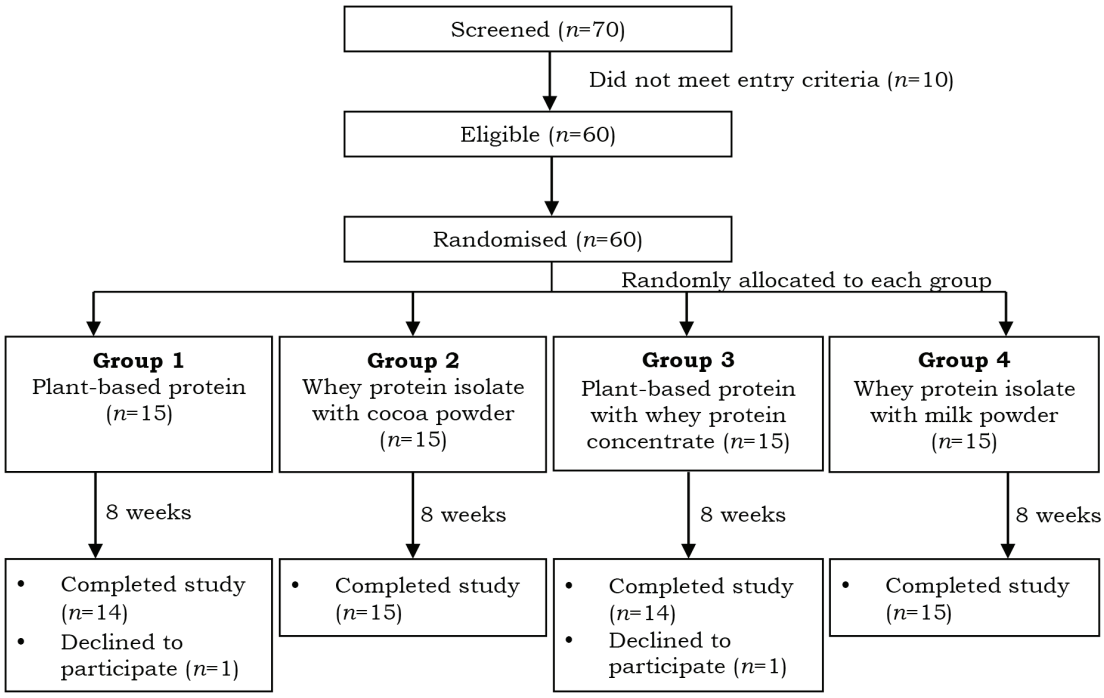

Figure 1. Flow diagram of the study

(3 males and 12 females), group 3 consisted of 14 participants (1 male and 13 females), and group 4 consisted of 15 participants (3 males and 12 females). Mean age of the participants was 43.1±4.1 years (group 1: 44.5±3.6 years; group 2: 43.4±5.0 years; group 3: 41.1±3.8 years; group 4: 42.6±3.6 years, p=0.239).

Effects of whey protein consumption on blood pressure and anthropometry

One hundred percent of the participants in each group consumed the supplement according to the study requirements. All participants underwent blood pressure and anthropometric assessment. Changes in blood pressure and anthropometric parameters are presented in Table 1. Blood pressure of the four study groups at pre-intervention and after the intervention was comparable (p>0.05 for all). Group 3 had significantly lower diastolic blood pressure at Week 8 (*MD*: -3.5±5.8 mmHg, p=0.049). WC of group 3 and group 4 was remarkably reduced after intake of the supplement for 8 weeks (*MD*: -3.3±3.9 cm, p=0.010 and -2.6±3.7 cm, p=0.017, respectively). A significant difference between the study groups existed regarding percentage visceral fat (p=0.024). Comparing within study groups, percentage visceral fat was significantly lower in group 3 after completion of the study (*MD*: -0.3±0.4%, p=0.019). All study groups showed a tendency towards lower fat mass and higher muscle mass after the intervention.

Effects of whey protein on biochemical parameters

FBG concentrations of group 1, group 2, and group 4 significantly increased after the study (*MD*: 0.2±0.4 mmol/L, p=0.048; 0.1±0.1 mmol/L, p=0.002; 0.2±0.3 mmol/L, p=0.035, respectively), while fasting insulin of group 2, group 3, and group 4 significantly decreased (*MD*: -28.0±41.0 pmol/L, p=0.024; -26.3±34.0 pmol/L, p=0.016; -15.2±24.4 pmol/L, p=0.036, respectively), as shown in Table 2. In addition, the HOMA-IR of group 3 was remarkably decreased at Week 8 (*MD*: -1.2±1.4, p=0.031). Regarding lipid profiles, group 2 presented a lower level of total cholesterol (*MD*: -0.4±0.5 mmol/L, p=0.007) and LDL-C (*MD*: -0.4±0.5 mmol/L, p=0.004) after the intervention. Likewise, after the study, group 4 showed a significantly lower level of TG (*MD*: -0.1±0.3 mmol/L, p=0.026). Regarding liver and kidney functions, comparing Week 8 with Week 0, AST of group 1 significantly dropped (*MD*: -0.0±0.0 μkat/L, p=0.034), similarly for ALT of group 3 (*MD*: -0.1±0.1 μkat/L, p=0.028). On the contrary, BUN of group 1 and group 4 significantly increased (*MD*: 0.8±1.2 mmol/L, p=0.040 and 0.4±0.8 mmol/L, p=0.042, respectively). A between-group difference in the level of uric acid was observed, with the highest level recorded in group 1 (p=0.008).

Effects of whey protein on markers of inflammation, antioxidants, and oxidative stress

Regarding inflammatory markers, there were significant changes in levels of hs-CRP, IL-6, and lactoferrin, as shown in Table 3. A remarkable increase in hs-CRP level was observed in group 1 after completion of the study (*MD*: 6.5±9.9 nmol/L, p=0.043), while the level of IL-6 of all study groups was reduced (p<0.05 for all). Likewise, lactoferrin significantly decreased in group 1, group 2, and group 3, and tended to decrease in group 4 (*MD*: -259.7±283.3 μg/L, p=0.004; -406.5±332.4 μg/L, p<0.001; -744.9±586.6 μg/L, p=0.001; -317.6±557.9 μg/L, p=0.063, respectively). A noteworthy increase in antioxidant capacity was found in group 2 (*MD*: 1.7±2.6 μM TE/ml, p=0.026), with other groups showing slight increments. Group 1 and group 4 presented a

Table 1. Comparison of blood pressure and anthropometric parameters between Week 0 and Week 8

Variables	*Group 1 (n=14)*	*Group 2 (n=15)*	*Group 3 (n=14)*	*Group 4 (n=15)*	$p^{\dagger}$
Systolic blood pressure (mmHg)					
Week 0	132.8±13.0	125.0±14.2	124.3±14.6	125.2±11.7	0.388
Week 8	129.2±11.1	121.0±12.2	119.6±12.9	123.8±7.9	0.175
$p^{\ddagger}$	0.319	0.208	0.112	0.581	
MD	-3.5±11.2	-3.9±11.0	-4.6±9.8	-1.3±9.0	
Diastolic blood pressure (mmHg)					
Week 0	89.9±11.1	81.1±12.3	80.4±10.5	80.0±9.5	0.105
Week 8	83.5±12.0	78.0±9.9	76.9±10.3	76.0±7.0	0.256
$p^{\ddagger}$	0.066	0.095	0.049*	0.072	
MD	-6.3±10.2	-3.1±6.5	-3.5±5.8	-4.0±7.9	
Body weight (kg)					
Week 0	78.4±16.9	71.1±9.6	70.2±10.5	71.7±10.9	0.340
Week 8	78.1±17.1	70.6±9.2	69.7±10.1	72.8±12.7	0.351
$p^{\ddagger}$	0.725	0.182	0.180	0.425	
MD	-0.2±2.3	-0.5±1.4	-0.4±1.2	1.0±5.0	
Body mass index (kg/m^2)					
Week 0	28.6±5.2	26.9±2.9	28.1±3.2	28.1±2.6	0.640
Week 8	28.5±5.4	26.7±3.1	27.9±3.1	28.5±3.3	0.565
$p^{\ddagger}$	0.793	0.242	0.203	0.421	
MD	-0.1±0.8	-0.1±0.5	-0.1±0.4	0.4±1.8	
Waist circumference (cm)					
Week 0	99.2±12.9	95.6±8.1	96.7±7.8	97.0±7.0	0.793
Week 8	99.0±12.3	92.7±9.2	93.4±7.7	94.3±6.0	0.316
$p^{\ddagger}$	0.754	0.069	0.010*	0.017*	
MD	-0.2±2.6	-2.9±5.8	-3.3±3.9	-2.6±3.7	
Fat mass (kg)					
Week 0	26.2±6.8	25.5±5.7	27.1±4.7	26.7±4.7	0.896
Week 8	22.5±7.7	24.7±6.2	26.4±5.3	26.2±4.5	0.451
$p^{\ddagger}$	0.207	0.077	0.109	0.063	
MD	-3.7±6.9	-0.8±1.5	-0.6±1.3	-0.4±0.8	
Visceral fat (%)					
Week 0	11.8±3.6	8.9±2.4	8.5±2.1	9.5±3.3	0.050
Week 8	11.7±3.5^{a}	8.6±2.2^{a,b}	8.2±2.0^{b}	9.5±3.2^{a,b}	0.024*
$p^{\ddagger}$	0.678	0.104	0.019*	1.000	
MD	-0.1±0.7	-0.2±0.5	-0.3±0.4	0.0±0.5	
Muscle mass (kg)					
Week 0	43.4±8.1	42.5±7.9	39.4±6.3	41.6±6.1	0.574
Week 8	43.7±8.0	42.9±7.9	39.6±6.2	41.8±5.6	0.523
$p^{\ddagger}$	0.223	0.136	0.053	0.321	
MD	0.3±0.7	0.3±0.9	0.2±0.3	0.1±0.6	

Group 1: PBP; group 2: WPI with cocoa powder; group 3: PBP with WPC; group 4: WPI with milk powder

Data are presented as mean±*SD*, *MD*=Mean difference.

†*p*-values were calculated using one-way ANOVA test.

‡*p*-values were calculated using paired *t*-test.

$^{a, b, c}$ Different alphabets denote significant difference between groups.

Table 2. Comparison of biochemical parameters between Week 0 and Week 8

Variables	*Group 1 (n=14)*	*Group 2 (n=15)*	*Group 3 (n=14)*	*Group 4 (n=15)*	$p^{\dagger}$
Fasting blood glucose (mmol/L)					
Week 0	5.2±0.4	5.0±0.5	5.9±3.6	5.0±1.2	0.582
Week 8	5.5±0.4	5.2±0.5	6.1±4.2	5.2±1.3	0.669
$p^{\ddagger}$	0.048*	0.002**	0.204	0.035*	
MD	0.2±0.4	0.1±0.1	0.2±0.6	0.2±0.3	
Fasting insulin (pmol/L)					
Week 0	34.5.0±27.4	55.0±33.5	53.5±36.3	48.1±30.8	0.383
Week 8	41.1±32.5	26.9±26.8	27.1±32.0	32.8±15.0	0.523
$p^{\ddagger}$	0.402	0.024*	0.016*	0.036*	
MD	6.6±26.3	-28.0±41.0	-26.3±34.0	-15.2±24.4	
HOMA-IR					
Week 0	1.4±1.0	1.9±1.0	1.9±1.2	1.5±0.4	0.477
Week 8	1.7±1.3	1.1±1.0	0.7±0.3	1.1±0.4	0.182
$p^{\ddagger}$	0.460	0.069	0.031*	0.084	
MD	0.2±1.1	-0.8±1.4	-1.2±1.4	-0.3±0.5	
Total cholesterol (mmol/L)					
Week 0	5.6±1.0	5.6±0.9	5.4±1.0	5.2±0.7	0.657
Week 8	5.3±0.9	5.1±0.7	5.3±1.0	5.0±0.6	0.776
$p^{\ddagger}$	0.310	0.007**	0.411	0.251	
MD	-0.2±0.8	-0.4±0.5	-0.1±0.5	-0.1±0.5	
Triglyceride (mmol/L)					
Week 0	1.7±0.7	1.2±0.6	1.3±0.6	1.5±0.7	0.331
Week 8	1.4±1.0	1.2±0.8	1.3±0.5	1.3±0.7	0.952
$p^{\ddagger}$	0.344	0.742	0.723	0.026*	
MD	-0.2±0.9	0.0±0.5	-0.0±0.5	-0.1±0.3	
HDL-C (mmol/L)					
Week 0	1.2±0.2	1.5±0.3	1.4±0.2	1.4±0.4	0.121
Week 8	1.2±0.2	1.5±0.3	1.4±0.2	1.4±0.3	0.124
$p^{\ddagger}$	0.467	0.924	0.896	0.344	
MD	0.0±0.1	0.0±0.0	-0.0±0.1	-0.0±0.2	
LDL-C (mmol/L)					
Week 0	3.5±0.9	3.5±1.0	3.4±0.9	3.0±0.7	0.461
Week 8	3.3±1.0	3.0±0.7	3.3±0.8	3.0±0.5	0.529
$p^{\ddagger}$	0.451	0.004**	0.556	0.867	
MD	-0.1±0.8	-0.4±0.5	-0.0±0.5	-0.0±0.5	
AST (μkat/L)					
Week 0	0.3±0.1	0.3±0.0	0.3±0.1	0.3±0.1	0.548
Week 8	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.870
$p^{\ddagger}$	0.034*	0.751	0.085	0.142	
MD	-0.0±0.0	-0.0±0.0	-0.0±0.0	-0.0±0.1	

Table 2. Comparison of biochemical parameters between Week 0 and Week 8 (continued)

Variables	*Group 1 (n=14)*	*Group 2 (n=15)*	*Group 3 (n=14)*	*Group 4 (n=15)*	$p^{\dagger}$
ALT (µkat/L)					
Week 0	0.3±0.1	0.2±0.0	0.4±0.3	0.4±0.2	0.277
Week 8	0.3±0.1	0.2±0.1	0.3±0.2	0.3±0.1	0.860
$p^{\ddagger}$	0.376	0.355	0.028*	0.176	
MD	-0.0±0.1	-0.0±0.0	-0.1±0.1	-0.0±0.1	
Blood urea nitrogen (mmol/L)					
Week 0	4.3±1.1	4.4±1.2	3.9±0.7	4.3±1.2	0.603
Week 8	5.1±1.4	4.9±1.2	4.0±1.0	4.8±1.2	0.118
$p^{\ddagger}$	0.040*	0.245	0.694	0.042*	
MD	0.8±1.2	0.4±1.4	0.1±1.1	0.4±0.8	
Creatinine (µmol/L)					
Week 0	70.8±15.6	63.7±9.8	62.0±11.7	71.8±9.3	0.079
Week 8	73.1±17.9	65.0±9.5	63.1±10.7	73.3±10.2	0.070
$p^{\ddagger}$	0.376	0.263	0.283	0.234	
MD	2.3±8.1	1.2±3.9	1.1±3.7	1.4±4.5	
Uric acid (µmol/L)					
Week 0	364.3±73.9	310.6±78.5	295.3±92.2	338.2±80.6	0.157
Week 8	391.1±87.4[a]	289.7±51.9[b]	282.5±79.7[b,c]	333.9±108.2[a,b,c]	0.008**
$p^{\ddagger}$	0.231	0.084	0.177	0.814	
MD	26.7±73.1	-20.8±41.5	-12.7±33.3	-4.3±70.5	

HOMA-IR, homeostatic model assessment of insulin resistance; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase

Group 1: PBP; group 2: WPI with cocoa powder; group 3: PBP with WPC; group 4: WPI with milk powder

Data are presented as mean±*SD*, *MD*=Mean difference

†*p*-values were calculated using one-way ANOVA test.

‡*p*-values were calculated using paired *t*-test.

[a, b, c] Different alphabets denote significant difference between groups.

significant decrease in the oxidative stress marker, malondialdehyde (MDA), (*MD*: -885.8±1044.5 nmol/ml, *p*=0.025; -750.9±979.8 nmol/ml, *p*=0.022, respectively), while MDA in the remaining groups tended to decrease.

Dietary intake of the participants

Comparing between study groups, differences were detected in energy intake, protein intake, and fat intake at week 8 (*p*=0.036, *p*=0.006, and *p*=0.002, respectively), as shown in Table 4. The dietary patterns at Week 0 and at Week 8 within group 1, group 2, and group 3 were similar (*p*>0.05 for all). However, at Week 8, group 1 and group 3 were likely to have lower energy intake, while group 2 and group 4 tended to have higher energy intake compared to pre-intervention. A significant difference within the study group was detected in group 4. After the intervention, the average fat intake of group 4 was greater than pre-intervention (*MD*: 9.7±11.7 g, *p*=0.015).

Table 3. Changes in markers of inflammation, antioxidants, and oxidative stress between Week 0 and Week 8

Variables	*Group 1 (n=14)*	*Group 2 (n=15)*	*Group 3 (n=14)*	*Group 4 (n=15)*	$p^{\dagger}$
hs-CRP (nmol/L)					
Week 0	18.7±14.9	27.8±19.4	38.1±22.7	30.6±26.8	0.200
Week 8	25.3±21.9	33.0±25.8	39.4±25.3	34.9±21.2	0.547
$p^{\ddagger}$	0.043*	0.234	0.827	0.229	
MD	6.5±9.9	5.1±14.1	1.2±18.9	4.2±11.6	
TNF-α (pg/ml)					
Week 0	198.5±189.9	94.7±52.8	318.8±238.9	222.4±225.0	0.080
Week 8	162.0±208.5	137.9±88.9	351.5±314.9	357.4±269.2	0.071
$p^{\ddagger}$	0.196	0.101	0.669	0.109	
MD	-36.5±91.9	43.1±74.6	32.7±246.2	135.0±207.8	
IL-6 (pg/ml)					
Week 0	310.2±50.9	326.6±36.9	297.0±76.2	265.4±93.7	0.156
Week 8	81.6±22.1	102.0±57.0	174.4±160.2	147.4±82.0	0.079
$p^{\ddagger}$	<0.001***	<0.001***	0.020*	0.002**	
MD	-228.6±56.4	-224.5±73.8	-122.5±173.5	-117.9±112.2	
Lactoferrin (μg/L)					
Week 0	2,266.2±409.0	2,277.5±240.7	2,309.8±433.8	2,158.7±412.1	0.758
Week 8	2,006.5±292.6[a]	1,871.0±264.6[a,b]	1,564.9±503.6[b]	1,841.0±410.5[a,b]	0.029*
$p^{\ddagger}$	0.004**	<0.001***	0.001**	0.063	
MD	-259.7±283.3	-406.5±332.4	-744.9±586.6	-317.6±557.9	
ORAC (μM TE/ml)					
Week 0	13.0±0.9	12.7±1.5	13.3±1.8	13.2±1.8	0.726
Week 8	13.3±2.2	14.4±2.1	13.9±1.7	13.8±3.6	0.755
$p^{\ddagger}$	0.641	0.026*	0.489	0.528	
MD	0.3±2.6	1.7±2.6	0.5±2.6	0.6±3.7	
MDA (nmol/ml)					
Week 0	1,994.0±726.0	2,083.6±1,172.2	2,071.7±1,161.1	1,864.9±580.0	0.940
Week 8	1,108.2±574.3	1,167.1±652.1	1,432.9±718.6	1,114.0±671.5	0.595
$p^{\ddagger}$	0.025*	0.064	0.186	0.022*	
MD	-885.8±1044.5	-916.5±1539.2	-638.7±1566.8	-750.9±979.8	

hs-CRP, high sensitivity C-reactive protein; TNF-α: tumour necrosis factor-alpha; IL-6: interleukin-6; ORAC: oxygen radical absorbance capacity; MDA, malondialdehyde
Group 1: PBP; group 2: WPI with cocoa powder; group 3: PBP with WPC; group 4: WPI with milk powder
Data are presented as mean±*SD*, *MD*=Mean difference.
†*p*-values were calculated using one-way ANOVA test.
‡*p*-values were calculated using paired *t*-test.
[a, b] Different alphabets denote significant difference between groups.

DISCUSSION

Inflammatory markers increase with fat accumulation (de Heredia, Gómez-Martínez & Marcos, 2012; WHO, 2000). An increase in these markers may interfere with the production and secretion of appetite regulating hormones such as leptin. This may result in raised appetite and probably stimulates fat accumulation, thus increasing blood lipids such as TG and LDL-C, and the risk of insulin

Table 4. Comparison of dietary intake between Week 0 and Week 8

Variables	*Group 1 (n=14)*	*Group 2 (n=15)*	*Group 3 (n=14)*	*Group 4 (n=15)*	$p^{\dagger}$
Energy intake (kcal/d)					
Week 0	1063±203	915±254	1018±227	1066±167	0.376
Week 8	1053±314	929±165	900±151	1167±257	0.036*
$p^{\ddagger}$	0.920	0.837	0.216	0.122	
MD	-10±210	14±235	-12±264	101±187	
Carbohydrate (g/d)					
Week 0	73.0±35.0	71.8±25.7	85.4±37.7	79.8±26.9	0.719
Week 8	92.5±43.0	89.8±24.9	81.1±29.4	90.3±23.4	0.830
$p^{\ddagger}$	0.096	0.073	0.601	0.300	
MD	19.5±33.2	18.0±31.5	-4.3±26.7	10.4±31.8	
Protein (g/d)					
Week 0	78.7±17.4	67.2±10.6	65.4±19.8	81.1±18.0	0.071
Week 8	82.8±17.0[a]	72.5±8.6[a,b]	63.6±16.6[b]	83.2±14.7[a]	0.006**
$p^{\ddagger}$	0.317	0.213	0.668	0.703	
MD	4.0±12.1	5.2±15.1	-1.7±14.4	2.1±15.9	
Fat (g/d)					
Week 0	43.8±15.9	33.5±13.0	29.1±4.6	42.8±16.5	0.084
Week 8	56.5±25.0[a]	40.7±11.5[a,b]	24.5±6.5[b]	52.6±16.8[a]	0.002**
$p^{\ddagger}$	0.169	0.152	0.139	0.015*	
MD	12.6±26.6	7.1±16.1	-4.6±7.1	9.7±11.7	

Group 1: PBP; group 2: WPI with cocoa powder; group 3: PBP with WPC; group 4: WPI with milk powder

Data are presented as mean±*SD*, *MD*=Mean difference.

†*p*-values were calculated using one-way ANOVA test.

‡*p*-values were calculated using paired *t*-test.

[a, b] Different alphabets denote significant difference between groups.

resistance, which can eventually lead to non-communicable diseases (Witard *et al.*, 2014). Whey protein is currently trendy among health-conscious people due to its advantages in maintaining a balance between muscle and fat mass. Therefore, this study aimed to evaluate the health effects of consuming 50 g of whey protein for 8 consecutive weeks in overweight and obese individuals.

The participants were likely to have reduced systolic and diastolic blood pressure after the study, especially those who consumed PBP with WPC, which significantly decreased diastolic blood pressure. Likewise, remarkable changes were also found in WC and visceral fat in the participants who consumed PBP with WPC. The effects of whey protein consumption on anthropometric parameters found in this study, especially abdominal obesity markers, were consistent with previous studies. A decrease in body weight, BMI, and WC was reported among the participants who consumed protein-fortified biscuits (total protein 50 g/day) for 8 weeks, compared to those who consumed biscuits fortified with wheat bran (Hassanzadeh-Rostamia, Abbasib & Faghiha, 2020). Similarly, a study conducted by Yang *et al.* (2014) also found a decrease in body weight, BMI, and WC in participants who consumed 30 g whey protein concentrate daily for 12 weeks. Additionally, previous studies

have also reported a decrease in fat mass and an increase in basal metabolic rate after consumption of whey protein (Zhou *et al.*, 2011; Acheson *et al.*, 2011).

About biochemical parameters, the participants who were supplemented with PBP, or WPI with cocoa powder, or WPI with milk powder, revealed an increasing trend of FBG. While intake of whey protein resulted in decrease of fasting insulin concentration, insulin tended to increase in those who consumed PBP. Regarding HOMA-IR, participants who consumed PBP with WPC saw reduced risk in insulin resistance, whereas the risk was more likely to increase in the group that consumed PBP. However, in an Australian study, obese participants had no remarkable change in serum glucose after supplementation with whey protein for 12 weeks. Nevertheless, there was a significant decrease in fasting insulin, HOMA-IR, total cholesterol, and LDL-C (Pal, Ellis & Dhaliwal, 2010). Similarly, glucose levels among hypertensive adults who consumed whey protein were also not altered, whereas insulin sensitivity was greater (Fekete *et al.*, 2018).

A meta-analysis conducted by Amirani *et al.* (2020) revealed a significant reduction in insulin and HOMA-IR, as well as blood lipids including TG, total cholesterol, and LDL-C after intake of whey protein. In this study, atherogenic blood lipids including total cholesterol and LDL-C were outstandingly reduced after consumption of WPI with cocoa powder. A significant alteration was also found in the group which received WPI with milk powder, in the form of decreased TG after the study. Dietary intake may be the reason for explaining the cause of the alterations in both anthropometric parameters and biochemical parameters. The subject in this study had no difference in dietary intake between pre- and post-intervention, except group 4 who had increased fat intake. According to Thai recommended dietary intake (Thai RDI), the subjects consumed approximately 1 g protein/kg body weight/day, which was enough for their daily requirement. Since there were no differences in the amount of dietary intake found in this study and the subjects did not report changes in their physical activities, the alterations may have been a result of the quality of protein intake.

The effects of prolonged consumption of whey protein on liver and kidney functions are still doubted. A previous study reported that whey protein supplementation decreases levels of AST and uric acid (Chen *et al.*, 2014). In this study, AST level, an indicator of liver damage, was significantly lower after ingestion of PBP. Besides, the participants who consumed PBP with WPC showed a supportive effect on the liver as the level of ALT, an enzyme that is generally released when liver cells are damaged, was remarkably reduced. However, a marker for measuring the waste products of protein metabolism in the body, BUN, was significantly increased among the participants who consumed PBP and WPI with milk powder after completing the study. A trend towards elevated BUN was also found among those who consumed WPI with cocoa powder or PBP with WPC. Therefore, the alterations probably resulted from higher protein consumption and insufficient intake of water. The interventions had no significant effects on levels of creatinine and uric acid. However, consumption of whey protein tended to lower the risk of precipitation of monosodium urate around tissues surrounding the joints and kidneys, but consumption of plant-based protein presented an increasing trend.

Although protein supplements resulted in a positive alteration of IL-6 inflammatory marker, PBP revealed a significant negative effect on inflammation by increasing levels of hs-

CRP. In addition, lactoferrin, a protein found in mammalian milk that plays a role in regulating inflammatory response by stimulating pro-inflammatory cytokine secretion, promoting the digestive tract, and preventing oxidative stress (Czosnykowska-Łukacka *et al.*, 2019; Demmelmair *et al.*, 2017; Queiroz, Assis & Júnior, 2013; Actor, Hwang & Kruzel, 2009), was decreased after intake of the given supplement. A previous study reported that after consumption of 15 g whey protein for 12 weeks, hs-CRP and IL-6 levels in overweight and obese participants were more likely to reduce (Yang *et al.*, 2019). In accordance, decreased levels of CRP, IL-6, and TNF were also presented after consuming 54 g WPI for 12 weeks (Pal & Ellis, 2010). In addition, a study also found alterations in inflammatory markers after the intake of a 60 g whey protein with 30 g high-fibre wheat bran product for 12 weeks. The study reported that TNF-α was significantly reduced, whereas hs-CRP did not alter (Rakvaag *et al.*, 2019). Similarly, there was no difference found in hs-CRP level after consumption of a high or low protein diet (Santesso *et al.*, 2012).

Regarding the effect of whey protein on antioxidant capacity, an increasing trend was found in all participants, especially among those who consumed PBP with WPC. This group had significantly elevated ORAC. Reasonably, the oxidative stress marker, malondialdehyde, was remarkably decreased after consecutively consuming PBP and WPI with milk powder. A reduced trend was also observed in those who consumed PBP with WPC or WPI with cocoa powder. However, a previous study reported that daily consumption of 40 g whey protein for 12 consecutive weeks had no effect on oxidation process, inflammatory response, and blood glucose regulation, although fat mass was significantly reduced (Flaim *et al.*, 2017).

This study had limitations on gender-based outcomes. There were no data regarding gender differences due to the small sample size in each group. Therefore, further study should include analysis and results regarding the differences between males and females.

CONCLUSION

Whey protein can potentially decrease the risk of non-communicable diseases by promoting proper fat mass and muscle mass, as well as regulating atherogenic forms of blood cholesterol. The positive effects of whey protein on health seem to be greater than that of PBP as whey protein consumption did not interfere with kidney and liver functions. Consumption of protein supplements was found to reduce the secretion of the inflammatory marker, IL-6, while PBP resulted in increased hs-CRP. In addition, antioxidant capacity was significantly higher and oxidative stress marker level was significantly decreased after intake of whey protein. Thus, proper consumption of whey protein will be beneficial to health in terms of regulating body composition and inflammation, as well as promoting antioxidant function in the body.

List of abbreviations

PBP: plant-based protein; WPI: whey protein isolate; WPC: whey protein concentrate; BMI: body mass index; hs-CRP: high sensitivity C-reactive protein; IL-6: interleukin-6; TNF-α: tumour necrosis factor-alpha; WC: waist circumference; FBG: fasting blood glucose; HOMA-IR: homeostatic model assessment of insulin resistance; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglyceride; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; ORAC: oxygen radical absorbance capacity; MD: mean difference.

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Authors' contributions

CP, principal investigator, conceptualised and designed the study, supervised data collection, advised on data analysis and interpretation, prepared the draft of the manuscript, and reviewed the manuscript; CP, led the data collection, data analysis and interpretation, assisted in drafting of the manuscript, and reviewed the manuscript; CH, conceptualised and designed the study, advised on data analysis and interpretation, and reviewed the manuscript; PPP, led the data collection and reviewed the manuscript; KK, advised on data analysis and interpretation, and reviewed the manuscript.

Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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